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Analgesic, Antioxidant Potential of Alcoholic (AL) Stem Bark Extract of *Albizia lebbek* (L.) Benth.

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ABSTRACT

Albizia lebbek (L.) Benth. is a medicinal plant. The plant is considered a most potent alexipharmic and every part of it is prescribed for the treatment of bites and stings from venomous animals. AL stem bark extract of *Albizia lebbek* was screened to evaluate the analgesic and free-radical scavenging effect. The stem bark extract exhibited significant analgesic activity, reducing power and free radical scavenging effect on DPPH free radical, superoxide radical, and hydrogen peroxide. The analgesic activity of AL extract showed significant evaluation in pain threshold in comparison of control at dose of 250 mg/kg and 500mg/kg ($p<0.01$) at 90 min after extract administration. In addition to this, the antioxidant activities were concentration dependent which were compared with standard antioxidants such as BHA and ascorbic acid. The highest free radical scavenging of the stem bark extract was observed at concentration of 2500µg/ml.

Key words:*Albizia lebbek, Antioxidant activity, Analgesic activity/ Nociceptive*.

INTRODUCTION

The medicinal properties of plants have been investigated through various scientific developments all around the world since decades due to their therapeutic potentials and economic viability. Various plants which have been reported to contain flavonoids and phenolic compounds exert multiple biological effect, including anti-carcinogenic[1], antioxidant and free radical scavenging abilities[7], analgesic and anti-inflammatory[2, 8, 11] etc. Similarly *Albizia lebbek* (L.) Benth. is a medicinal plant which also contains flavonoids and phenolic compounds along with other chemical constituents. The plant is considered a most potent alexipharmic and every part of it is prescribed for the treatment of bites and stings from venomous animals. Although a number of synthetic remedies are available to treat pain as well as inflammation but they have various side effects such as prolonging bleeding and causing gastric ulcers. For example aspirin and other $NSAID_S$ can trigger or aggravate an asthmatic attack[12]. In a similar fashion, aspirin can initiate or aggravate urticaria. These effects are most likely not mediated by antibodies but are due to a direct effect of thioether leukotrienes [13]. Due to these side effects of synthetic drugs, herbal medicines are usually preferred over synthetic drugs. The use of herbs to treat disease is almost universal among non-industrialized societies. On the other hand medicinal plants also play an important role to treat majority of the diseases/disorders which are mainly linked to oxidative stress due to free radicals[6]. A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted into more aggressive radical species that can cause extensive cellular damage[14]. In treatment of these diseases, antioxidant therapy has gained an immense importance. Antioxidants have been reported to prevent oxidative damage caused by free radical and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers[6]. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite[3]. Currently available synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs [10]. The aim of the present investigation was to evaluate in vivo analgesic and in vitro antioxidant, free radical scavenging activity of the *Albizia lebbek* stem bark extract. The bark of *Albizia lebbek* is bitter, cooling, alexiteric, anthelmintic and is useful in diseases of blood, leucoderma, itching, skin diseases, excessive perspiration and inflammation [4, 5].

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MATERIALS AND METHODS:

Chemicals

Chemicals used in this study were 1,1-diphenyl-2-picrylhydrazyl (DPPH), Potassium ferricyanide, Trichloroacetic acid, Iron chloride, Nitro blue tetrazolium (NBT), Nicotinamide adenine dinucleotide (NADH), Phenazine methosulfate (PMS), Hydrogen peroxide, Phosphoric acid, Butylated hydroxyl anisole (BHA), Ascorbic acid, Pentazocin etc. All reagents used for the study were of analytical grade.

Plant materials

Stem bark of *Albizia lebbek* were collected from the Kurukshetra University, Kurukshetra (Haryana), India during October-November 2008 and were positively identified by Mr. S.K. Sharma, President, The Environment Society of India, Chandigarh (India). The stem bark of *Albizia lebbek* were cleaned, dried in the shade, then powdered, sieved through mesh size 40 and stored in an airtight container at 25°C.

Extraction

Powdered *Albizia lebbek* stem bark (800g) was extracted with alcohol by using soxhlet for 48 h. The extract was filtered and last traces of the solvent were evaporated under reduced pressure in a rotary evaporator. The percentage yield of AL extract was 6.61%.

ANALGESIC ACTIVITY:

Tail immersion test [1]

Tail immersion was conducted as described by Aydin et al. (1999). Six mice were administered orally with pentazocine (30mg/kg), extract (250 and 500mg/kg). The distal part of the tails of the animals was immersed in hot water maintained at 55.0± 1.0 °C. The time taken to withdraw the tail was noted as reaction time. A cut off time of 10sec was maintained at 55°C to prevent tissue damage. The reaction time was measured at 15, 30, 60 and 120 min after treatment, respectively.

Radiant heat tail-flick method [8]

A radiant heat automatic tail flick algesiometer report was used to measure response The central analgesic activity of the plant material was studied by measur-

ing drug-induced changes in the sensitivity of the pre-screened (reaction time: 2-4 sec) mice to heat stress applied to their tails by using algesiometer. Briefly, the current intensity passing through the naked nicrome wire was maintained at 5 ampere. The distance between the heat source and the tail skin was 1.5cm and cut-off reaction time was fixed at 10 sec to avoid any tissue damage. Pentazocin was used to compare the analgesic effect of the plant extract.

Acetic acid induced writhing test [5]

The peripheral analgesic activity of bark extract of AL was measured by the acetic acid induced writhing test. Briefly, the inhibition of writhing produced by the plant extract was determined by comparing with the inhibition produced by the control group. Pentazocin at oral dose of 30 mg/kg was used as standard analgesic agent. Intra-peritoneal injection of acetic acid (0.7%) at a dose of 0.1ml/10g of body weight was used to create pain sensation. The number of writhing was calculated for 10min, immediate after the application of acetic acid.

ANTIOXIDANT ACTIVITY:

Total Phenolic Content [10]

The total phenolic contents were extracted and determined spectrometrically. 1 ml of Folin-Ciocalteu's reagent, previously diluted (1:20), was added to 1 ml of sample (250µg/ml) and mixed thoroughly. To the mixture, 4 ml of sodium carbonate (75g/L) and 10 ml of distilled water were added and mixed well. The mixture was allowed to stand for 2 hrs at room temperature. Contents were then centrifuged at 2000 rpm for 5 min and the absorbance of the supernatant was taken at 760nm. A standard curve was obtained using various concentrations of gallic acid. Results were expressed as percentage of gallic acid equivalents (GAE) per 100g of fresh mass.

Total Flavonoid Assay [6]

Total flavonoid content was measured by aluminium chloride colorimetric assay. 1ml of extract or standard solution of catechin (500µg/ml) was added to 10ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3ml of 10% $AlCl_3$ was added. At 6^{th} min, 2ml of 1M NaOH was added and total volume was made up to 10ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm. Total flavonoid content of the bark was expressed as percentage of catechin equivalent per 100g of fresh mass.

DPPH free radical scavenging activity [6]

The free-radical scavenging activity of extract was measured by decrease in the absorbance of alcohol solution of 1,1-di phenyl ,2-picryl hydrazyl (DPPH). A stock solution of DPPH (33mg/L) was prepared in alcohol, which gave initial absorbance (Abs) of 0.493 and 5ml of this stock solution was added to 1ml of extract solution at different concentrations (100–2500µg/ml). After 30 min, absorbance was measured at 517nm and compared with standards (10-50µg/ml). Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

% Anti-radical activity = (Control Abs - Sample Abs / Control Abs) × 100

Superoxide radical scavenging assay [6]

The reaction mixture consisting of 1ml of nitro blue tetrazolium (NBT) solution (156mM NBT in phosphate buffer, pH 7.4), 1 ml NADH solution (468mM NADH in phosphate buffer, pH 7.4), and 1ml of sample solution of extract was mixed. The reaction was started by adding 100 ml of phenazine methosulfate (PMS) solution (60mM PMS in phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min and the absorbance was measured at 560nm against blank sample and compared with standards. Decreased absorbance of reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

% inhibition = $[(A_0-A_1)/A_0]$ x100

Where A_0 was the absorbance of the control and A_1 was the absorbance of extract or standard compounds.

Scavenging of Hydrogen Peroxide (H_2O_2) [6]

A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of extract (100-2500µg/ml) were added to a hydrogen peroxide solution (0.6 ml, 40mM). Absorbance of hydrogen peroxide at 230nm was determined after 10 min. against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of extract and standard compounds was calculated using the following formula:

% scavenged $[H_2O_2]$ = $[(A_0-A_1)/A_0]$ x 100

Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample and standards.

Reducing power assay [6]

The reducing power of extract was determined as per the reported method. Different concentrations of extract (250–2500µg/ml) in 1ml of alcohol were mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferrocyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20min. A portion (2.5ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5ml, 0.1%). The absorbance was measured at 700nm and compared with standards. Increased absorbance of the reaction mixture indicated increased reducing power.

Statistical Analysis

Values were represented as mean ± SD of three parallel measurements and data were analyzed using the *t*-test and in case of analgesic activity values are represented as mean ± S.E.M and data were analyzed using ANOVA followed by Dunnet's t-test.

ANALGESIC ACTIVITY

Table 1: Tail flick method

Group	No. of Animals	Dose (mg/kg)	B.R.T (sec.) Mean±S.E.M	After Drug (sec.) Mean ± S.E.M 30 min	60 min	90min	120 min
Control	6	_	1.33 + 0.33	1.00 + 0.00	1.33 + 0.33	1.33 + 0.33	1.66 + 0.33
Pentazocin	6	30	2.66 + 0.33**	3.66 + 0.33*	4.33 + 0.66*	4.33 + 0.33*	4.00 + 0.57*
Alcohol	6	250	1.33 + 0.33	1.33 + 0.33	3.66 + 0.33**	4.33 + 0.33*	2.33 + 0.33
Alcohol	6	500	1.66 + 0.33	1.66 + 0.33	4.00 + 0.57*	4.66 + 0.33*	3.33 + 0.33**

(*) P<0.01, (**) P<0.05, n = 6

Table 2: Tail Immersion Method

Group	No. of Animals	Dose (mg/kg)	B.R.T (sec.) Mean±S.E.M	After Drug (sec.) Mean ± S.E.M 30 min	60 min	90min	120 min
Control	6	-	1.66 ± 0.33	2.00 ± 0.57	2.33±0.33	2.33 ± 0.33	2.00 ± 0.00
Pentazocin	6	30	5.66 ± 0.33*	7.00 ± 0.57*	8.66±0.33*	9.00 ± 0.57*	6.33 ± 0.66*
Alcohol	6	250	3.66 ± 0.33*	4.33 ± 0.33**	6.66±0.33*	7.66 ± 1.45*	4.33 ± 0.88
Alcohol	6	500	3.33 ± 0.33**	4.33 ± 0.33	6.33±0.66**	8.00 ± 0.00*	4.33 ± 0.33*

(*) P<0.01, (**) P<0.05, n = 6

Table 3: Writhing Test

Group	No. of Animals	Dose (mg/kg)	No. of Wriths (Mean ± S.E.M)	% Inhibition
Control	6	-	40.00± 1.15*	-
Pentazocin	6	50	8.00 ± 0.57*	80.00%
Alcohol	6	250	14.33 ± 1.45*	64.17%
Alcohol	6	500	14.00 ± 3.05*	65%

n = 6, P < 0.01 significant from control.

ANTIOXIDANT ACTIVITY

Table 4. Comparative antioxidant profile of the *Albizia lebbek* stem bark extract

Sample	Conc. (µg ml^{-1})	DPPH Radical scavenging(%)	Hydrogen Peroxide Scavenging (%) (mean ± SD)	Superoxide Anion Scavenging(%)	Reducing power activity (absorbance)
AL	(2500)	71.80 ± 0.20	69.09 ± 1.00	82.90 ± 1.12	1.679
Ascorbic acid	(50)	65.91 ± 0.10	86.1 ± 1.1	80.62 ± 1.17	1.000
BHA	(50)	71.88 ± 0.07	89.3 ± 3.1	71.54 ± 2.68	1.581

n = 3.

Figure 1: **Comparison of control at dose of 250 mg/kg and 500mg/kg (p<0.01)**

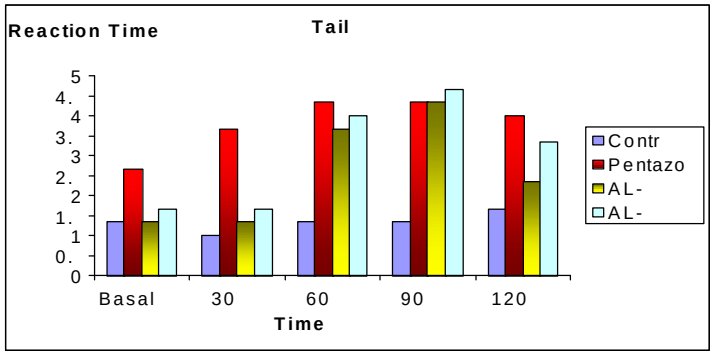


Figure 2: **comparison of control at dose of 250 mg/kg and 500mg/kg (p<0.01)**

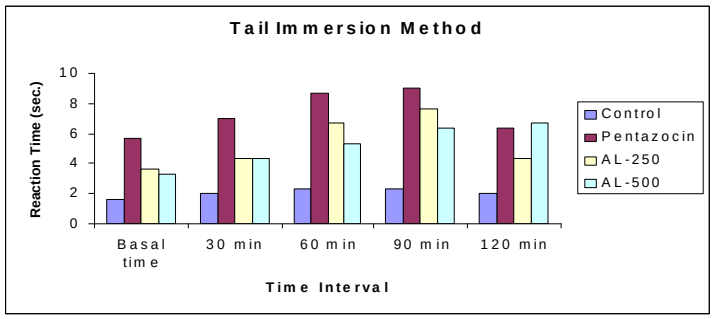


Figure 3: **Writhing Method comparison of control at dose of 250 mg/kg and 500mg/kg (p<0.01)**

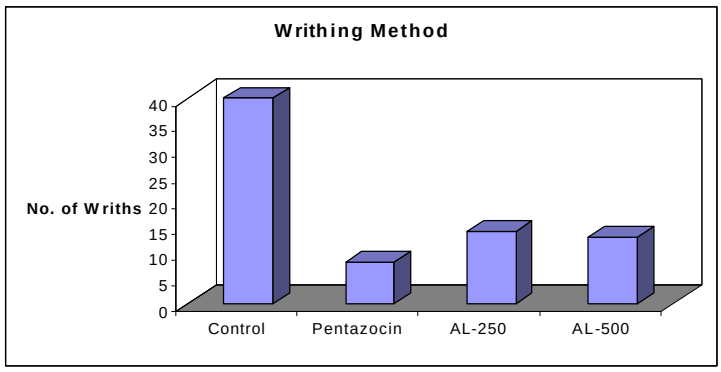


Figure 4: **The scavenging effect of *Albizia lebbek* AL extract on the DPPH radical, Hydrogen peroxide and Superoxide radicals**

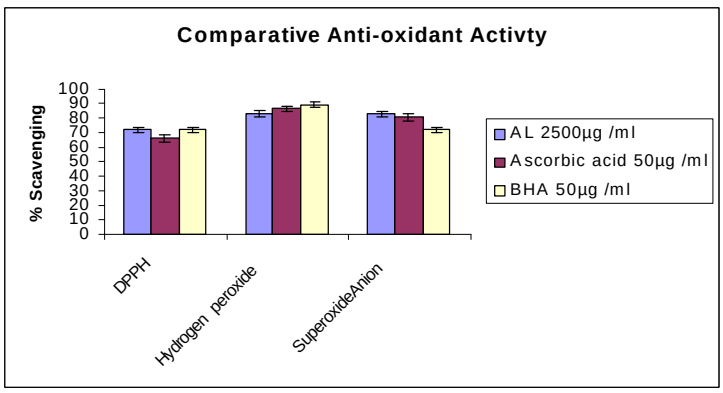
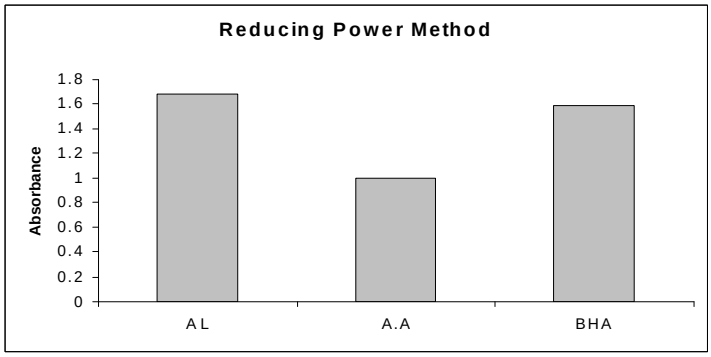


Figure 5: **Reducing power method of AL stem bark extract of *Albizia lebbek*. Results are mean ± SD of three parallel measurements.**



RESULTS AND DISCUSSION:

For the determination of analgesic activity, we used following methods i.e. tail-flick method (Table 1), tail immersion method and writhing method (Table 2 & 3). The analgesic activity profile of AL extract at doses 500mg/ kg showed significant (P<0.01) analgesic activity when compared with control and Pentazocin treated animals. In tail flick method the analgesic activity of AL extract showed significant evaluation in pain threshold after 90 min of administration of dose in comparison of control and standard group at dose of 250mg/kg and 500mg/kg (p<0.01). The results obtained were comparable to the analgesic activity of pentazocin (standard). (Table 1) During tail immersion method the analgesic activity of AL extract also showed significant effect i.e. p<0.01 in comparison of control and standard group at dose of 250mg/kg and 500mg/kg. (Table 2) When the AL extract tested in writhing test it showed 64% and 65% inhibition of writhes at dose of 250mg/kg and 500mg/kg (p<0.01) respectively in contrast to 80 % inhibition by standard drug (50mg/kg) after extract administration. (Table 3) The limited response of stomach muscles twisted by acetic acid is a responsive process for preliminary analgesic agents, which might be due prostaglandin pathways. The present study on extract of *Albizia lebbek* has demonstrated that this plant has significant analgesic properties and it justifies the traditional use of this plant in the treatment of various types of pains.

The free radical scavenging activity was evaluated by various *in vitro* assays. DPPH radical was used as a substrate to evaluate free radical scavenging activities of extract. The scavenging effect of *Albizia lebbek* AL extract on the DPPH radical was 71.80±0.20%. These results indicated that extracts have significant effect on scavenging of the free radicals. Figure 4 illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of *Albizia lebbek* AL stem bark extract. BHA and ascorbic acid were taken as standards. Since H_2O_2 is highly important for antioxidant activity due to its ability to penetrate biological membranes. However H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Thus, removal of H_2O_2 is very important for protection of food systems. Figure 4 shows the H_2O_2 scavenging activity by AL stem bark extract of *Albizia lebbek*. This was compared with 50µg/ml of BHA and Ascorbic acid. The percentage scavenging activity of AL stem bark extract of *Albizia lebbek*, BHA and Ascorbic acid was found as 69.09±0.11%, 89.30±3.1% and 86.1±1.1%, respectively. The superoxide anion radical scavenging activity of extracts was assayed by the non-enzymatic Phenazine methosulfate-Nicotinamide adenine dinucleotide (PMS-NADH) system. Figure 4 shows the percentage inhibition of superoxide radical generation by extracts and comparison with Ascorbic acid and BHA. The percentage inhibition of superoxide generation by AL stem bark extract of *Albizia lebbek* was found as 82.90±0.12%. On the other hand, ascorbic acid and BHA at concentration of 50µg/ml have 80.62±0.75% and 71.54±0.68% inhibition of superoxide radical. Similarly to the other methods the measurements of the reduction of ferric ion to ferrous ''Fe^{3+}– Fe^{2+} transformation' in the presence of AL stem bark extract of *Albizia lebbek* was found. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reductive capability of extracts was found to be 1.679 as compared with BHA and Ascorbic acid 1.000 and 1.581.

On the basis of the data obtained from results of this study, it was clearly indicative that the AL extract of *Albizia lebbek* stem bark have significant analgesic (Figure 1-3) and *in-vitro* antioxidant activity. The antioxidant activity may be due to flavonoid and phenolic content in plant. However, the components responsible for the antioxidative activity of AL stem bark extract of *Albizia lebbek* are currently unclear. Therefore, it is suggested that further work be performed on the

isolation and identification of the antioxidant components of *Albizia lebbek (L.)* Benth. Further the AL extract exhibited significant reducing power and free radical scavenging effect by DPPH free radical, superoxide radical, and hydrogen peroxide methods. The antioxidant activities were concentration dependent which were compared with standard antioxidants such as BHA and ascorbic acid. The highest free radical scavenging activity of extracts was observed at concentration of 2500µg/ml, in which AL extract showed maximum activity.

REFERENCES:

1. Aydin S, Demir T, Ozturk, Y, Baser KHC, Analgesic activity of Nepeta italica L., Phytotherapy Research 13, 1999, 20–23.
2. Bose A, Mondal S, Gupta JK, Ghosh T, Dash GK, Analgesic, anti-inflammatory and antipyretic activities of the fractions of Cleome Rutidosperma. Journal of Fitoterapia 78, 2007, 515-520.
3. Halliwell B, Gutteridge JMC, Free Radicals in Biology and Medicine. 3rd Ed, Volume 189, Oxford university press, 1999, 617–783.
4. Kirtikar KR, Basu BD, Indian medicinal plants. 2nd Ed. Sri Satguru publications, 2003, 2585-2590.
5. Koster R, Anderson M, De Beer EJ, Acetic acid for analgesic screening. Fed. Proc.18, 1959, 412.
6. Prajapati ND, Purohit SS, Sharma AK, Kumar T, A Handbook of Medicinal Plants. Ist Ed. Agrobios publication, 2003, 26-27.
7. Kumar S, Kumar D, Singh N, Vasisht BD, In vitro free radicals scavenging and antioxidant activity of Moringa oleifera Pods. Journal of Herbal Medicine and Toxicology 1(2): 2007, 17-22.
8. Lotito SB, Frei B, Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans. Free Radic. Biol. Med. 41(12), 2006, 1727–46.
9. Delporte C, Backhouse N, Inostroza V, Aguirre MC, Peredo N, Silva X, Negrete R, Miranda HF, Analgesic activity of Ugni molinae (murtilla) in mice models of acute pain. Journal of Ethnopharmacology 112, 2007, 162–165.
10. Ramarathnam N, Ochi H, Takeuchi M, Antioxidant defence system in vegetable extracts. In Natural Antioxidants: Chemistry, Health Effects and Applications; F. Shahidi, Ed. AOCS Press: Champaign, IL, 1997, 76-87.
11. Rylski M, Duriasz-Rowinska H, Rewerski W, The analgesic action of some flavonoids in the hot plate test. Acta Physiol Pol.30 (3): 1979, 385-388.
12. Settipane GA, Adverse reactions of aspirin and related drugs. Arch. Intern. Med. 141: 1981, 328-332.
13. Settipane RA, Constantine HP, Aspirin intolerance and recurrent urtcaria in normal adults and children. Epidemilogy and Review. Allergy. 35: 1980, 149-154.
14. Valko M, Morris H, Cronin MT, Metals toxicity and oxidative stress. Curr. Med. Chem.12 (10): 2005, 1161–208.

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