Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 2, 2013

Research Article

ANTI-INFLAMMATORY AND *IN VITRO* ANTIOXIDANT POTENTIAL OF EXTRACTS LEAVES OF *LUFFA ACUTANGULA (VAR) AMARA* IN RODENT MODEL (RATS)

ULAGANATHAN IYYAMPERUMAL*, NAPPINNAI MOHANAVELUA, SHANMUGAPANDIYAN PITCHAIMUTHU[A], SOMA RAHAB, MUTHUSAMY PERIYANNANC AND RAJU ILAVARASAND

[*a] Mohamed Sathak A.J. College of Pharmacy, Sholinganallur, Chennai 600119. India, [b]C.L.Baid Metha College of Pharmacy, Chennai 96. India, [c]Department of Pharmacy, Madras Medical College of Pharmacy, Chennai 600003, [d]Captain Srinivasa Murti Drug Research Institute for Ayurveda and Siddha, Arumbakkam, Chennai- 600106. Email: nathan_cology@yahoo.co.in and shanmugapandiyan@gmail.com

Received: 07 Jun 2012, Revised and Accepted: 10 Jan 2013

ABSTRACT

Objective: The aim of the present study was to evaluate the anti-inflammatory effect and *in vitro* antioxidant potential of ethyl acetate (EAELA) and ethanol (EELA) extracts of dried leaves of *Luffa acutangula (var) amara*.

Methods: Anti inflammatory effect was evaluated by carrageenan induced hind paw edema and cotton pellet granuloma models. Anti oxidant effect was evaluated applying *in-vitro* models were the free radical scavenging activity was measured. Models applied were 1, 1-diphenyl-2-picrylhydrazyl hydrochloride (DPPH) reduction method, lipid Peroxidation method, reduced glutathione and nitric oxide scavenging method. Two dose levels 250 and 500 mg/kg, p.o were applied for both the extracts.

Results: Both extracts at both dose levels were found to possess significant anti-inflammatory effect in acute and chronic models. The EELA and EAELA extracts at 25 to 800 mcg/ml concentrations showed significant anti-oxidant effect in Nitric Oxide and DPPH models. Significant inhibitory activity on lipid peroxidation and glutathione reduced assay were observed. Reactive oxygen species scavenging and lipid peroxidation inhibition activities indicate that *Luffa acutangula* might be valuable natural antioxidant source.

Conclusion: The results obtained from this study indicate that the tested extracts have potential anti-oxidant and anti-inflammatory activity.

Keywords: *Luffa acutangula (var) amara,* Carrageenan, Cotton pellet granuloma, Anti-oxidant.

INTRODUCTION

Current treatment for inflammatory diseases is limited to use of steroidal and Non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs exerts their effects by inhibiting the metabolism of arachidonic acid, by both cyclooxygenase and lipoxygenase enzyme pathway.[1] Despite their widespread use, NSAIDs are often associated with severe adverse effects; the most common being gastrointestinal bleeding[2] For this reason, safer compounds with fewer side effects are needed.

Luffa acutangula (var) amara is an annual herb found in all parts of India, especially on the western peninsula and Ceylon[3]. This plant is used as a laxative, carminative and intestinal tonic. Moreover, it serves as a cure for "vata", "kapha", biliousness, anemia, liver complaints, leucoderma, piles, bronchitis and acites. It also assuages the effects of uterine, vaginal tumors and tuberculous glands. It is also said to be useful in asthma. The kernel of the seeds is used to treat dysentery[4,5]. The plant has pharmacological actions like demulcent, diuretic, bitter tonic, nutritive and expectorant[6] and immuno-modulatory and anti-tumor activities[7]. It is also effective in leprosy, glandular conjunctivitis, ring worm infestation and dermatopathy[8]. Review of literature did not show any report about the potential anti-inflammatory and *in vitro* antioxidant effect. Hence, in the present study, the possibility of anti-inflammatory and *in-vitro* antioxidant effect of leave extracts of *Luffa acutangula (var) amara* were evaluated in Carrageenan-induced paw edema and Cotton pellet granuloma in acute and chronic inflammation models in rats and free radical scavenging activity on different *in vitro* models like DPPH, lipid peroxidation, reduced glutathione and nitric oxide scavenging models.

MATERIALS AND METHODS

Plant collection and identification

The fresh leaves of *Luffa acutangula (var) amara* were collected from the gardens in Courtallum, Tirunelvelli dist, in July and August-2004. The plant was identified and authenticated by Dr. P. Jayaraman, Botanist, Plant anatomy Research Centre, West Tambaram, Chennai-600045. A voucher specimen was deposited in the Department of Pharmacology, C. L. Baid Metha College of pharmacy, Chennai, Tamil Nadu. (Voucher no-205/ 2004).

Chemicals required

All the chemicals used in this study were of analytical grade. Carrageenan, 1,1-Diphenyl-2-picrylhydrazyl hydrochloride (DPPH), 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), naphthyl ethylene diamine dihydrochloride (NEDDH) were obtained by Sigma Chemicals Company, USA. Thiobarbituric acid (TBA), trichloro acetic acid (TCA) and sulphonilamide were obtained from SD fine Research Lab, Bombay, India.

Preparation of extract

Leaves were collected, dried under shade, coarsely powdered and extracted with ethanol and ethyl acetate using soxhlet extractor. Extracts were dried under reduced pressure using a rotary flash evaporator and stored between 0-4°C protected from sunlight until further use. The percentage yield of ethanol (EELA) and ethyl acetate (EAELA) extract were found to be 11 % w/w and 9 % w/w. The extracts were used for the pharmacological studies by dissolving in 0.5 % v/v Tween 80.

Phytochemical Study

The EELA and EAELA leaves were subjected to preliminary phytochemical screening for various plant constituents[9,10].

Animals

Colony in bred strains of Wistar rats of both sex weighing (180-250g) were used for the pharmacological studies. The animals were kept under standard conditions (day/night rhythm) 8.00 am to 8.00 pm (12 hrs), 22°C room temperature, standard pelleted diet (Hindustan Lever, Bangalore) and water *ad libitum*. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. Rats were divided into groups. Each group containing six animals and were kept in different cages. Animals were selected at random and both sexes were used. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC). Approval No: IAEC XI-

20/CLBMCP/2004-2005; Dated 7.7.2004.

ANIMALS STUDIES

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method),[11]. Wistar rats (n = 6) of either sex selected by random sampling technique were used for acute toxicity study. Selected rats were fasted overnight from food providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg body weight by gastric intubation and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight.

***In vivo* anti-inflammatory evaluation**

Carrageenan induced paw edema

Rats were divided into six groups of six animals each. Inflammation was induced as described previously[12,13]. All rats were fasted overnight with water *ad libitum* and grouped as mentioned below. Edema was induced by injecting 0.1 ml of 1 % w/v carrageenan in normal saline into the sub plantar site of the right hind paw of each rat. Group I served as normal control rats receiving 5 ml/kg of 0.5 % v/v Tween 80 per oral, Group II and Group III were administered with EELA 250 and 500 mg/kg, p.o. respectively, Group IV and Group V given EAELA 250 and 500 mg/kg, p.o. respectively, and Group VI Standard drug (Indomethacin 5mg/kg) 1h prior to carrageenan administration. The different dose levels were administered after dispersing in 0.5% v/v Tween 80 orally. The swelling of paw volume was measured every hour up to 5 hrs by dipping the right hind paw of all rats into mercury column and measuring the mercury displacement by using plethysmograph (Ugo Basile, Italy).

Cotton pellet granuloma

Chronic inflammation was induced as described previously in literature[14]. The effect of the extracts on exudative and proliferative inflammation by forming the granuloma pouch was evaluated. The granuloma was produced by implanting the sterilized cotton (weight 20 ± 1mg) pellet subcutaneously in the ventral region of the groin under ether anaesthesia. Group I rats were given 5ml/kg of 0.5 % v/v Tween 80 per oral, Group II and III were given EELA 250 and 500 mg/kg, Group IV and V administered EAELA 250 and 500 mg/kg orally, and Group VI received standard drug (Indomethacin 5 mg/kg) The animals were treated for 7 days. The animals were sacrificed on 8th day the pellets surrounded by granuloma tissue were removed. The moist pellets were weighed then dried at 60oC for 24hrs. Dried pellets were weighed and the anti-proliferative effect was compared with that of positive and negative control groups.

***In vitro* antioxidant studies**

Preparation of liver homogenate:

Rats from the chronic model study were used for this procedure. At end of the above study, the liver was dissected immediately, removed and washed with ice-cold saline, and a 10% w/v homogenate was prepared using 0.1M Tris-Hydrochloride buffer, pH 7.4 and centrifuged at 3000 rpm for 10 minutes. The supernatant was used for the assay of lipid peroxidation and reduced glutathione.

Lipid peroxidation

Lipid peroxidation in the liver homogenate was determined by measuring the amounts of malondialdehyde produced[15]. Lipid peroxidation was initiated by adding 100 µl of 15 mM ferrous sulphate (FeSO4) and 3 ml of liver homogenate. After 30 min, 100 µl of this reaction mixture was taken in a tube containing 1.5 ml of 10 % Trichloro acetic acid. After 10 min, the tubes were centrifuged and supernatant was separated and mixed with 1.5 ml of 0.67 % thiobarbituric acid (TBA) in 50 % acetic acid. The mixture was heated in boiling water bath. The intensity of pink coloured complex was measured at 535 nm in a spectrophotometer (Schimadzu1601).

Assay of Reduced glutathione

Reduced glutathione was determined according to the method[16]. Liver homogenate with different concentrations of extract (25 - 800 µg/ml) were mixed with 0.5 ml of trichloro acetic acid in 0.1 mM sodium ethylene diamine tetra acetate (EDTA). The sample was mixed and centrifuged at 3000 rpm for 10 min. Supernatant was mixed with 2.5 ml of 0.1 M Phosphate buffer (pH 8). The colour developed by adding 100 µl of 0.01 % 5, 5-dithio-bis-(2-nitrobenzoic acid) (DTNB). Absorbance was measured at 412 nm.

DPPH (1, 1 Diphenyl, 2 picryl hydrazyl) Radical Scavenging Activity

DPPH scavenging activity was measured according to the method[17]. To an ethanolic solution of DPPH (0.2mM, 2.7ml), 0.3 ml of test extracts in ethanol at different concentration (25-800 µg/ml) were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 1 h. Decolorization of DPPH was determined by absorbance at 517 nm. Equal amount of ethanolic solution of DPPH was using as a blank.

Nitric oxide scavenging;

Nitric oxide scavenging activity was determined according to the method[18]. Sodium nitroprusside (5 mM) in phosphate buffer solution was mixed with different concentrations of extracts (25 to 800 µg/ml) and incubated at 25oC for 30 min. Vehicle used devoid of the test substance was used as control. After 30 min, 1.5 ml of Griess reagent (1 % sulphonilamide, 2 % phosphoric acid, and 0.1 % naphthyl ethylene diamine dihydrochloride) the absorbance of the chromospheres formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthyl ethylene diamine was measured at 546 nm.

STATISTICAL ANALYSIS

The data represents mean ± SEM. Results were analysed statistically by ANOVA followed by Dunnet's't' test using SPSS software student's version. The difference was considered significant when $p<0.05$.

The percentage inhibition was calculated as –

$$\% \text{ inhibition} = \frac{\text{Control-Test X 100}}{\text{Control}}$$

RESULTS

Phytochemical screening

Preliminary phytochemical screening of the EELA and EAELA showed the presence of alkaloid, carbohydrate, flavanoids, glycoside, saponin, terpenes, protein, tannins, phenols, gums and mucilage.

Acute toxicity study

EELA and EAELA were screened for acute toxicity and were found to be free from toxicity at the dose of 2000 mg/kg. p.o.

Anti-inflammatory activity

EELA and EAELA were found to exhibit significant action in both phases of acute inflammation. The mean increase in paw edema volume was about 0.40 ± 0.009 ml in the Group I- vehicle treated control rats. The percentage inhibition observed after treatment with (Group II and III) EELA at doses of 250 and 500 mg/kg were 67.6% and 72.5% respectively after 5th hrs after Carrageenan administration. At a dose of 250 and 500 mg/kg of EAELA (Group IV and V), edema formation was inhibited to an extent of 62.5% and 65% at 5th hrs after Carrageenan administration, respectively when compared with the control group. However, the standard drug, Indomethacin 5mg/kg, p.o. (Group VI) showed inhibition of edema to an extent of 75% at 5thhrs after Carrageenan administration. Results in Table 1.

Table 1: Anti-Inflammatory activity of EELA and EAELA on Carrageenan Induced Paw Edema

Groups	Paw volume in ml/h					% inhibition
	1	2	3	4	5	
I	0.18 ± 0.005	0.26 ± 0.006	0.32 ± 0.007	0.36 ± 0.004	0.40 ± 0.009	--
II	0.15 ± 0.01	0.18 ± 0.005	0.23 ± 0.02	0.21 ± 0.07**	0.13 ± 0.03***	67.5
III	0.15 ± 0.003	0.19 ± 0.002	0.18 ± 0.014*	0.16 ± 0.004**	0.11 ± 0.03***	72.5
IV	0.17 ± 0.006	0.21 ± 0.004	0.20 ± 0.09*	0.18 ± 0.04**	0.15 ± 0.007***	62.5
V	0.16 ± 0.007	0.18 ± 0.002	0.16 ± 0.002*	0.14 ± 0.004**	0.14 ± 0.032***	65
VI	0.14 ± 0.04	0.17 ± 0.006	0.15 ± 0.007*	0.13 ± 0.005**	0.10 ± 0.006***	75

Units: ml/h the values are expressed as mean ± SEM from 6 animals in each group. % Inhibition shown in result of comparison Groups II, III, IV, V and VI versus Group I. *p<0.05, **p<0.01 and ***p<0.001.

Table 2: Anti-Inflammatory Activity of EELA and EAELA on Cotton Pellet-Granuloma

Groups	Weight of cotton pellet granuloma in (mg)		% Inhibition
	Wet weight	Dry weight	
I	369.3 ± 6.75	298.8 ± 3.04	--
II	344 ± 4.03	168.17±7.9***	43.5
III	324.2 ± 1.62	128.6 ± 3.05***	56.9
IV	334.2 ± 4.87	189.5 ± 4.86**	36.5
V	342.2 ± 1.62	142.6 ± 3.05***	52
VI	287.4 ± 4.92	108.2 ± 1.88***	63.8

Values are expressed as ml, are mean ± SEM from 6 animals in each group.% inhibition shown 5 comparison of Groups II, III, IV, V and VI versus Group I. **p<0.01 and ***p<0.001.

The effects of EELA and EAELA and standard on cotton pellet granuloma in rats shown in Table 2. At dose of 250 and 500 mg/kg of EELA (Group II and III) and EAELA (Group IV and V) and standard 5 mg/kg (Group VI), the granuloma formation was inhibited markedly when compared with the control group. On dried weight basis, EELA exhibited a maximum inhibition of 43.5%, 56.9%. EAELA exhibited a maximum inhibition of 36.5%, 52% and standard drug 63.8 % respectively.

Table 3: *In-Vitro* Antioxidant Potential of EELA and EAELA

Concentration (µg/ml)	Free radical scavenging in rat liver homogenates (% inhibition)				Free radical scavenging activity (% inhibition)			
	Lipid-Peroxidation		Reduced glutathione		DPPH		Nitric oxide scavenging	
	EELA	EAELA	EELA	EAELA	EELA	EAELA	EELA	EAELA
25	5.28 ± 0.73	5.78 ± 0.52	5.08 ± 0.02	4.04 ± 0.15	4.08 ± 0.03	3.61 ± 0.15	5.00 ± 0.24	4.62 ± 0.11
50	13.77 ± 0.98	10.79 ± 0.35	14.94 ± 0.04	10.40 ± 0.11	15.06 ± 0.98	9.91 ± 0.24	10.80 ± 0.39	14.40 ± 0.35
100	26.00 ± 0.80	18.86 ± 0.69	22.67 ± 0.04	17.29 ± 0.03	25.98 ± 0.80	16.8 ± 0.14	22.54 ± 0.84	20.07 ± 0.32
200	36.60 ± 0.36	27.69 ± 0.93	30.22 ± 0.08	23.61 ± 0.17	35.3 ± 0.36	22.8 ± 0.66	35.44 ± 0.31	28.67 ± 0.15
400	47.71 ± 0.24	34.70 ± 0.54	38.85 ± 0.05	31.95 ± 0.12	44.27 ± 0.24	34.8 ± 0.23	43.83 ± 0.32	38.95 ± 0.22
800	60.95 ± 0.91	44.98 ± 0.24	51.09 ± 0.04	38.41 ± 0.08	62.56 ± 0.91	42.6 ± 0.24	59.60 ± 0.23	45.68 ± 0.32
IC_{50} (µg/ml)	560.76±1.50	824.32±4.56	702.29±0.54	988.65±1.41	538.28±0.53	824.11±0.36	575.88±1.62	784.00±3.03

Values are expressed as %, are mean ± SEM from 6 replicate

***In vitro* antioxidant studies**

The percentage inhibition after EELA and EAELA were found to be 60.9% and 44.9% respectively. The percentage inhibition of GSH assay for EELA and EAELA were found to be 51.1% and 38.4% respectively. DPPH scavenging activity showed the percentage inhibition of EELA and EAELA were found to be 62.6% and 44.6% respectively. The scavenging of nitric oxide EELA and EAELA was concentration dependent and the percentage inhibition was found to be 59.6% and 45.7% respectively. Results in Table 3.

DISCUSSION

The preliminary phytochemical screening of *Luffa acutangula (var) amara* showed the presence of phytoconstituents like flavanoids, alkaloids, saponin and glycosides .In both extracts the biological activities of plant constituents are complex. Hence the anti-inflammatory and antioxidant activity of both extracts may be attributed to the complex pharmacological action of phytoconstituents present in the extracts, particularly flavanoids.

Leaves extracts of *Luffa acutangula (var) amara* has been widely used as a folkloric medicine for the treatment of diseases. Carrageenan induced paw oedema, a classical models of acute inflammation has been widely used in the study of steroid and non-steroidal anti-inflammatory drugs[19,20]. The primary test to acute phase of inflammation screen new anti-inflammatory agents to measures the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent [21]. The edema depends on the participation of kinins and polymorphonuclear leukocytes with their pro-inflammatory factors including prostaglandins[22]. The paw edema induced by sub planter injection of carrageenan in rat is biphasic response[23]. The initial phase observed around 1hr, is attributed to release of histamine, serotonin. The second, accelerating, phase of swelling is due to the release of prostaglandins like substances. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents[24]. The extracts of *Luffa acutangula (var) amara* is effective in that it inhibited the 3rdhr of the carrageenan induced inflammation. The release of prostaglandins at the 3rdh is already documented hypothesis. Thus the plant mediated it anti-inflammatory activity by inhibiting the PG synthesis. It was also observed that, both extracts evaluated are effective in reducing chronic inflammation. Cotton-pellet induced granuloma in rats a chronic model of inflammation has been utilized to assess the activity of anti-inflammatory drugs on proliferative phase of inflammation[25]. Chronic inflammation is a reaction arising when the acute response is insufficient to eliminate pro-inflammatory agents. Chronic inflammation induces a proliferation of fibroblast and the infiltration of neutrophils and exudation[26,27]. Chronic inflammation occurs by means of the development of proliferative cells. NSAIDs cause decrease in granuloma tissue arising as a result of cellular reaction, which is released by inhibiting granulocyte infiltration following foreign body implantation[28]. Therefore in chronic anti-inflammatory model of

cotton pellet, effect of EELA and EAELA may be by inhibiting the activity of macrophages and the formation of fibrosis. The EELA and EAELA possess significant anti-inflammatory effects in laboratory animals at the doses investigated. The results support the traditional use of this plant in some painful and inflammatory conditions and also suggest the presence of biologically active principals i.e. flavanoid glycosides, cucurbitacin, saponins, proteins and carbohydrates. Several flavonoids and saponin glycosides isolated from medicinal plants have been discovered to possess significant analgesic and/or anti-inflammatory effects[29]. Therefore, it is possible that the anti-inflammatory effects observed with *Luffa acutangula (var) amara* may be attributed to its flavonoids and saponin glycoside components, shown to be present during phytochemical screening. Further studies are in progress to isolate and characterize the active principles of the extract. The protective effect of EELA and EAELA against oxidative stress induced cell damage *in-vitro* experiments are carried out. Lipid peroxidation (LPO) is a free radical induced process leading to oxidative deterioration of polyunsaturated lipids. A large number of toxic byproducts are formed by lipid peroxidation, which acts as toxic second messengers[30]. The free radical species formed are capable of oxidizing sulfhydryl moieties of protein leads to protein fragmentation and loss of cell viability. The extract showed significant protection against ferrous sulphate induced LPO. The peroxides formed can further be degraded by Fenton type reaction in presence of ferrous ions. It is generally assumed that ability of the plant phenolic compounds such as flavanoids to chelate iron in the LPO system is essential for their antioxidant property. Hence the tested extracts may have the ability to chelate ferrous ion.

Reduced glutathione is a non-enzymatic mode of defense against the free radicals. The thiol groups especially of cysteine and glutathione are important for lymphocyte function[31]. The extracts are significantly inhibited the oxidation of reduced glutathione which mediated by free radicals. The free radical scavenging activity was evaluated based on the ability to quench the synthetic DPPH. This assay provided useful information on the reactivity of the compounds with stable free radical[32]. Because of the odd electrons DPPH shows an absorption band at 517nm in the visible spectrum (deep violet color). As this electron becomes paired off in the presence of free radical scavenger, the absorption vanishes and the resulting discoloration is stoichiometric with respective to the capacity of the test compound to scavenge free radical independently. The results revealed that the test extract have compounds which may be electron donors and could react with free radicals to convert them to more stable product and terminate chain reaction. Nitric oxide (NO) has been implicated in a number of pathogenesis. Excess of NO has been associated in a several aliments like circulatory shock, stroke, and inflammation. NO generated at high levels by activated macrophages and neutrophils are important cytotoxic molecule in human immune defense[33]. NO generated from sodium nitroprusside in aqueous solution spontaneously generates nitric oxide that interacts with oxygen (O2) to produce nitric ions which is estimated by Griess reagent. Extracts inhibits nitrite formation by competing with O2 to react with nitric oxide[34]. The chromophore formation is not complete in the presence of both extracts, which scavenges NO and hence absorbance decreases as concentration of EELA and EAELA increases in dose dependent manner. Flavanoids have certain health effects and their anti-oxidant, free radical scavenging, anti mutagenic and anti-carcinogenic properties are well known[35,36]. Therefore, it is possible that the anti-oxidant effects observed with *Luffa acutangula (var) amara* may be attributed to its flavonoids and saponin glycoside components, shown to be present during phytochemical screening. The results of the present study showed that EELA and EAELA are potent inhibitors of acute and chronic inflammation. The mechanism of the effects may depend on the formation of several inflammation mediators. Further studies are in progress to isolate and characterize the active principles of the extract.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Vaithilingaraja Arumugaswami, Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, for his comment of the manuscript preparation. We thank Stacy Hu for carefully editing this manuscript.

REFERENCES

1. Insel PA, Hardman JG, Limbird LE: Analgesic Antipyretic and Anti- inflammatory agents and Drugs Employed in treatment of Gout. McGraw-Hill, New York: 1996, p.617-657.
2. Fung HB, Kirshenbaum HL: Pharmacological analysis of acute anti-inflammatory process induced in the rats paw by local injection of Carrageenan and by heating. Clinical Therapeutics1999; 21:1131-1134.
3. Chopra RN, Badhwar RL, Ghosh S: Poisonus plants of India. Indian Council of Agricultural Research, New Delhi: 1996; (1):253-255.
4. Kirtikar KR, Basu BD: Indian medicinal plants. 2nd Ed, New Delhi: Publication and Information Directorate, CSIR; 2006; (2):1123-1125.
5. Chopra, R.N., Chopra, I.C., Handa, K.L., Kapur LD: Indigenous Drugs of India. 2nd Ed, Academic Publishers, Calcutta: 1958; 249-250.
6. Aswal BS, Bhakuni DS, Goel AK, Mehrotra BN, Mukherjee KC: Screening of Indian plants for biological activity. Ind. J. Exp. Biol.1984; 22: 312-313.
7. Ng TB, Chan WY: Proteins with abortifacient, ribosome inactivating immune-modulatory, anti-tumor and anti-AIDS activities from Cucurbitaceous plants. General Pharmacology 1992; 23(4):575-590.
8. Chanda S, Dave R, Kaneria M: In vitro antioxidant property of some Indian Medicinal Plants. Res. J. Medicinal Plant 2011; 5(2); 169-179
9. Kokate CK: Practical Pharmacognosy. 6th ed. Delhi; Vallabh Prakashan; 1991, p.107-121.
10. Horbone JB: Phytochemical methods.1st ed.London; Cheppman and hall; 1973, p. 60- 66.
11. Ecobichon DJ: The basis of toxicology testing, CRC press, New York; 1997, p.43-86.
12. Lee J, Kim KA, Jeong SH, Lee SG, Park SJ: Anti-inflammatory, anti-nociceptive and anti-psychiatric effects by the rhizomes of Alpinia officinarum on complete Freund's adjuvant-induced arthritis in rats. J. Ethnopharmacol 2009; 126:258–264.
13. Begum S, Saxena B, Goyal M, Ranjan R, Joshi VB, Rao CV, Krishnamurhty S, Sahai M: Study of anti-inflammatory, analgesic and antipyretic activities of seeds of Hyoscyamus niger and isolation of a new coumarinolignan. Fitoterapia 2010; 81:178–184.
14. Chen-Xiao Zhang, Zi-Ru Dai, Qiu-Xing Cai: Anti-inflammatory and anti-nociceptive activities of Sipunculus nudus L. extract. J. Ethnopharmacol 2011; 137:1177–1182.
15. Okhawa H, Onishi N, Yagi K: Assay of lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal. Biochem 1979; 95:351-358.
16. Sreejayan N, Rao MNA: Free radical scavenging activity of curcuminoids. Drug Research 1996; 49:169-172.
17. Amarowicz R, Naczk M, Shahidi F: Antioxidant activity of various fractions of non-tanin phenolics of canola hulls. J. Agri. and Food Chem 2000; 48:2755–2759.
18. Sreejayan N, Rao MNA: Nitric Oxide scavenging of curcuminoids. J Pharma Pharmacol 1997; 49:105-107.
19. Chen-Xiao Zhang, Zi-Ru Dai, Qiu-Xing Cai: Anti-inflammatory and anti-nociceptive activities of Sipunculus nudus L. extract. J. Ethnopharmacol 2011; 137:1177–1182.
20. Arunachalam K, Parimelazhagan T, Manian S: Analgesic and Anti-inflammatory effects of merremia Tridentata(L) Hallier F. Int. J. Pharm and Pharmaceu. Sci 2011; 3(1): 75-79.
21. Ojewaole AOJ: Evaluation of anti-inflammatory property of sclerocarya birrea (A. Rich) Hochst (family; anacardiaceae) stem bark extracts in rats. J. Ethnopharmacol 2003; 85:217-220.
22. Damas J, Remeacle VC, Deflandre E: Furthur studies of the mechanism of Counter-irritation by turpentine. Arch. Pharmacol 1986; 332 :196-200.
23. Vinegar R, Scheiber W, Hugo R: Biphasic development of carrageenan edema in rats. Pharmacol. Exp. Ther. 1969; 150:328-334.

24. Zhan-Zhou Zhu, Ke-Jia Ma, Xia Ran, Hong Zhang, Cheng-Jian Zheng, Ting Han, Qiao-Yan, Zhang, Lu-Ping Qin: Analgesic, anti-inflammatory and antipyretic activities of the petroleum ether fraction from the ethanol extract of Desmodium podocarpum. J. Ethnopharmacol 2011; 133:1126–1131.
25. Selye H: On the mechanism through which hydrocortisone affect the resistance of tissue to injury an experimental study with granuloma pouch techniques. J. Amm. Assoc. 1953; 152 :1207-1213.
26. Ismail TS, Gopalakrishnan S, Begum VH, Elango V: Anti-inflammatory activity of Salacia oblonga Wall. and Azima tetracantha Lam. J. Ethnopharmacol 1997; 56:145–152.
27. Vilela FC, Bitencourt AD, Cabral LDM, Franqui LS, Soncini R, Giusti-Paiva A: Anti-inflammatory and antipyretic effects of Sonchus oleraceus in rats. J. Ethnopharmacol 2010; 127: 737–741.
28. Ionac M, Parnham MJ, Plauchithiu M, Brune K: Oxacepine atypical inhibitor of inflammation and joint damage. Pharmacol. Res. 1996; 33: 367-373.
29. Duke JA. Handbook of Biologically Active Phytochemicals and their Activities, (CRC Press, Boca, Raton, FL); 1992.
30. Gutteridge JMC: Age pigments and free radicals fluorescent lipid complexes formed by copper containing proteins. Biochem. Biophys. Act 1985; 34: 144-146.
31. Graf E, Mahoney JR, Bryant RG, Eaton JW: Iron catalyzed hydroxyl free radical formation stringent requirement for free iron coordination site. J. Biol. Chem. 1984; 259: 362-365.
32. Oyaizu M: Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. Jap. J. Nutri. 1986; 44: 307–315.
33. Braugghler JM, Duncan C, Chase LR: The involvement of iron in lipid Peroxidation, importance of ferrous to ferric ratio in initiation. J. Biol. Chem. 1986; 261: 02-107.
34. Blois W: Antioxidant determinations by the use of stable free radical. Nature 1954; 26: 119-121.
35. Havsteen BH: The biochemistry and medical significance of the flavanoid. Pharmacol and Therapeutics 2002; 96: 67-202.
36. Middleton E, Kandaswamy C, Theoharides TC: The effects of plant flavanoids on mammalian cells; implications for inflammation, Heart disease and Cancer. Pharmacol Rev 2000; 52: 673-751.