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Sedative, membrane stability, cytotoxic and antioxidant properties of methanol extract of leaves of *Protium serratum* Wall.

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ABSTRACT

Objective: To study the sedative, membrane stability, cytotoxic and antioxidant properties of the leaves of *Protium serratum* extracted using methanol.

Methods: Sedative test was performed using hole cross and open field methods at 200 and 400 mg/kg. Membrane stability of red blood cell was used for anti-inflammatory test at different concentrations. Cytotoxic study was performed using brine shrimp lethality test. Total flavonoid contents, total phenol contents and reducing power were used to assess antioxidant properties of the extract.

Results: Extract showed better sedative action at lower doses in both experiments. Maximum 73.33% locomotion reduction was found at 200 mg/kg at 120 min and that was 89.29% for diazepam in hole cross test. In membrane stability test, extract and standard drug diclofenac have 35.66% and 91.20% stability, respectively. LC_{50} value of the extract was 22.91 μg/mL. Total phenol and flavonoid contents were (55.53±14.63) mg gallic acid equivalent per gram of extract and (106.33±7.35) mg of quercetin equivalent per gram of extract, respectively per gram of extract. Significant reducing power was observed as compared to ascorbic acid.

Conclusions: Extract possesses good pharmacological properties. Hence, further extensive study is essential to find out possible active constituents for the treatment of anxiety, inflammation or sickle cell disease, cancer and free radical mediated abnormalities.

1. Introduction

Plants play very vital roles in our daily life. In earth, plants are known in pharmacy. People are using various kinds of plants before thousands years ago for the treatment of ailments. The World Health Organization estimates that 80% of African and Asian populations use traditional medicine as the first source for their health care needs[1]. Many modern drugs which are derived directly or indirectly from the medicinal plants are now used successfully. High class ingredients are preferred in medicinal plants which can be used as a drug development and drug synthesis. Plants contain diverse phytoconstituents and they possess distinct and unique properties. So, variant chemical structures and pharmacological actions of the plant constituents help us to find out effective drugs.

Protium serratum (Wall. ex Colebr.) Engl. (*P. serratum*) is a genus of more than 140 species of flowering plants in the family Burseraceae. The synonym of the plant is *Bursera serrata*. It is known as Chitrika, Hiliabhadi, Gutgutya, Neul and Neuor in Bengali. The local name of *P. serratum* is Gutgutiya (Chakma), Shu Dui Shi (Marma), Thai Cherem (Tripura). It is native in Bangladesh, Assam and Philippines. This large, evergreen and perennial tree found in the hill tracks of Bangladesh. Leaf blades are oblong, oblong-lanceolate, acuminate. Flowers are green and drupes are bright pink. The mature fruit is edible. They are also used in incense and perfumes. Bark paste is applied on boils and scabies[2]. Most of the members

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of Burseraceae are known for their aromatic resins or gums and turpentine[3]. Turpentine compounds have sedative and anti-inflammatory effects. Some species of the Burseraceae family reported to have pharmacological properties. Hexane extracts of *Bursera simaruba* (L.) Sarg. leaves display anti-inflammatory activity on the adjuvant-carrageenan-induced inflammation in rats[4]. *Bursera schlechtendalii* (Burseraceae) has shown antitumor activity against the 9KB (adenocarcinoma of nasal pharynx) test system[5]. The extract of the plant *Bursera fagaroides* contains 3 compounds, which apparently are glycosides with a potent activity upon agglutination -immobilization and a low effect upon spermatocytes viability, which might be used as contraceptives[6]. Aqueous methanol extract of the leaves and fruits of the plant possess antioxidant activity[7]. Rashid *et al.*, isolated two terpenoids, β-amyrin (1) and β-sitostenone (2) and a coumarin, scopoletin (3) from petroleum ether and dichloromethane soluble extracts of the stem bark of *P. serratum* Wall. and dichloromethane extract was also reported to have antimicrobial and cytotoxic activity[8].

β-Amyrin, β-sitostenone and scopoletin present in *P. serratum* also isolated from different plant sources and possessed diverse pharmacologic actions. The mixture of α- and β-amyrin, pentacyclic triterpenes isolated from the stem bark resin of *Protium heptaphyllum* evidenced sedative and anxiolytic effects that might involve an action on benzodiazepine-type receptors and also an antidepressant effect where noradrenergic mechanisms will probably play a role[9]. β-Amyrin palmitate isolated from the leaves of *Lobelia inflata* possesses sedative action[10]. α and β-Amyrin retard acute inflammation in rat model of periodontitis[11]. *Quassia amara* (Family: Simaroubaceae) was reported to have antinociceptive, anti-inflammatory, muscle-relaxant, and sedative effects in rats and mice, and β-sitostenone, β-sitosterol, β-carbolines, β-dehydroquassins were main phytochemicals in it[12,13]. Antidepressant-like effect of scopoletin, a coumarin from *Polygala sabulosa* is dependent on the serotonergic [5-HT(2A) receptors], noradrenergic [(α1)- and (α2)-adrenoceptors] and dopaminergic [dopamine D(1) and D(2) receptors] systems[14]. Scopoletin shows anti-inflammatory activities through inhibition of eicosanoid biosynthesis, cell influx, and peroxidation[15]. *P. serratum* is medicinally important and contains active constituents (terpenoids and coumarin), so as a part of our ongoing research with medicinal plants from Bangladesh, we investigated sedative, membrane stability, cytotoxic and antioxidant activities of methanol extract of the plant.

2. Materials and methods

2.1. Plant material

The plant *P. serratum* was collected from Cox's Bazar, Chittagong, Bangladesh in 2013. The plant was identified by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Bangladesh. A voucher specimen has been retained in the Chittagong Forestry with accession number 38015.

2.2. Preparation of extract

The collected plants were washed thoroughly with water, chopped and then dried under shade. The leaves were heated at 35 °C for 1 h and pulverized in a mechanical grinder. The powder obtained was successively extracted in methanol. The extract was made to dry by using rotary evaporator under reduced pressure.

2.3. Animals

Swiss albino mice of either sex having weight 25-30 g were collected from International Centre for Diarrhoeal Disease and Research, Bangladesh. The animals were housed under standard laboratory conditions [relative humidity 55%-65%, room temperature (23.0±2.0) °C and 12 h light: dark cycle] and acclimatized for 7 d. The animals were fed with standard diet and water.

2.4. Sedative activity

2.4.1. Hole cross test

The test was carried out as described by Takagi *et al*[16]. Hole cross apparatus is a wooden box having a dimensions of 30 cm×20 cm×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of a partitioning wall in the cage. Each mouse was immediately placed on one side of the cage after oral administration of test drugs. The number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 90 and 120 min.

2.4.2. Open field test

The experiment was performed according to the methods described by Gupta *et al*[17]. The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the mice was counted for 3 min on 0, 30, 60 and 120 min during the study period.

2.4.3. Membrane stability test

The test was performed with the approval of Ethics Committee of Pharmacy Department, International Islamic University Chittagong (approval No. ECPD-IIUC2013/09). Fresh blood was collected from healthy human volunteer under supervision of a physician. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at

3 000 r/min and packed cells were washed with isosaline (0.85% NaCl or NaOH, pH=7.2) for three times until clear supernatant solution was obtained. Red blood cell (RBC, 10%) suspension was then prepared with normal saline and kept at 4 °C undisturbed before use. The assay mixtures consisted of 2 mL of hyposaline (0.25%, w/v) sodium chloride, 1.0 mL of 0.15 mol/L sodium phosphate buffer, pH=7.4, 0.5 mL of RBC (10%, v/v) suspension, and 1.0 mL of drugs (extract/standard). Two controls were prepared, one with 1.0 mL of isotonic saline with RBCs, (blood control) and the other one with 1.0 mL of extract solution without RBCs (test control). All the assay mixtures were incubated at 37 °C for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm. The percentage of haemolysis was calculated by using the following formula.

$$\%\ \text{Stabilizing activity}=100-\frac{\text{Test absorbance}-\text{Test control absorbance}}{\text{Blood control absorbance}}\times 100$$

2.5. Brine shrimp lethality bioassay

The cytotoxicity of crude extract was performed at concentrations of 10, 50, 100, 200, 300 and 500 μg/mL on brine shrimp nauplii using Meyer method[18]. Ten matured shrimps were applied to each of all experimental test tubes and the number of survived nauplii in each test tube was counted after 24 h. From this data, the percentage (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\%\ \text{Mortality}=\frac{N_t}{N_0}\times 100$$

Where, N_t refers to number of killed nauplii after incubation for 24 h; N_0 is number of total nauplii transferred *i.e.* 10.

The LC_{50} was then determined using regression analysis after plotting % mortality versus log concentration of the drug.

2.6. Estimation of antioxidant constituents

Polyphenols are considered to be the main phytoconstituents responsible for *in vitro* antioxidant activity. So before examining the antioxidant activity, total phenol and flavonoid content in the extract of the leaves were estimated as follows.

2.6.1. Total phenolic content

This was determined by the using Folin–Ciocalteu reagent as described by Slinkard and Singleton[19]. For this test, 0.5 mL extract (200 μg/mL) was added with 2.5 mL Folin–Ciocalteu reagent (diluted 10 times with water) that means 2 mL Folin–Ciocalteu reagent added with 8 mL water and 2.5 mL sodium carbonate solution (7.5%). Then the test tube was incubated for 20 min at 25 °C. Then absorbance was taken 760 nm using a spectrophotometer. Gallic acid was used for a calibration standard curve.

2.6.2. Total flavonoid contents

Flavonoid presence in the plant extract was determined by using aluminum chloride colorimetric method. Plant extract (1 mL, 200 μg/mL) was mixed with 3 mL methanol and then mixed 10% aluminum chloride solution (200 μL) after that adding 200 μL of 1 mol/L potassium acetate solution and diluting with 5–6 mL water. The mixture was incubated for 30 min at room temperature and taken absorbance at 420 nm against the blank solution and recorded it.

2.6.3. Reducing power capacity

Reducing power of the extract was determined at different concentrations (1 000, 500, 250, 125 μg/mL) according to the method previously described[20]. Extract solution (1 mL) was mixed with 2.5 mL phosphate buffer and 2.5 mL potassium ferricyanide which was incubated at 50 °C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid solution was added in it. The mixture was centrifuged at 3 000 r/min for 10 min. After that, 2.5 mL supernatant solution was withdrawn and mixed with 2.5 mL distilled water. At last, 0.5 mL ferric chloride was added. Absorbance was measured at 700 nm against blank solution. A blank solution contained the same solution mixture without extract or standard and it was incubated under the same conditions as the rest of the samples solution. Here ascorbic acid is used as a standard.

3. Results

3.1. Sedative activity

3.1.1. Hole cross test

The sedative effect of methanol extract of *P. serratum* leaves was investigated at two doses (200 and 400 mg/kg body weight *p.o.*). The results were compared with control and standard groups and were statistically significant ($P<0.05$). Locomotion of the mice in all groups was found to decrease till observation period with variance degrees and it was more profound in test control (200 mg/kg) and diazepam (1 mg/kg) groups. The extract showed better activity at lower dose (Table 1). Maximum locomotion reduction was 73.33% for extract (200 mg/kg) at 120 min and was 89.29% for diazepam at the same time. The number of hole crossed by the 400 mg/kg extract treated mice was higher than the control group at all observation time except at 30 min. So, the extract did not show significant action at 400 mg/kg dose.

3.1.2. Open field test

The results obtained in the open field test were almost similar to those of hole cross test. The number of squares visited by the mice was reduced considerably in all groups throughout the study periods. From Table 2, it was found that the extract showed significant central nervous system (CNS) depressant activity at 200 mg/kg dose compared to control group and the result was comparable to standard drug diazepam.

Table 1

CNS depressant activity of methanolic extract of leaves of *P. serratum* on hole cross test in mice.

Group	Treatment	Dose (mL/kg, *p.o.*)	Number of movements				
			0 min	30 min	60 min	90 min	120 min
Group I (Control)	1% Tween 80 in water	10	17.800±3.470	13.000±2.160	9.600±3.020	8.300±2.170	6.900±1.130
Group II (Standard)	Diazepam	1	16.800±2.300	6.000±1.580*	4.000±1.870*	2.400±1.820*	1.800±0.840*
Group III (Extract)	MEPS	200	15.000±2.650	7.200±2.640*	5.330±1.530*	5.010±2.100*	4.000±2.370*
Group IV (Extract)	MEPS	400	15.330±2.520	12.670±4.620*	11.670±3.789*	10.000±4.000*	10.330±4.230*

All values are expressed as mean±SD (n=5); One-way analysis of variance (ANOVA) followed by Dunnet's test. *: P<0.05, significant difference compared to control. MEPS: Methanolic extract of leaves of *P. serratum*.

Table 2

CNS depressant activity of methanol extract of leaves of *P. serratum* on open field test in mice.

Group	Treatment	Dose (mL/kg, *p.o.*)	Number of movements				
			0 min	30 min	60 min	90 min	120 min
Group I	1% Tween 80 in water	10	83.40±4.70	78.00±3.92	66.40±4.14	49.20±2.51	47.00±3.68
Group II	Diazepam	1	79.85±2.45	59.60±2.21*	36.80±3.30*	20.70±4.92*	11.80±2.84*
Group III	MEPS	200	87.40±4.14	67.67±3.27*	31.67±3.60*	21.67±4.37*	19.68±4.21*
Group IV	MEPS	400	91.62±3.23	72.70±3.56*	63.00±2.67*	52.33±3.75*	40.67±1.93*

All values are expressed as mean±SD (n=5); One way analysis of variance followed by Dunnet's test. *: P<0.05, significant difference compared to control. MEPS: Methanolic extract of leaves of *P. seraatum*.

3.2. Membrane stability test

The extract showed dose dependent membrane stability effect on RBC. The percentage of hemolysis was proportionally decreased with extract concentration and maximum 35.66% membrane stability was obtained at 1 000 μg/mL whereas diclofenac sodium was 91.20% stability shown in Table 3.

Table 3

Membrane stability of methanol extract of leaves of *P. serratum*.

Group	Concentration (μg/mL)				
	62.5	125.0	250.0	500.0	1 000.0
Test	4.250±0.694	10.910±1.510	15.460±0.513	19.840±1.510	35.660±0.870
Standard	72.606±2.193	76.676±1.065	80.680±1.407	84.150±1.360	91.200±4.284

3.3. Brine shrimp lethality bioassay

In this test, the toxicity of extract was studied by measuring the effect of different concentrations ranging from 25-600 μg/mL on the nauplii. Percentage mortality is shown in Figure 1. The extract showed concentration dependent cytotoxicity. LC_{50} value of the crude extract found in the study was 22.91 μg/mL.

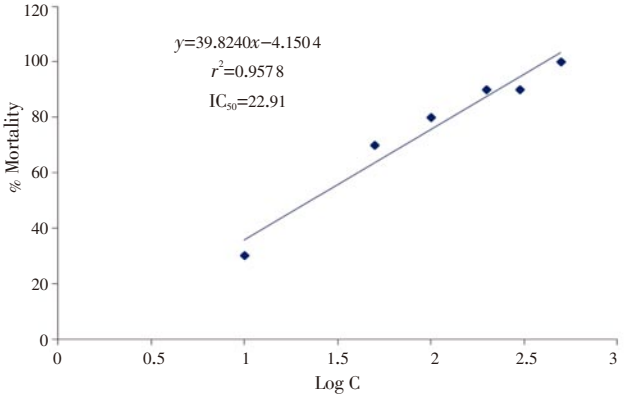

Figure 1. Cytotoxic activity of *P. serratum*.

3.4. Estimation of antioxidant constituents

3.4.1. Total phenolic content

The Folin-Ciocalteu reagent was used to estimate total phenols present in the extract. Phenol content of plant extract was determined from the calibration curve of gallic acid and the result was expressed as gallic acid equivalent (GAE) per gram of extract. Total phenol content in the extract was (55.53±14.63) mg GAE/g of extract.

3.4.2. Total flavonoid content

The amount of total flavonoid was determined by spectrophotometry. The total flavonoid content of the extract was calculated from the calibration curve of quercetin and the value was (106.33±7.35) mg of quercetin equivalent per gram of extract.

3.4.3. Reducing power capacity

Reducing power of the extract and ascorbic acid is shown in the Figure 2. High absorbance value of the reaction mixture indicated strong reducing power of the sample. Therefore, we observed greater reducing capacity of the sample at higher concentration. The extract showed significant reducing power activities as compared to ascorbic acid.

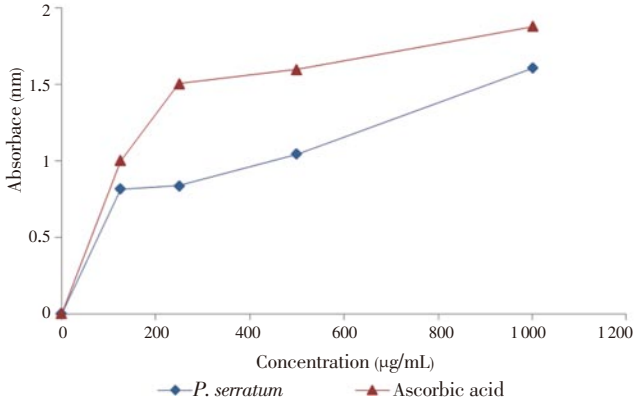

Figure 2. Reducing power capacity of leaves extract of *P. serratum*.

4. Discussion

The data presented in this study showed that the methanol extract of leaves of *P. serratum* possessed CNS depressant, membrane stability, cytotoxic and antioxidant properties. The activities of this extract were dose dependent in all experiments. Plant extract generally contains diverse phytoconstituents and it is very difficult to make assumption on mechanism actions of the extract. Locomotion reduction of mice in both hole cross and open field tests indicated sedative activity of crude extract. Flavonoids and terpenoids are active phytoconstituents of herbal hypnotics known to stimulate γ-aminobutyric acid (GABA) inhibitory neurotransmitter in the CNS[21]. Besides, it is believed that inhalation of fragrance is a powerful means of relaxation, reducing stress and workload and some of the odors are used in the treatment against bipolar disease, depression, anxiety and tension[22,23]. The plant has traditional uses in perfumes and incense and contains flavonoids, terpenoids and turpentine. So, the extract may possibly act by interacting with GABA-mediated synaptic transmission and with these phytoconstituents.

Vitality of cells depends on the integrity of their membranes[24]. Exposure of RBC to injurious substances such as hypotonic medium and phenylhydrazine results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin[25]. Compounds with membrane stability effects could be applied in the management of inflammation and sickle cell disease. Injury to RBC membrane will render the cell more susceptible to secondary damage through free radical induced lipid peroxidation. This notion is consistent with the observation that the breakdown of bio-membranes leads to the formation of free radicals which in turn enhance cellular damage[26,27]. The progression of bone destruction seen in rheumatoid patients for example, has been shown due to increased free radical activity[28,29]. It is expected that pharmacological agents with membrane-stabilizing action may offer anti-inflammatory activity through the prevention of the release of phospholipases or other chemical mediators[30]. Stability of RBC by these agents can also be explained to withstand higher concentrations of NaCl by increasing its volume, reverting the shape of the sickling to produce some biconcave shape, and thereby maintain membrane integrity. Such effects have been reported that homoserine inhibits *in vitro* sickling of erythrocytes in hypotonic solutions[31].

The low cost and ease of performing the assay and the commercial availability of cheap brine shrimp eggs makes brine shrimp lethality assay a very useful indicator for general toxicity and the identification of antitumor and pesticidal compounds. LC_{50} value of the methanol extract of *P. serratum* leaves in the present investigation was 22.91 μg/mL. LC_{50} value of dichloromethane soluble extract of stem bark as previously reported was 9.64 μg/mL. So, these toxicity variations may be relevant to different plant parts. The brine shrimp mortality might be due to the toxic compounds present in the plant extracts that possess larvicidal properties.

Free radicals or reactive oxygen species have direct or indirect effects on almost all disease states in our body. On the contrary, our pathophysiologic conditions generally increase free radical production beyond optimum and ultimately potentiate physiologic abnormalities. The compounds with antioxidant properties have beneficial effect against harmful action of free radicals. The present investigation shows that the methanol extract of *P. serratum* leaves possesses antioxidant activity indicative by good flavonoids contents and reducing capacity. Total flavonoids content (55.53 mg GAE/g of extract) of the extract in our study is comparable with previous reports (69.4 and 55.53 mg GAE/g). This variation may be due to geographical sites, season, collection process of plant part or experimental process.

On the basis of present investigation, we have concluded that methanol extract of *P. serratum* leaves possesses good sedative, membrane stability, cytotoxic and antioxidant properties. Since, extract contains diverse phytochemicals, so further extensive study is essential with the extract to find out possible active constituents for the treatment of anxiety, inflammation or sickle cell disease, cancer and free radical mediated abnormalities. We have greatly intended to isolate active compounds from the extract and investigate their pharmacological actions.

Conflict of interest statement

The authors declare that they have no competing interests.

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Pharm students.

References

[1] WHO. Traditional medicine. Geneva: WHO; 2008. [Online] Available from: http://www.who.int/mediacentre/factsheets/2003/fs134/en/ [Accessed on 20th March, 2014]

[2] Kandi B, Dhal NK, Mohanty RC. A case study on indigenous phytotherapy for skin diseases in Nuapada district, Odisha, India. *Int J Herb Med* 2013; **1**(2): 117-123.

[3] Huq AM, Hasan H. *Flora of Bangladesh* (*Burseraceae*). Dhaka: Bangladesh Agricultural Research Council; 1987.

[4] Carretero ME, López-Pérez JL, Abad MJ, Bermejo P, Tillet S, Israel A, et al. Preliminary study of the anti-inflammatory activity of hexane extract and fractions from *Bursera simaruba* (Linneo) Sarg. (Burseraceae) leaves. *J Ethnopharmacol* 2008; **116**: 11-15.

[5] McDoniel PB, Cole JR. Antitumor activity of *Bursera schlechtendalii* (burseraceae): isolation and structure determination of two new lignans. *J Pharma Sci* 1972; **61**: 1992-1994.

[6] Huacuja RL, Delgado NM, Carranco LA, Reyes LR, Rosado GA. [Agglutinating and immobilizing activity of an ethanol extract of *Bursera fagaroides* on human and other mammalian spermatozoa]. *Arch Invest Med* 1990; **21**: 393-398. Spanish.

[7] Prakash D, Upadhyay G, Gupta C. Total phenol and antioxidant activity of some fruits and their under-utilized parts. *Int Food Res J* 2013; **20**(4): 1717-1724.

[8] Ara K, Rahman MS, Rahman AHMM, Hasan CM, Rashid MA. Terpenoids and coumarin from *Bursera serrata* Wall. *Dhaka Univ J Pharm Sci* 2009; **8**(2): 107-110.

[9] Aragão GF, Carneiro LM, Junior AP, Vieira LC, Bandeira PN, Lemos TL, et al. A possible mechanism for anxiolytic and antidepressant effects of alpha- and beta-amyrin from *Protium heptaphyllum* (Aubl.) March. *Pharmacol Biochem Behav* 2006; **85**(4): 827-834.

[10] Subarnas A, Tadano T, Oshima Y, Kisara K, Ohizumi Y. Pharmacological properties of beta-amyrin palmitate, a novel centrally acting compound, isolated from *Lobelia inflata* leaves. *J Pharm Pharmacol* 1993; **45**(6): 545-550.

[11] Holanda Pinto SA, Pinto LM, Cunha GM, Chaves MH, Santos FA, Rao VS. Anti-inflammatory effect of alpha, beta-Amyrin, a pentacyclic triterpene from *Protium heptaphyllum* in rat model of acute periodontitis. *Inflammopharmacology* 2008; **16**(1): 48-52.

[12] Toma W, Gracioso JS, Hiruma-Lima CA, Andrade FD, Vilegas W, Souza Brito AR. Evaluation of the analgesic and antiedematogenic activities of *Quassia amara* bark extract. *J Ethnopharmacol* 2003; **85**(1): 19-23.

[13] Leslie Taylor. *Technical data report for amargo, Quassia amara.* 2nd ed. Los Angeles: Sage Press, Inc.; 2003.

[14] Capra JC, Cunha MP, Machado DG, Zomkowski AD, Mendes BG, Santos AR, et al. Antidepressant-like effect of scopoletin, a coumarin isolated from *Polygala sabulosa* (Polygalaceae) in mice: evidence for the involvement of monoaminergic systems. *Eur J Pharmacol* 2010; **643**: 232-238.

[15] Ding Z, Dai Y, Hao H, Pan R, Yao X, Wang Z. Anti-inflammatory effects of scopoletin and underlying mechanisms. *Pharm Biol* 2008; **46**(12): 854-860.

[16] Takagi K, Watanabe M, Saito H. Studies on the spontaneous movement of animals by the hole cross test: effect of 2-dimethylaminoethane. Its acylates on the central nervous system. *Jpn J Pharmacol* 1971; **21**: 797.

[17] Gupta BD, Dandiya PC, Gupta ML. A psycho-pharmacological analysis of behaviour in rats. *Jpn J Pharmacol* 1971; **21**: 293-298.

[18] Meyer BN, Ferrigni NR, Putnam JE, Jacobsen JB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plants constituents. *Planta Med* 1982; **45**: 31-34.

[19] Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. *Am J Enol Vitic* 1977; **28**: 49-55.

[20] Oyaizu M. Studies on product of browning reactions: antioxidative activities of browning reaction prepared from glucosamine. *Jpn J Nutr* 1986; **44**: 307-315.

[21] Johnston GA. GABA(A) receptor channel pharmacology. *Curr Pharm Des* 2005; **11**: 1867-1885.

[22] Cooke B, Ernst E. Aromatherapy: a systemaic review. *Br J Gen Pract* 2000; **50**: 493-496.

[23] Buchbauer G, Jirovetz L. Aromatherapy-use of fragrances and essential oils as medicaments. *Flavour Fragr J* 1994; **9**: 217-222.

[24] Ferrali M, Signorni C, Ciccoli L, Comporti M. Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and isouramil. *Biochem J* 1992; **285**: 295-301.

[25] Augusto O, Kunze KL, Ortiz de Montellano PR. N-phenylprotoporphyrin IX formation in the hemoglobin-phenylhydrazine reaction. Evidence for a protein-stabilized iron-phenyl intermediate. *J Biol Chem* 1982; **257**: 6231-6241.

[26] Halliwell B, Hoult JR, Bllake DR. Oxidants, inflammation and anti-inflammatory drugs. *FASEB J* 1988; **2**: 2867-2873.

[27] Maxwell SR. Prospects for the use of antioxidant therapies. *Drugs* 1995; **49**: 345-361.

[28] Cotran RS, Kumar V, Collins T. Acute and chronic inflammation. *Robbins pathologic basis of disease*. 6th ed. Philadelphia: W. B. Saunders Company; 1999, p. 50-88.

[29] Pattison DJ, Silman AJ, Goodson NJ, Lunt M, Bunn D, Luben R, et al. Vitamin C and the risk of developing inflammatory polyarthritis: prospective nested case-control study. *Ann Rheum Dis* 2004; **63**: 843-847.

[30] Aitadafoun M, Mounieri C, Heyman SF, Binistic C, Bon C, Godhold J. 4-Alkoxybenzamides as new potent phosholipase A_2 inhibitors. *Biochem Pharmacol* 1996; **51**: 737-742.

[31] Kang SS, Benjamin G. Homoserine inhibition of *in vitro* sickling of erythrocytes. *Exp Mol Pathol* 1975; **22**: 220-224.