Pharmacognosy Research [Phcog Res]
Vol 1, Issue 6, Nov-Dec, 2009 Page 381-386
(An Official Publication of Pharmacognosy Network Worldwide)
Received: 10 09, 2009
Modified: 15 09, 2009
Accepted: 08 10, 2009

PHCOG MAG.: Research Article

Antinociceptive activity of aqueous leaf extract of *Tetracera sarmentosa* L. in rats

Fernando T. R. G. W.[1], Ratnasooriya W. D.[2]* and Deraniyagala S. A.[1]

[1]*Department of Chemistry, University of Colombo, Colombo 03, Sri Lanka.*
[2]*Department of Zoology, University of Colombo, Colombo 03, Sri Lanka.*

*** Author of Correspondence :** *Professor W. D. Ratnasooriya : wdr@zoology.cmb.ac.lk*

ABSTRACT

In Sri Lankan traditional medicine mature leaves of *Tetracera sarmentosa* L. (Family : Dilleniaceae) is used as a healing agent in the treatment of bone fractures. A strong possibility exists that these leaves could possess antinociceptive activity but this has not been scientifically tested and validated. The aim of this study was to investigate the antinociceptive potential of an aqueous leaf extract (ALE) of *T. sarmentosa* in rats (doses used : 500, 750, and 1000 mg/kg, given orally) using three algesiometric methods (hot plate, tail flick and formalin tests). The results showed that ALE possesses marked and significant ($p<0.05$) antinociceptive action when evaluated in hot plate (in a dose dependant manner) and formalin tests (only the highest dose tested) but not in tail flick test. The antinociceptive action of ALE had a quick onset (within 1 h) and a moderately long duration of action (up to 4 h). The antinociceptive action of ALE was not blocked by atropine (a muscarinic receptor antagonist) or metoclopramide (a dopamine receptor antagonist). Moreover, the ALE did not have sedative (in terms of hole board test) or membrane stabilizing (in terms of *in vitro* heat induced haemolysis of rat erythrocyte test) activities. ALE contained a wide variety of chemical constituents of which alkaloids, polyphenols, flavonoids, steroids may have contributed to antinociceptive action of ALE. Collectively, these observations suggest that ALE – induced antonociception was mediated both centrally, at supraspinal level, and peripherally. The antinocicetion action was not due to cholinergic, dopaminergic, sedative and membrane stabilizing mechanisms but possibly due to opioidergic mechanisms (by indirect evidences). The results also suggest that ALE is effective against both neurogenic and inflammatory pains. In conclusion, this study shows, for the first time, that ALE of *T. sarmentosa* possesses moderately strong pain relieving activity, which is beneficial in the treatment of bone fractures.

Keywords: *Tetracera sarmentosa,* Antinociception, Pain inhibition, Opioid mechanism, Nociception, Antinociception.

INTRODUCTION

Tetracera sarmentosa L. (Family Dilleniaceae) 'Korasa Wel' in Sinhala is an evergreen woody climber in tall trees (1, 2). It is found in scrab along roadsides, hedges, thickets and in primary forests, from sea – level up to 1500 m altitude in several South Asian countries including Sri Lanka (3). The leaves are pinnately compound and usually spirally arranged. Its base is acute, apex acute to obtuse, margin entire to distinctly denate, rough and the petiole is 4 – 15 mm in length. Leaf blade is leathery, very scabrous, later abaxial surface glabrous or only pubescent with protuberance, secondary veins obviously prominent on abaxial surface (3). The inflorescence terminal; 10 – 25 × 5 – 15 cm and includes 30 – 150 flowers which are found in the basal part often with 1 – 4 leaves. Its flower is about 1 cm in diameter and bisexual. The sepals are 5, with 2 outer ones and 3 inner ones and rough outside. There are 5 petals and are yellow. The stamens are whitish yellow (3). Branches are rough. Branches (small branches arising from branches) scabrid with 1 mm long hairs (3). They are hairy when young, later glabrous. Young branches are reddish brown. (1)

In Sri Lankan traditional medicine, leaves of *T. sarmentosa* is used as a healing agent in the treatment

of bone fractures. Since bone fracture is associated with moderate to severe pain (4), pain relieving drugs are often recommended in its treatment (5). So, if *T. sarmentosa* leaves possess antinociceptive action in addition to its healing properties it would be beneficial in bone fracture treatment. However, it is unknown whether *T. sarmentosa* leaves possess antinociceptive activity.

The aim of this study was to experimentally investigate antinociceptive potential of *T. sarmentosa* leaves. This was tested in rats using an aqueous leaf extract and three different algesiometric models which are scientifically validated and widely used to evaluate potential antinociceptive agents.

MATERIALS AND METHODS

Plant Collection and Authentication

The leaves of *T. sarmentosa* were collected from Kelaniya, Meerigama in the Gampaha District of Sri Lanka in July 2007 and identified and authenticated by Dr. S. Ranwala of the Department of Plant Sciences, University of Colombo. Voucher specimen (wdr/sad 1007) is deposited at the museum, Department of Zoology, University of Colombo.

Preparation of the aqueous leaf extract of (ALE) of T. sarmentosa

Air shade dried mature leaves of *T. sarmentosa* (350 g) was refluxed with distilled water (5200 ml) for 20 h in a round bottom flask fitted with a Leibig condenser. A brown coloured ALE was obtained by filtering. It was boiled to obtain a concentrated extract by reducing the volume. The concentrated extract was freeze-dried for 5 days and the freeze dried powder (yield : 40.5 g) was stored air tight at 4 °C. The freeze dried powder was suspended in 1% (w/v) gum acacia for oral administration. The 3 doses were used : 500, 750 and 1000 mg/kg. The highest dose tested was 7.5 times higher than what is usually recommended by the traditional practitioners of Sri Lanka is prescribing decoctions, which is within the accepted range for rat models.(6)

Experimental Animals

Healthy adult albino male rats (weight: 230–250g) were used in the study. The animals were kept in plastic cages (3 per cage) under standardized animal house conditions (temperature: 28–31°C, photoperiod: approximately 12 hours natural light per day; relative humidity : 50% – 55%). All rats had free access to water and pelleted food (Master Feed Ltd., Colombo, Sri Lanka). Animal experiments were conducted in accordance with the internationally accepted principles for animal use and care and rules of the Faculty of Science, University of Colombo.

Evaluation of Analgesic activity

Hot Plate and Tail Flick tests

Twenty six rats were randomly divided into five groups (n = 6/group in treatment groups and n = 8/group in control group). The rats in group 1 were orally treated with 500 mg/kg of freeze dried ALE. Groups 2 and 3 were orally administrated with 750 and 1000 mg/kg of freeze dried ALE respectively. The last group severed as the control and 1ml of 1% gum acacia solution was orally administrated per rat. The reaction times of these rats were measured 1 day prior to the treatment and 1 to 6 h after the treatment at hourly intervals using hot plate and tail flick test methods (6). In the hot plate test, rats were placed in a hot plate (Model MK 35 A, Muromachi Kikai Co.Ltd., Tokyo, Japan) at 50 °C and the time taken to lick either of the hind paws or to jump up was recorded. The rats showing a pretreatment reaction time greater than 15 s in the hot plate test were not used in the experiment (7). A cut off time of 20 s was set to avoid tissue damages (6). In the tail flick test, the tail of the rat up to 5 cm from its tip was immersed in a water bath at 55 °C and time taken to flick the tail was recorded using a stopwatch (6). Rats showing a pretreatment reaction time greater than 5 s in the tail flick test were not selected for the experiment (7). A cut off time of 5 s was set to avoid tissue damages (8).

Formalin test

Twelve rats were divided into 2 groups and were orally administered with ALE or vehicle as follows; Group 1 (n = 6) with 1000 mg/kg of ALE; Group 2 (n = 6) with 1ml of vehicle. 3 h after administration, each rat was subcutaneously injected with 0.05 ml of 2.5% formalin solution (BDH Chemicals, Poole, UK) into the sub plantar surface of the left hind paw. Rats were then observed for 1 h and the numbers of licking, flinching, lifting and time spend on licking the injected paw were recorded in two phases : 1st phase, 0–5 min and 2nd phase, 20 – 60 min.(9)

Evaluation of the mechanisms of antinociceptive activity

Investigation for muscarinic receptor mediation

Twelve rats were divided into two equal groups. (n = 6/ group) Pretreatment values were taken 1 hour prior to the treatment. Those in group 1 were intraperitonially injected with 5 mg/kg of atropine sulphate (Laboratory Renaudin, Paris, France), a muscarinic receptor antagonist

and those in group 2 were injected with 1 ml of isotonic saline (0.9% w/v Sodium chloride solution).After 10 min, the rats in both groups were orally administered with 1000 mg/kg of ALE. These rats were subjected to the hot plate test 3 h after *T. sarmentosa* ALE treatment (10).

Investigation for dopamine receptor mediation

Twelve rats were divided into two groups (n=6/group). Group 1 was orally treated with 1.5 mg/kg of metochlopramide (Ipca Laboratories Limited, Mumbai, India), a dopamine antagonist, in 1 ml of 1% methylcellulose. Group 2 was orally treated with 1ml of 1% methylcellulose. One hour later, both groups of rats were orally treated with 1000 mg/kg dose of ALE and the nociception was determined before treatment with dopamine antagonist and 3 h post treatment, using the hotplate technique (10).

Evaluation of effects on muscle coordination and strength

Twelve rats were orally treated either with 1000 mg/kg of ALE (n = 6) or 1ml of 1% gum acacia solution (n = 6).Three hour after treatment, these rats were subjected to the bar holding test and Bridge test and respective latencies were measured (11).

Evaluation of sedative activity

Twelve rats were randomly divided into two equal groups. (n = 6/group) The rats in group 1 were orally administered with 1ml of 1% gum acacia solution and those in group 2 with of 1000 mg/kg of ALE. After 3 h, each of these rats was individually placed on a rat hole board apparatus and was observed for 7.5 min. During this period, number of crossings, number of rearings, number of dippings, cumulative dipping duration, and number of faecal boluses produced were recorded (11).

Evaluation of membrane stabilization

The investigation of neuronal membrane stabilizing activity of ALE, was investigated on heat - induced haemolysis of rat erythrocyte *in-vitro* model. (6). Briefly, 20 µl of uncoagulated fresh rat blood (EDTA was used as the anticoagulant) was added to vials containing 1 ml of 0.15 M phosphate buffered saline (pH 7.4). To this, 20 µl of ALE of concentrations 0.01 mg/ml (n = 16), 0.1 mg/ml (n = 16), 0.2 mg/ml (n =16) and phosphate buffered saline (n = 16) was added respectively. Thereafter, the vials were mixed thoroughly and incubated at 37 °C for 15 min followed by 25 min at 54 °C. The vials were centrifuged at 3200 rpm for 5 min. (HERMLE Centrifuge Z 233 M – 2, UK) The absorbance was measured at 540 nm using a UV/VIS spectrophotometer (Jasco V 500, Jasco Corporation, Tokyo, Japan). Heat-induced haemolysis in terms of the absorbance values of control and treatment groups were compared (12).

Phytochemical analysis

ALE was subjected to phytochemical screening. Phytochemical screening procedures were carried out for the screening of four different chemical groups (13) ; alkaloids, polycyclic compounds, flavonoids and leucoanthocyanins and tannins and poly phenols. The ALE was subjected to column chromatography on reverse phase C-18 silica gel (Fluka Chemie G). The column was eluted with water, mixtures of methanol and water, methanol, mixtures of methanol and ethyl acetate, ethyl acetate, mixtures of ethyl acetate and dichloromethane, dichloromethane, mixtures of dichloromethane and hexane and hexane. Fractions showing similar thin layer chromatography (TLC) spots were combine after inspecting under UV light. (at 254 nm and 366 nm) The combined fraction were subjected to TLC (Aldrich silica gel precoated on aluminium sheets and Fluka Chemie G reverse phase C-18 pre coated glass plates). The mobile phases were 20% methanol in ethylacetate, 25% ethylacetate in dichloromethane 20% hexane in dichloromethane and 50% dichloromethane in hexane for normal phase TLC and 10% water in methanol for reversed phase TLC plates. TLC plates were sprayed with colour reagents specific for various classes of compounds (14).

Statistical analysis

The data were expressed as the means ± S.E.M. Statistical analysis was performed using Mann-Whitney *U*-test. Significant values were set at $P \leq 0.05$. Linear regression analysis was preformed to assess dose-dependencies.

RESULTS

Hot plate and Tail flick tests

As shown in Table 1, 500 mg/kg dose of the ALE caused a significant ($P < 0.05$) prolongation of the reaction time in the hot plate test in the 4th h (by 8%) compared to the control values. The 750 mg/kg of the ALE significantly increased the reaction time at 3rd and 4th h (3 h by 19% and 4 h by 7%). In contrast, the 1000 mg/kg dose of ALE significantly prolonged the reaction time from the 1st h to the 4th h (1 h by 16%, 2 h by 21%, 3 h by 38% and 4 h by 31%). These effects at 1st ($r^2 = 0.8424$), 2nd h ($r^2 = 0.75$), 3rd h ($r^2 = 0.9796$) and 4th h ($r^2 = 0.7085$), were dose-dependent. In contrast, none of the doses of the ALE prolonged the reaction time in tail flick test (data not shown).

Table 1. ***The effect of oral administration of different doses of the ALE of T. sarmentosa on the hot plate reaction time (mean ± SEM).***

Treatment	Hot Plate reaction time (s)						
	Pre – Treatment	Post Treatment					
	(s) ±	1h	2h	3h	4h	5h	6h
Control (n=8)	8.6± 0.8	8.7± 0.5	8.7± 0.5	8.1± 0.5	8.1± 0.3	8.7± 0.4	8.1± 0.5
500 mg/kg ALE (n=6)	7.8±0.2	8.5±0.4	8.9±0.3	8.8±0.4	8.7±0.3*	7.5±0.2	6.6±0.3
750 mg/kg ALE (n=6)	8.4±0.1	8.7±0.2	8.9±0.2	9.7±0.2*	8.6±0.2*	7.6±0.3	6.8±0.3
1000 mg/kg ALE (n=6)	8.6±0.3	10.1±0.7*	10.4±0.3*	11.2±0.5*	10.5±0.5*	8.6±0.4	8.8±0.9

ALE = aqueous leaf extract

*Values are significant at $P < 0.05$ vs. control

Table 2. ***The effect of oral administration of different doses of the ALE of T. sarmentosa on formalin test parameters (mean ± SEM).***

Treatment (n=6/group)	1st Phase (0–5 min)					2nd Phase (20–60 min)				
	Mean number of licking	Cumulative linking duration(s)	Mean number of lifting	Cumulative lifting duration(s)	Mean number of flinching	Mean number of licking	Cumulative linking duration(s)	Mean number of lifting	Cumulative lifting duration(s)	Mean number of flinching
Control	12.8±1.4	103.33±6.51	6.7±1.2	32.2±10.6	6.7±2.2	78±8.3	511.01±58.17	43.8±8.0	134.51±11.06	21.5±6.0
1000 Mg/kg ALE	7.0*±1.8	61.28*±16.35	4.8±0.7	27.96±9.94	4.7±0.7	37.2*±7.4	231.52*±48.53	18.0*±3.3	81.83*±16.13	20.8±5.6

ALE = aqueous leaf extract

*Values are significant at $P < 0.05$ vs. control

Formalin Test

As shown in the Table 2,1000 mg/kg dose ALE significantly (P < 0.05) impaired most of the test parameters both in the early and late phases (early phase: mean number of licking by 45%, cumulative licking duration by 40% and in the late phase: mean number of licking by 52%, cumulative licking time by 55%, mean number of lifting by 59%, cumulative lifting duration by 39%,) compared to the control values.

Mechanisms of antinociceptive activity

Muscarinic receptor mediation

Intraperitonial administration of atropine did not significantly (P > 0.05) impair the prolongation of reaction time induced by 1000 mg/kg of *T. sarmentosa* ALE. (control vs treatment : 9.43 ± 0.19 vs 9.25 ± 0.39)

Dopamine receptor mediation

Metochlopramide did not significantly ($P \geq 0.05$) impair the prolongation of reaction time induced by 1000 mg/kg of ALE. (control vs treatment : 11.61 ± 0.41 vs 11.93 ± 0.47)

Effects on muscle coordination and strength

Both the latencies in the Bridge and bar holding tests were not significantly ($P > 0.05$) altered by the 1000 mg/kg of ALE. (control vs treatment : Bridge test : 25.68 ± 0.84 vs 25.49 ± 2.06, bar holding test : 26.70 ± 1.16 vs 26.80 ± 1.55)

Sedative activity

None of the rat hole board test parameters were significantly ($P > 0.05$) altered by the treatment with 1000 mg/kg of ALE as compared to control. (control vs treatment : number of crossings : 38.6 ± 2.7 vs 32.3 ± 2.9, number of rearings : 22.3 ± 1.7 vs 21.8 ± 0.9, number of dippings : 10.0 ± 1.1 vs 9.0 ± 1.4, cumulative dipping duration : 20.51 ± 3.87 vs 23.49 ± 5.07, and number of faecal boluses : 0.2 ± 0.2 vs 0.6 ± 0.4)

Evaluation of membrane stabilization

All the concentration of ALE tested failed to induce a significant (P > 0.05) change in the percentage of inhibition in the heat induced-haemolysis test of rat blood cells. (control vs 0.01 mg/ml, 0.1mg/ml , 0.2 mg/ml : 0.256 ± 0.005 vs 0.243 ± 0.017, 0.265 ± 0.019, 0.225 ± 0.009)

Phytochemical analysis

Phytochemical screening of the ALE showed the presence of primary, secondary and/or tertiary alkaloids. Quaternary base alkaloids and/or amine oxides were not present. The ALE also showed the presence of free triterpenes, diterpenes, sterols or related polycyclic substances, unsaturated sterols, flavonoids, leucoanthocyanins, tannins

of pyrogallol type and polyphenols. The TLC of water fractions showed the presence of steroids (R_f = 0.85 and 0.80), methanol/water fractions showed the presence of alkaloids (R_f = 0.85, 0.69 and 0.5 and 0.46), flavonoids (R_f = 0.42), steroids (R_f = 0.84, 0.68, 0.64, 0.5 and 0.48), phenols (R_f = 0.83), triterpene glycosides and cholesterol (R_f = 0.78, 0.68 and 0.42), and amino acids (R_f = 0.73), methanol fractions showed the presence of alkaloids (R_f = 0.47 and 0.40) and amino acids (R_f = 0.36), methanol/ ethylacetate fractions showed the presence of alkaloids (R_f = 0.69), ethylacetate fractions showed the presence of alkaloids (R_f = 0.82, 0.65 and 0.4) flavonoids (R_f = 0.23), steroids (Rf = 0.22), phenols (R_f = 0.75), triterpene glycsides and cholesterol (Rf = 0.69) on spraying with characteristic reagents. ethylacetate/ dichloromethane fractions did not show the presence of any phytochemical compound. Dichloromethane fractions showed the presence of alkaloids(R_f = 0.68 and 0.67), flavonoids (R_f = 0.45), steroids (R_f = 0.46), triterpene glycsides and cholesterol (R_f = 0.46), and amino acids (R_f = 0.41),Dichloromethane/ hexane fractions showed the presence of alkaloids (R_f = 0.64) and hexane fractions showed the presence of alkaloids(R_f =0.0.76 and 0.19), flavonoids (R_f = 0.21), steroids (R_f = 0.16 and 0.18), triterpene glycsides and cholesterol (R_f = 0.92), and amino acids (R_f = 0.83) on spraying with characteristic reagents.

DISCUSSION

This study examined the antinociceptive potential of ALE of *T. sarmentosa* , a plant which is used in the traditional medicine of Sri Lamka for the treatment of bone fractures. The results showed, for the first time, that ALE of *T. sarmentosa* possesses acute, moderately strong, quick acting oral antinociceptive activity with a fairly long duration of action. This ALE – induced antinociception was genuine and not secondary manifestations resulting from muscle relaxation (as evident by the bar holding test and unsuppressed locomotary activity in the rat hole board test) (7) or impaired muscle co-ordination (as judged by the Bridge test) (7). Further, the antinociception action was treatment related and possibly receptor mediated since the effect was dose-related. The results also suggest that ALE is active against both neurogenic (as shown by hot plate test and first phase of formalin test) (14) and continuous inflammatory pain (as shown in the second phase of formalin test) (15). In the formalin test, ALE suppressed all the four parameters in the second phase whilst impaired only two parameters in the first phase. This suggest that ALE could be more effective against inflammatory pain than neurogenic pain.

The positive results in the hot plate test suggest that antinociception is mediated centrally at supraspinal level: hot plate test predominately measures supraspinally organized responses (16). Lack of an effect in the tail flick test suggest that spinal mechanisms are unlikely to be operative in the induction of antonociception by ALE: tail flick test predominately measures antinociception mediated *via* spinal mechanisms (16). On the other hand, impairment of both phases of formalin test indicates that peripheral mechanisms also play a substantial role in ALE – induced antonociception (6).

Stress induce antinociception (17) but, ALE was not stressogenic (in terms of fur erection and expothalamia) Hence, in this study, antinociception is unlikely to be due to stress. Antinociception can be induced by dopaminergic mechanisms (17). Mediation of dopaminergic mechanisms are unlikely in this study as metaclopramide, a dopamine receptor antagonist, failed to inhibit ALE – induced antinociception. Cholinergic mechanisms can also evoke antinociception (18). However, this mode of action is also unlikely to be operative since atropine, a muscarinic receptor antagonist failed to curtail ALE – evoked antinociception. Sedation is known to impair pain (19) and several sedatives have pain relieving actions (17). Sedative mode of action is however unlikely to be operative with ALE since it failed to inhibit, at least, one of the parameters of the rat hole board test : hole board test is a validated and widely used model to evaluate potential sedatives (12). Antinociception can result from a membrane stabilizing effect and / or rasing of a nociception threshold as reported with some herbal drugs (12). However, ALE is unlikely to mediates its antinociception by membrane stabilization as it failed to inhibit heat – induced haemolysis of rat erythrocytes *in vitro.* It is well recognized that opioid mechanisms play a vital role in inhibiting pain (17) and opioid receptor agonists are potent antinociceptives (17). Although, we have no direct evidence, it appears that ALE induced antinociception is possinly mediated *via* opiodergic mechanisms : opioids inhibit both phases of the formalin test (20) and a similar effect was evident with ALE. Several plant alkaloids induce antinociception by opioid mechanisms (21) and ALE also contained alkaloids as evident from phytochemical and chromatographic analysis. ALE could act *via* opioid mechanisms to bring antinociception by having opioidmimetic activity and / or by releasing endogenous peptides such as endophines and enkephalines which act on the opioid receptors (17).

The ALE also contained sterols, polyphenols and flavonoids. These constituents are known to inhibit the early and late phases of the formalin test. Thus, it is possible that these constituents play, atleast, an auxillary role in mediating antinociceptive action. Another

potential mechanism of antinociceptive action ALE is *via* antioxidant action as oxygen free radicals are implicated in pain (22). Further, potent antioxidant activity and strong free radical scavenging action has been reported with a related species, *T. loureiri* (23). These evidences support the proposed antioxidant mode of action of ALE.

In conclusion, this study, shows for the first time, moderately strong oral antinociceptive activity of ALE of *T. sarmentosa*. This action is beneficial and may widens its therapeutic use in bone fracture. Further, this study points out that *T. sarmentosa* leaves may contain lead compound/s that may be developed as potent antinociceptives.

ACKNOWLEDMENT

We appreciate technical assistance given by Mr. T. S. P. Fernando and Mr. J.R.A.C. Jayakody Department of Zoology, University of Colombo, in conducting the animal experiments. This investigation received financial support from the grant to SAD for consumables from the University of Colombo (AP/3/2/2000/S/05) is gratefully acknowledged.

REFERENCES

1. Dassanayake M.D., *Revised handbook to the flora of Ceylon*, Volume **X**, (Oxford & IBH Publishing CO. Pvt. Ltd. Culcutta, India, 1978) pp.109, 116–119.
2. Senarathna L.K., *A Checklist of Flowering Plants of Sri Lanka*, (National Science Foundation of Sri Lanka, 2001) pp. 116
3. Trimen H., *A Hand-book of the Flora of Ceylon*, part 1, (Periodical Experts, Delhi, 1972) pp.154
4. Snell R.S., *Clinical Anatomy*, Lippincott Williams and Wilkiins (2004) pp. 37.
5. Mace S.E., Ducharme J., Murphy M.F., Pain Management and Sedation: Emergency Department Management, (The McGrew-Hill Companies, New York, 2006).
6. Ratnasooriya W.D., Deraniyagala S.A., Peiris S.K.J.S.. Antinociceptive Potential of the Sri Lankan Endemic Plant *Vernonia zeylanica*. *Pharm. Biol.* **45**: 525–535 (2007).
7. Vasudewa N.S., Abeytunga D.T.U., Ratnasooriya W.D.. Antinociceptive activity of *Pleurotus ostreatus*, an edible mushroom, in rats. *Pharm. Biol.* **45**: 530–540 (2007).
8. Kudahewa D.D., Abeytunga D.T.U., Ratnasooriya W.D.. Antinociceptive activity of *Pleurotus cystidiosus*, an edible mushroom in rats. *Phcog Mag.* **4**: 37–41, (2008).
9. Goonasekara C.L., Ratnasooriya W.D., Deraniyagala S.A.. Antinociceptive effect and toxicological study of aqueous bark extract of *Barringtonia racemosa* on rats. *J. Ethnopharmacol.* **86**: 21–26 (2003).
10. Ratnasooriya W.D., Dharmasiri M.G.. Water extract of leaves and stem of *Psychotria sarmentosa* has analgesic and antihyperalgesic activity in rats. *Med. Sci. Res.* **27**: 715–718 (1999).
11. Arawwala L.D.A.M., Arambewela L.S.R., Ratnasooriya W.D.. Antinociceptive Activities of aqoueous and ethanolic exstracts of *Alpinia calcarata* in rats. *J. Ethnopharmacol.* **95**: 311–316 (2004).
12. Ratnasooriya W.D., Deraniyagala S.A., Bathige S.D.N.K., Galhena G., Liyanage S.S.P., Jayakody J.R.A.C.. Anti-inflammatory activity and analgesic activities of mature fresh leaves of *Vitex negundo* in rats. *J. Ethnopharmacol.* **87**: 199–206 (2003).
13. Fransworth N.R., Phytochemical Screening, College of Pharmacy, University of Illonois, Chicargo. (1978) pp.32–65
14. Stahl E., *Thin Layer Chromatography, A laboratory Hand Book*, Academic Press Publishers, New York, (1965) pp.483–502
15. Farsam H., Amanlou M., Dehpour A.Z., Jahaniani F.. Anti-inflammatory and analgesic activity of *Biebersteinia multifida* DC. Root extract. *J. Ethnopharmacol.* **71**: 443–447 (2000).
16. Wong C.H., Day P., Yarmush J., Wu W., Zbuzek U.K.. Nifedipine-induced analgesic after epidural injections in rats. *Anesth. Analg.* **79**: 303–306 (1994).
17. Rang H.P., Dale M.M., Ritter J. M., *Pharmacology*, Churchill Livingstone, London. (2007) pp. 147, 478, 600.
18. Anonymous: *British National Formulary*. London, (The British Medical Association and the Royal Phamaceutical Society of Great Britain 2000) Pp.571
19. Nadeson R., Goodchild C.S.. Antinociceptive Properties of propofol : Involvement of spinal cord γ-amino-butyric $acid_A$ receptors. *J. Pharmacol. Exp. Ther* **282**: 1181–1186 (1997).
20. Tjolsen A., Berge D.G., Hunskaar S., Rosland J.H., Hole K.: The formalin test : An evaluation of the method, Pain **51**: 5–17 (1992).
21. Elisabetsky E., Amador T.A., Albuquerque R.R., Nunes D.S., Ana do C.T. Carnalho. Analgesic activity of *Psychotria colorata* (Willd. Ex R. & S.) Muell Arg. alkaloids, *J. Ethnopharmacol.* **48**: 77–83 (1995).
22. Halliwell B.. Free radical antioxidants in human disease : Curiosity, cause of consequence. *Lancet* **344**: 721–724.
23. Kukongviriyapan V., Janyacharoen T., Kukongviriyapan U., Laupattarakasaem P., Konokmedhakul S., Chantaranothai P.. Hepatoprotective and antioxidant activities of *Tetracera loureiri*. *J. Phytothe. Res.***17** 717–721 (2003).