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Analgesic and Antioxidant Properties of Ethanolic Extract of *Terminalia catappa* L. Leaves

H.V. Annegowda, M.N. Mordi, S. Ramanathan and S.M. Mansor
Centre for Drug Research, Universiti Sains Malaysia, 11800 Penang, Malaysia

Abstract: The aim of the present study was to investigate the analgesic and antioxidant activities of ethanolic extract of *Terminalia catappa* (TCSE) leaves obtained by soxhlet extraction. The analgesic effects of TCSE extract was studied by formalin induced pain, hot plate and tail flick tests where as antioxidant activity was evaluated by ABTS radical scavenging and metal chelating assays. In formalin test, 80 mg kg^{-1} (p.o.) dose of TCSE extract inhibited both the phases ($p<0.05$) of animal's nociception, but TCSE extracts (40 mg kg^{-1}, p.o.) inhibited only late phase. TCSE extract (80 mg kg^{-1}, p.o.) showed a significant ($p<0.05$) increase in the reaction time in hot plate test at the time interval of 60, 90 and 120 min. In contrast, both the doses of TCSE extracts did not show any analgesic effect in tail flick test, but morphine (5 mg kg^{-1}, s.c.) shown significantly ($p<0.05$) higher analgesic effect at all time intervals. TCSE extracts showed moderate ABTS free radical scavenging activity compared to standard gallic acid and higher activity compared to BHT (88.07, 96.35 and 68.76% of inhibition, respectively) but shown less ability to chelate ferrous ion. It was concluded from our studies that TCSE extracts have analgesic and anti-inflammatory properties as well as better radical scavenging ability.

Key words: *Terminalia catappa*, analgesic, antiinflammation, ABTS, metal chelation

INTRODUCTION

Terminalia catappa L. (*T. catappa* L.) is a Combretaceous plant which grows commonly in tropical and subtropical countries. The leaf, bark and fruit of this plant have long been used in folk medicine for antidiarrheic, antipyretic and haemostatic purposes in India, Phillippines, Malaysia and Indonesia (Lin, 1992). *T. catappa* leaf has been reported to possess antioxidative, hepatoprotective, antidiabetic, anti-inflammatory and anti-HIV reverse transcriptase activity (Chyau *et al*., 2002; Tang *et al*., 2004; Nagappa *et al*., 2003; Middleton *et al*., 2000; Fan *et al*., 2004; Tan *et al*., 1991). Leaves of *T. catappa* contains a number of hydrolysable tannins such as punicalagin, punicalin, chebulagic acid, corialgin, geranin, terflavins A and B, tergallagin, gratin B (Tanaka *et al*., 1986), flavonoids like, isovitexin, vitexin, isoorientin, rutin (Lin *et al*., 2000) and triterpenoids such ursolic acid and 2α, 3β, 23-trihydroxyurs-12-en-28 oic acid (Fan *et al*., 2004).

The undesired effects of the analgesic compounds available in the market provided an opportunity for new and better analgesic compounds (Katzung, 2007). Bioactive compounds derived from plants have been utilised since from the earlier time for the various purposes including the treatment of pain. Moreover, numerous plant species were found to possess analgesic and anti-inflammatory activities (Gill *et al*., 2010; Jothimanivannan *et al*., 2010; Mills and Bone, 2000). The plants belonging to the family *Combretaceae* comprise of about 200 species of *Terminalia*, which are most widely used for medicinal purposes (McGaw *et al*., 2001).

The presence of wide number of bioactive compounds with variety of biological activities in *T. catappa* attracted us to select this plant for our analgesic study. Earlier researchers have reported the analgesic activity of aqueous juice prepared by maceration from the tender leaves of *T. catappa* to support its folklore use (Ratnasooriya *et al*., 2002). The amount bioactive constituents present in the plant extract depend on the method of extraction and type of solvent employed which directly influence the biological activity of the plant extracts (Hayouni *et al*., 2007). In this regard, our main objective was to evaluate the analgesic activity of *T. catappa* leaf ethanolic extract obtained by soxhlet extraction. Free radicals are involved during pain stimulation and antioxidants shown to reduce such pain (Kim *et al*., 2004; Das and Maulik, 1994). Hence, an attempt also been made to evaluate the antioxidant activity of *T. catappa* leaf extract.

MATERIALS AND METHODS

Plant material: Fresh leaves of *Terminalia catappa* were collected from the campus of Universiti Sains

Corresponding Author: H.V. Annegowda, Centre for Drug Research, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia
Tel: +60 46532121 Fax: +60 46568669

Malaysia (USM), Penang, Malaysia in November 2008. The plant was authentified by a botanist and the voucher specimen (No. 11048) was deposited in Herbarium, School of Biological Sciences, USM. Collected leaves were then washed with running water to remove the dirt and adherent then dried and grinds to get powder of 40 mesh size.

Preparation of extract: Hundred gram of ground sample was refluxed with 1000 mL of ethanol (99.5%) at 70°C for 48 h using soxhlet apparatus. The liquid extract was separated from solid residue by vacuum filtration through Whatman filter paper (No. 1), filtered ethanolic was then concentrated to small volume using rotary evaporator (Buchi Rotavapor R-215, Switzerland) followed by drying in freeze dryer (LABCONCO, Free Zone 6 Liter, USA) to get constant mass and kept in air tight container at 4°C until further analysis.

Chemicals: Potassium persulfate, 2,2-azinobis (ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), gallic acid, butylated hydroxytoluene (BHT), aspirin, ferrozine, formaldehyde and ethylenediamine tetraacetic acids (EDTA) were purchased from Sigma Aldrich, Germany. Morphine was obtained from Hospital USM, Kelantan, Malaysia. All solvents used were of analytical grade and purchased from Fisher Scientific (M) Sdn. Bhd, Malaysia.

Animals: Male Sprague Dawley rats (150-200 g) and Swiss albino mice (20-25 g) were obtained from animal house, USM. These animals were acclimatised to the laboratory one week before the experimental procedure. All animals had free access to water and food pellets and were fasted overnight prior to use. The experimental procedures were approved by Animal Ethics Committee, USM.

Analgesic activity

Formalin-induced pain: Modified method described by Santos and Calixto (1997) was used in this study. The pain was induced by injecting 0.05 mL of 2.5% formaldehyde (40%) in saline in the subplantar region of rat hind paw. Preliminary oral LD_{50} dose of TCSE in mice was 800 mg kg^{-1} hence rats (six in a group) were given with one-tenth LD_{50} dose of TCSE extract (40 and 80 mg kg^{-1}, p.o.), morphine (5 mg kg^{-1}, s.c.), aspirin (100 mg kg^{-1}, p.o.) and co-solvent. The time spent in licking and biting the injected paw was considered as indicative of nociceptive behaviour was recorded. Two successive phases are involved following the injection of formalin, first phase (0 to 5 min) representing neurogenic pain and second phase (15 to 30 min) representing tonic and inflammatory pain response (Hunskaar and Hole, 1987). The percentage inhibition for each rat and each group was obtained as follows:

$$\text{Percentage inhibition} = [(A_0 - A_1/A_0)\times 100]$$

where, A_0 is the reading of the co-solvent and A_1 is the reading of samples.

Hot plate: The hot plate test to measure the latency response was performed according to the method described by Woolfe and MacDonald (1994), with slight modification (Santos *et al*., 1998). Rats were placed on the heated surface of a hot plate analgesia meter (IITC Life Science Series 8, Model PE34, Victory Blvd, Woodland Hills, CA 91367, USA) maintained at 55±0.2°C. The time taken for licking, shaking of forepaws or jumping was recorded as the index of response latency. Animals were tested before administration of samples in order to obtain the baseline latency and an automatic cut-off time of 45 sec was used in order to prevent rat paw tissue damage. Rats (six in a group) were given with TCSE extract (40 and 80 mg kg^{-1}, p.o.), morphine (5 mg kg^{-1}, s.c.), aspirin (100 mg kg^{-1}, p.o.) and co-solvent (propylene glycol: Tween 80: water = 4:1:4, v/v/v) served as control. The response latency was measured 30 min before and 30, 60, 90 and 120 min after the administration of samples.

Tail flick: The method described by D'Amour and Smith (1941) was used to measure response latency in the tail flick test. A radiant heat analgesiometer (IITC Life Science Series 8, Model 33T, Victory Blvd, Woodland Hills, CA 91367, USA) was used. Rats responded to a focused beam of light by flicking their tail. An automatic cut-off time of 10 s was used to avoid tissue damage. Animals were tested before administration of samples in order to obtain the baseline latency. Rats (six in a group) were given with TCSE extract (40 and 80 mg kg^{-1}, p.o.), morphine (5 mg kg^{-1}, s.c.), aspirin (100 mg kg^{-1}, p.o.) and co-solvent. The latency for flicking the tail was determined 30 min before and 30, 60, 90 and 120 min after the administration of samples and quantified by an average of three measurements.

Antioxidant activity

ABTS radical scavenging assay: ABTS radical scavenging activity of TCSE was determined according to the previously described procedure (Re *et al*., 1999). Fresh ABTS radical was prepared by adding 5 mL of a 4.9 mM potassium persulfate solution to 5 mL of a 14 mM ABTS solution and this solution was kept for 16 h in dark. This solution was diluted with methanol to yield an absorbance of 0.700±0.02 at 734 nm and the same solution was used for the antioxidant assay. The final reaction mixture (1 mL) of standard and extract comprised 950 μL of ABTS

solution and 50 μL of the extract at variable concentration (0.5-10 μg mL^{-1}). This reaction mixture was vortex for 10 sec, after 6 min absorbance was recorded at 734 nm and compared with the control ABTS solution.

ABTS radical scavenging activity of different extracts was calculated using the following formula:

$$\text{ABTS radical scavenging activity (\%)} = [(A_0 - A_1/A_0)\times 100]$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of samples.

Metal chelating activity: The chelating ability of ferrous ions by TCSE extract was evaluated by the method described by Dinis *et al.* (1994). To the 2.5 mL of TCSE extract (0.250-2 mg mL^{-1}) and EDTA served as the positive control (0.0025-0.04 mg mL^{-1}), 0.05 mL of $FeCl_2 \cdot 4H_2O$ (2 mM) was added. The reaction was initiated by the addition of 0.2 mL of ferrozine solution (5 mM) then the mixture was vortexed and kept for 10 min at room temperature. Absorbance of the resulting solution was then measured at 562 nm against the blank prepared in the same way using $FeCl_2$ and methanol. Sample without extract served as the negative control. All tests were done in triplicate and the percentage inhibition was calculated using the formula used for the ABTS assay.

Statistical analysis: The results of antioxidant and analgesic activity were expressed as means±SD and means±SEM respectively. Statistical analysis of variance was performed with one way ANOVA, followed by Tukey's HSD (Honestly Significant Difference) using SPSS 17 (SPSS Inc., Wacker Drive, Chicago, USA). $p<0.05$ were consider to be statistically significant when compared to control.

RESULTS

Analgesic activity

Formalin induced pain: The effect of TCSE extract, aspirin and morphine in early phase (0-5 min, neurogenic pain) and late phase (15-30 min, inflammatory pain) of formalin test are shown in Table 1. TCSE extract significantly ($p<0.05$) blocked the neurogenic pain only at the dose of 80 mg kg^{-1} and at the same dose it inhibited the inflammatory pain (46.9%, $p<0.05$) better than neurogenic pain (16.39%, $p<0.05$). TCSE extracts shown significant ($p<0.05$) activity during the late phase. Similarly, morphine produced significant inhibition ($p<0.05$) of both early and late phase. In contrast, aspirin showed significant inhibition ($p<0.05$) only during the late phase.

Hot plate: The result of hot plate test was shown in Table 2. The analgesic effect of TCSE extract was

Table 1: Effect of TCSE extract, morphine and aspirin on formalin-induced pain

		Total time spent in licking (sec)			
Treatment	Dose (mg kg^{-1})	0-5 min	Inhibition (%)	15-30 min	Inhibition (%)
Control	0	52.21±2.20		95.00±2.61	
TCSE extract	40	48.93±2.32	6.28	71.24±1.96*	25.0*
TCSE extract	80	43.65±1.03*	16.39*	50.42±3.26*	46.9*
Morphine	5	15.44±1.33*	70.42*	21.05±1.14*	77.8*
Aspirin	100	49.48±2.13	5.23	34.18±3.42*	64.0*

Each value represents mean±SEM in seconds for six rats treated with TCSE extracts or reference drugs. *$p<0.05$, significant from control

Table 2: Effect of TCSE extract and morphine on pain induced by hotplate

		Latency period (min)			
Treatment	Dose (mg kg^{-1})	30	60	90	120
Control	0	5.19±0.51	5.54±0.31	5.91±0.21	5.02±0.29
TCSE extract	40	5.52±0.26	6.82±0.27	7.03±0.45	6.71±0.25
TCSE extract	80	6.49±0.48	9.67±0.21*	10.27±0.19*	9.15±0.21*
Morphine	5	18.03±0.56*	22.08±0.65*	12.53±2.10*	13.40±1.19*

Each value represents mean±SEM in seconds for six rats treated with TCSE extracts or reference drugs. *$p<0.05$, significant from control

Table 3: Effect of TCSE extract and morphine on pain using tail flick test

		Latency period (min)			
Treatment	Dose (mg kg^{-1})	30	60	90	120
Control	0	4.06±0.30	4.99±0.17	4.48±0.26	4.45±0.09
TCSE extract	40	4.06±0.26	5.14±0.53	5.08±0.53	4.75±0.56
TCSE extract	80	4.56±0.33	5.59±0.12	5.63±0.41	4.38±0.56
Morphine	5	10.00±0.00*	9.39±0.26*	8.11±0.42*	7.76±0.54*

Each value represents Mean±SEM in seconds for six rats treated with TCSE extracts or reference drugs. *$p<0.05$, significant from control

significantly ($p<0.05$) more with the dose of 80 mg kg^{-1} at the time interval of 60, 90 and 120 min whereas 40 mg kg^{-1} found to be not effective at all the time intervals. Whereas, morphine (5 mg kg^{-1}) considerably ($p<0.05$) increased the reaction time to the nociceptive response in comparison with the control, TCSE extract and aspirin at all the tested time intervals. It was also found that the antinociceptive activity of morphine was three and two and half times more than that of 80 mg kg^{-1} TCSE extract at 30 and 60 min, respectively and was found to be almost equal at 90 min time interval.

Tail flick: Results of tail flick test (Table 3) revealed that TCSE extracts did not show any analgesic effect in this test at the different intervals of time tested, whereas morphine showed significantly ($p<0.05$) higher analgesic activity at all the tested time interval. It was also found that the activity of morphine was significantly higher at 30 and 60 min and was found to decrease at 90 and 120 min.

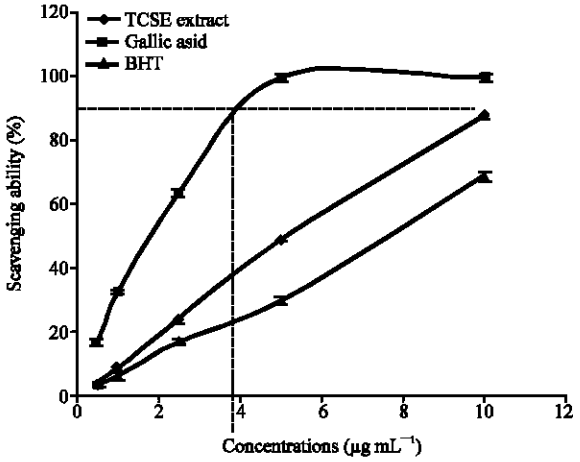

Fig. 1: ABTS radical scavenging ability of TCSE and reference standards. Each value is expressed mean±SD (n = 3)

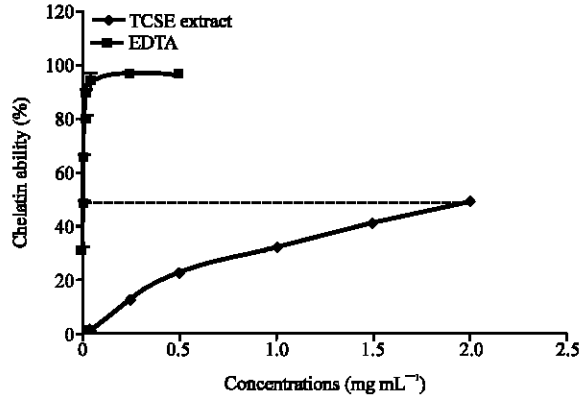

Fig. 2: Chelating ability of TCSE and EDTA. Each value is expressed mean±SD (n = 3)

Antioxidant activity: The free radical scavenging activity of TCSE extract was investigated using ABTS assay. TCSE extract inhibited the ABTS radicals in a concentration-dependent manner (Fig. 1). Scavenging activity of TCSE extract was marked ($p<0.05$) than the synthetic antioxidant BHT in all the concentration but was less than gallic acid. TCSE extract at the concentration of 10 µg mL^{-1} showed similar activity as that of gallic acid 4 µg mL^{-1} concentrations. TCSE extract was found to possess weak chelating ability in comparison with the positive control EDTA (Fig. 2). Chelating ability of TCSE extract at the concentration of 2 mg mL^{-1} was similar to that of EDTA at the concentration of 0.005 mg mL^{-1}.

DISCUSSION

In this study, we evaluated the potential antinociceptive activity of TCSE extract using chemical model (formalin induced pain) and thermal models (hot plate and tail flick) of nociception in rats. The antinociceptive models used were such that both centrally and peripherally mediated effects were investigated. Antioxidant models used were to evaluate the radical scavenging and metal chelating ability of TCSE extract.

Formalin method was normally employed to elucidate the mechanism of pain and analgesia which consists of early phase (0-5 min) because of the stimulation of nociceptive receptors in the paw and the late phase probably reflects the inflammation process (Tjolsen *et al*., 1992; Coderre and Melzack, 1992). Centrally acting drugs inhibit both phases of formalin induced pain, where as drugs like aspirin and which acts peripherally inhibit only the late phase (Chan *et al*., 1995). Oral administration of TCSE extract (80 mg kg^{-1}) inhibited the early phase of formalin test but the percentage of inhibition was four times less than that of morphine, which shows that central mechanism may be involved in the analgesic activity of TCSE extract. The second phase of formalin test is related to a peripheral inflammatory process. Both the doses (40 and 80 mg kg^{-1}) significantly ($p<0.05$) inhibited the late phase. Hence, it can be deduced that peripheral mechanism might also be involved in the anti-inflammatory effect. This result was in agreement with the findings of Fan *et al.* (2004) in which they isolated triterpenic acids which are responsible for anti-inflammatory activity from *T. catappa* leaf. But our results were contrary to the previous findings of Ratnasooriya *et al.* (2002) in which administration of macerated aqueous juice of *T. catappa* inhibited only the early phase not the late phase. The reason may be the extraction method and the solvent we employed might have extracted out the bioactive compounds responsible for the anti-inflammatory activity.

Hot plate test is normally employed to find out the involvement of central analgesic activity (Paulino *et al*., 2003). In the present study, TCSE extract showed analgesic activity at higher dose (80 mg kg^{-1}) thus, the extract may act via central mechanism. Morphine (5 mg kg^{-1}) a centrally acting analgesic produced significant ($p<0.05$) analgesic activity than TCSE extract (80 mg kg^{-1}). Thus, TCSE extract may have lower potency than morphine. Present findings were in agreement with the previous findings of Ratnasooriya *et al.* (2002) in which oral administration of macerated aqueous juice of *T catappa* increased the antinociceptive reaction time.

Tail flick test is a very well known test used for discriminating morphine like analgesics from non opiate analgesics (Vogel, 2002). Both the doses of TCSE extracts (40 and 80 mg kg^{-1}) were unable to produce analgesic effect. We suspect it might be because of the dose of the TCSE extract used was too low to induce

the antinociception in this test. Present results were in agreement with the previous findings of Ratnasooriya *et al.* (2002) in which oral administration of macerated aqueous juice of *T. catappa* did not exhibit analgesic effect in tail flick test.

Since, the TCSE extract inhibited both the early and late phase of formalin test and analgesic activity in hotplate test, both central and peripheral mechanism may be involved which implies that it has not only antinociceptive but also anti-inflammatory activity.

Production of oxyradicals as well as nitric oxide plays an important role in various models of inflammation (Cuzzocrea *et al.*, 1998; Moncada *et al.*, 1991). TCSE extract was found to possess good radical scavenging activity and may partly contribute for the anti-inflammatory activity of the extract. Certain flavonoids like quercetin were found to inhibit cyclooxygenase, 5-lipooxygenase pathways and even phospholipidase A_2 responsible for the antiinflammatory response (Williams *et al.*, 1995). A study by Filho *et al.* (1996) reported on the antinociceptive effect of quercetin through a central mechanism. Flavonoids may increase the amount of endogenous serotonin or may interact with 5-HT_2A and 5-HT_3 receptors (Lee *et al.*, 2005) which may be involved in the mechanism of central analgesic activity (Colpaert *et al.*, 2002). Previous researchers reported the presence of several therapeutically valued flavonoids from the leaves of *T. catappa* (Lin *et al.*, 2000). Even though TCSE extract was found to possess good radical scavenging activity, it had weak metal chelating ability in comparison with the reference drug EDTA.

CONCLUSIONS

Based on the results of present study, we conclude that TCSE extract possess antinociceptive activity at higher dose and anti-inflammatory activities at lower dose. This activity may be related to the presence of phytochemicals such as flavonoids, tannins and triterpenes reported in leaf extract. This study also justifies the influence of extracting solvent and the method of extraction with respect to the biological activity of the plant extract. Taking into account its potent bioactivity, the plant is an ideal candidate for progress into advanced studies.

Further studies are currently underway to isolate and characterise the bioactive compounds and their antinociceptive mechanisms.

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REFERENCES

Chan, T.F., H.Y. Tsai and W. Tian-Shang, 1995. Anti-inflammatory and analgesic activities from the roots of *Angelica pubescens*. Plant Med., 61: 2-8.

Chyau, C.C., S.Y. Tsai, P.T. Ko and J.L. Mau, 2002. Antioxidant properties of solvent extracts from *Terminalia catappa* leaves. Food Chem., 78: 483-488.

Coderre, T.J. and R. Melzack, 1992. The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. J. Neurosci., 12: 3665-3670.

Colpaert, F.C., J.P. Tarayre, W. Koek, P.J. Pauwels and L. Bardin *et al.*, 2002. Large-amplitude 5-HT1A receptor activation: A new mechanism of profound, central analgesia. Neuropharmacology, 43: 945-958.

Cuzzocrea, S., B. Zingarelli, E. Gilard, P. Hake, A.L. Salzman and C. Szabo, 1998. Anti-inflammatory effects of mercaptoethylguanidine, a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger, in carrageenan-induced models of inflammation. Free Radic. Biol. Med., 24: 450-459.

D'Amour, F.D. and D.L. Smith, 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Therapeut., 72: 74-79.

Das, D.K. and N. Maulik, 1994. Antioxidant effectiveness in ischemia-reperfusion tissue injury. Methods Enzymol., 233: 601-610.

Dinis, T.C.P., V.M.C. Madeira and L.M. Almeida, 1994. Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys., 315: 161-169.

Fan, Y.M., L.Z. Xu, J. Gao, Y. Wang, X.T. Tang, X.N. Zhao and Z.X. Zhang, 2004. Phytochemical and anti-inflammatory studies on *Terminalia catappa*. Fitoterapia, 75: 253-260.

Filho, V.C., A.S. Santos, R.O.P. Decmpos, O.G. Miguel and R.A. Yunes *et al.*, 1996. Chemical and pharmacological studies of *Phyllatus caroliniensis* in mice. J. Pharm. Pharmacol., 48: 1231-1236.

Gill, N.S., K. Dhiman, J. Bajwa, P. Sharma and S. Sood, 2010. Evaluation of free radical scavenging, anti-inflammatory and analgesic potential of *Benincasa hispida* seed extract. Int. J. Pharmacol., 6: 652-657.

Hayouni, E.A., M. Abedrabba, M. Bouix and M. Hamdi, 2007. The effects of solvents and extraction method on the phenolic contents and biological activities *in vitro* of tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. Food Chem., 105: 1126-1134.

Hunskaar, S. and K. Hole, 1987. The formalin test in mice: Dissociation between inflammatory and non-inflammatory pain. Pain, 30: 103-114.

Jothimanivannan, C., R.S. Kumar and N. Subramanian, 2010. Anti-inflammatory and analgesic activities of ethanol extract of aerial parts of *Justicia gendarussa* Burm. Int. J. Pharmacol., 6: 278-283.

Katzung, B.G., 2007. Basic and Clinical Pharmacology. 10th Edn., McGraw Hill Companies Inc., New Delhi.

Kim, H.K., S.K. Park, J.L. Zhou, G. Taglialatela, K. Chung, R.E. Coggeshall and J.M. Chung, 2004. Reactive oxygen species (ROS) play an important role in a rat model of neuropathic pain. Pain, 111: 116-124.

Lee, B.H., S.M. Jeong, J.H. Lee, J.H. Kim and I.S. Yoon *et al*., 2005. Quercetin inhibits the 5-hydroxytriptamine type 3 receptor-mediated ion current by interacting with pre-transmembrane domain I. Mol. Cells, 20: 69-73.

Lin, T.C., 1992. Study on the tannins and related compounds in the fruit of *Terminalia catappa* L. J. Chin. Med. Pharm. Res., 14: 165-174.

Lin, Y.L., Y.H. Kuo, M.S. Shiao, C.C. Chen and J.C. Ou, 2000. Flavonoid glycosides from *Terminalia catappa* L. J. Chin. Chem. Soc., 47: 253-256.

McGaw, L.J., T. Rabe, S.G. Sparg, A.K. Jager, J.N. Eloff and J. van Staden, 2001. An investigation on the biological activity of *Combretum* species. J. Ethanopharmacol., 75: 45-50.

Middleton, Jr. E., C. Kandaswami and T.C. Theoharides, 2000. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. Pharmacol. Rev., 52: 673-751.

Mills, S. and K. Bone, 2000. Principles and Practice of Phytotherapy-Modern Herbal Medicine. 1st Edn., Churchill Livingstone, New York.

Moncada, S., R.M.J. Palmer and E.A. Higgs, 1991. Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmacol. Rev., 43: 109-142.

Nagappa, A.N., P.A. Thakurdesai, N. Venkat Rao and J. Singh, 2003. Antidiabetic activity of *Terminalia catappa* Linn fruits. J. Ethnopharmacol., 88: 45-50.

Paulino, N., A.P. Dantas, V. Bankova, D.T. Longhi, A. Scremin, S.L. De Castro and J.B. Calixto, 2003. Bulgarian propolis induces analgesic an anti-inflammatory effects in mice and inhibits *in vitro* contraction of airway smooth muscle. J. Pharmacol. Sci., 93: 307-313.

Ratnasooriya, W.D., M.G. Dharmasiri, R.A.S. Rajapakse, M.S. De Silva and S.P.M. Jaywardena *et al*., 2002. Tender leaf extract of *Terminalia catappa* antinociceptive activity in rats. Pharm. Biol., 40: 60-66.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med., 26: 1231-1237.

Santos, A.R. and J.B. Calixto, 1997. Further evidence for the involvement of tachykinin receptor subtypes in formalin and capsaicin models of pain in mice. Neuropeptides, 31: 381-389.

Santos, A.R., E.M. Vedana and G.A. De Freitas, 1998. Antinociceptive effect of meloxicam, in neurogenic and inflammatory nociceptive models in mice. Inflamm. Res., 47: 302-307.

Tan, G.T., J. Pezzuto, A.D. Kinghorn and S.H. Hughes, 1991. Evaluation of natural products as inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. J. Nat. Prod., 54: 143-154.

Tanaka, T., G. Nonaka and I. Nishioka, 1986. Tannins and related compounds. XLII. Isolation and characterization of four new hydrolysable tannins, terflavins A and B, tergallagin and tercatain from the leaves of *Terminalia catappa* L. Chem. Pharm. Bull., 34: 1039-1049.

Tang, X.H., L. Gao, J. Gao, Y.M. Fan, L.Z. Xu, X.N. Zhao and Q. Xu, 2004. Mechanism of hepatoprotection of *Terminalia catappa* L. extract on D-galactosamine-induced liver damage. Am. J. Chin. Med., 2: 509-519.

Tjolsen, A., O.G. Berge, S. Hunskaar, J.H. Rosland and K. Hole, 1992. The formalin test: An evaluation of the method. Pain, 51: 5-17.

Vogel, H.G., 2002. Drug Discovery and Evaluation: Pharmacological Assays. 2nd Edn., Springer-Verlag, New York, pp: 694-696.

Williams, C.A., J.R. Hoult, J.B. Harborne, J. Greenham and J. Eagles, 1995. A biologically active lipophilic flavonols from *Tanacetum parthenium*. Phytochemistry, 38: 267-270.

Woolfe, G. and A.D. MacDonald, 1994. The evaluation of the analgesic action of pethidin hydrochloride (DEMEROL). J. Pharmacol. Exp. Ther., 80: 300-307.