Journal of Medicinal Plants Research Vol. 6(36), pp. 4995-5001, 19 September, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.431
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

In-vitro and *in-vivo* anti-inflammatory activity of *Syzygium alternifolium* (wt) Walp.

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Accepted 19 June, 2012

The study was aimed to evaluate the analgesic and anti-inflammatory activity (by both *in-vitro* and *in-vivo*) of both chloroform and methanol root extracts of *Syzygium alternifolium* (wt) Walp. Methods used for the studies are *In-vitro* measurement of 5-lipoxygenase inhibition and *in-vivo* measurement of rat paw edema and ear edema in rats, acetic acid induced writhing response and heat induced pain. Our findings clearly indicate that *S. alternifolium* chloroform root extract showed 5-LOX inhibition activity similar with the standard drug, Zileutin. In *in-vivo* anti-inflammatory studies, *S. alternifolium* chloroform root extract exhibited better anti-inflammatory activity in comparison to the *S. alternifolium* methanolic root extract. Similar results were observed in the acetic acid induced writhing model and radiant heat induce model. Hence, it was concluded that *S. alternifolium* chloroform root extract exhibited more degree of efficacy in comparison to the *S. alternifolium* methanolic extract and good *in-vitro* and *in-vivo* anti-inflammatory effect.

Key words: *Syzygium alternifolium,* 5-lipoxygenase (5-LOX), analgesia, inflammation.

INTRODUCTION

Tirumala Hills (Rayalaseema region, Andhra Pradesh, India), which lies geographically in the South Eastern Ghats, are well-known for the rich heritage of flora. *Syzygium alternifolium* (wt) Walp. (SA) is one of those plants which belongs to the family Myrtaceae and is locally known as Mogi or Adavinerudu. It is used for treatment of fevers and skin diseases (Thammanna et al., 1990). Recent reports demonstrated the anti-hyperglycemic and anti-hyperlipidemic activities of methanol: water (4:1) fraction isolated from aqueous extract of *S. alternifolium* roots in streptozotocin induced diabetic rats (Kasetti et al., 2010). Due of ethnobotanical usage of these plant products without any systematic scientific studies, an attempt was made to evaluate the analgesic and anti-inflammatory activity (both *in-vitro* and *in-vivo*) of different extracts of *S. alternifolium* (wt) Walp.

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MATERIALS AND METHODS

Collection of plant material

Roots of *S. alternifolium* (wt.) Walp. were collected from surrounding areas of Tirupati and Tirumala hills and were identified

by the botanist, Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S. V. University, Tirupati. A voucher specimen (Herbarium accession no. 135) was deposited in the herbarium, Department of Botany, S.V. University, Tirupati.

Preparation of plant extracts and phytochemical screening

The fresh roots (2 kg) of *S. alternifolium* were collected from Tirumala Hills, Tirupati, Chittoor District. The roots were subjected for air dried and powdered. The air dried roots of *S. alternifolium* were extracted successively with petroleum ether, chloroform and methonal using soxhlet apparatus. All the extracts were filtered using cotton plug followed by Whatman filter paper. The extracts were concentrated using rotary vaccum evaporator (Buchi, USA) and then dried in lyophizier (Labconco USA) under reduced pressure. The dried extracts were stored in airtight container and placed in refrigerator. The root extracts of *S. alternifolium* were analyzed for the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins according to the standard methods (Harborne, 1973).

Experimental animals

Wistar rats (source: National Institute of Nutrition, Hyderabad, India) of either sex weighing 150 to 200 g and albino mice 20 to 30 g (source: National Institute of Nutrition (NIN), Hyderabad, India) were used for *in vivo* pharmacological studies. Animals were maintained under standard laboratory conditions at 25 ± 2°C, 50 ± 15% RH and normal photoperiod (12 h dark/ 12 h light). Commercial pellet diet (NIN, India) and water were provided *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee of Vaagdevi college of Pharmacy, Hanamkonda, Warangal (India) and by the Animal Regulatory Body of the Government (Regd. no. 1047/ac/07/CPCSEA).

Experimental design

Experimental designs for three different models of inflammations were given in Table 1. Rats were used for paw edema test and mice were used for writhing and ear edema test.

Acute toxicity studies of S. alternifolium

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Albino mice (n =6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight by providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg body weight by oral feeding needle and observed for 14 days. If mortality was observed in 2 to 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. Moreover, if mortality was not observed, the procedure was repeated for further higher dose such as 50, 300, 2000 mg/kg body weight (Ecobichon, 1997).

In-vitro 5-lipoxygenase inhibition

The assay mixture contained 80 mM linoleic acid and sufficient amount of potato 5-lipooxygenase enzyme in 50 mM phosphate buffer (pH 6.3). The reaction was initiated by the addition of enzyme buffer mixture to substrate (linoleic acid) and the enzyme activity was monitored as in increase in absorbance at 234 nm. The reaction was monitored for 120 s and measured using UV kinetic mode on Varian Cary-50 UV-visible spectrophotometer. In the inhibition studies, the activities were measured by incubating various concentration of test substance with enzyme buffer mixture for 2 min before addition of the substrate. The assay was performed in triplicate and mean values were used for the calculation. Percentage inhibition was calculated by comparing slope or increase in absorbance of the test substance with that of control enzyme activity. The activity of 5-LOX was compared with the standard positive control, Zileutin (Reddenna et al., 1990; Ulusu et al., 2002).

Acetic acid-induced writhing test

Albino mice weighing 18 to 25 g, were randomly divided into groups of six animals each. In this method, acetic acid was administered intraperitonialy to the experimental animals to create pain sensation. The extracts were solubilized in the ratios of (1:1) propylene glycol one drop and starch suspension one drop. The plant extract (*S. alternifolium*) in two different doses (500 and 1000 mg/kg, b.w) and vehicle were administered orally 30 min prior to intraperitoneal administration of 1% v/v acetic acid solution (0.1 ml/10 g), but diclofenac sodium was administered 15 min prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse from all groups were observed individually by counting the number of writhing they made in 15 min, commencing just 5 min after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal because sometimes the animals started to give writhing but they did not complete it; this incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group, while diclofenac sodium (40 mg/kg) was used as positive control.

Measurement of paw edema

The carrageenan assay procedure was carried out as follows: Edema was induced by injecting 0.1 ml of 1% solution of carragenan in saline to (sub plantar) right hind paw of rats. The plant extract, *S. alternifolium* in two different doses (250 and 500 mg/kg, b.w) and vehicle were administered orally 60 min prior to injection of carragenan. The volume of edema of injected and contra collateral paws were measured at 0.5, 1, 1.5, 2, 3, 4 and 5 h after induction of inflammation using a plethysmograph and the percentage of edema inhibitory activity was calculated.

TPA-induced mouse ear edema

Each mouse received 2.5 µg of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) dissolved in 20 µL of 70% ethanol (EtOH) (De Young et al., 1989). This was applied by an automatic pipette with TPA 20 µL volumes to both anterior and posterior surfaces of the right ear. The left ear (control) received the same volume of solvent (EtOH 70%). Diclofenac (0.5 mg/ear) was used as a standard drug. For the evaluation of the activity, two different ways were followed up, namely:

(1) The thickness of each ear was measured 4 h after induction of

Table 1. The three different experimental designs for various models of inflammation.

Groups	Experimental design for rat paw edema	Experimental design for writhing test in mice/Hot plate method in mice	Experimental design for ear edema test in mice
Group 1	Control	Control	Control group (0.5 mg/ear)
Group 2	Diclofenac 40 mg/kg p.o.	Diclofenac 40 mg/kg, p.o.	*Syzygium alternifolium* chloroform extract (SAC) (0.5 mg/ear)
Group 3	*Syzygium alternifolium* chloroform extract (SAC)- 250 mg/kg p.o.	*Syzygium alternifolium* chloroform extract (SAC)- 500 mg/kg p.o.	Syzygium alternifolium methanolic extract (SAM)-(0.5 mg/ear)
Group 4	SAC- 500 mg/kg,p.o.	SAC- 1000 mg/kg,p.o.	Diclofenac sodium (0.5 mg/ear)
Group 5	*Syzygium alternifolium* methanolic extract (SAM) 250 mg/kg p.o.	*Syzygium alternifolium* methanolic extract (SAM) 500 mg/kg p.o.	
Group 6	SAM-500 mg/kg p.o.	SAM-1000 mg/kg p.o.	

inflammation using a gauge calipers. The edema was expressed as the difference between right and left ears due to TPA application. (2) After 4 h, the animals were sacrificed under deep ether anesthesia. Disks of 6 mm diameter were removed from each ear and weighed in balance. The swelling was estimated as the difference in weight between the punches from right and left ears, and expressed as an increase in ear thickness.

Analgesic activity using Eddy's hot plate method

Albino mice of either sex were selected, weighed and divided into six groups of six animals each. The time of reaction to pain stimulus of the rat placed on the plate heated at 55 ± 0.5°C was recorded at 1, 2, 3, 4 and 5 h after the administration of the plant extract (*S. alternifolium*) in two different doses (500 and 1000 mg/kg, b.w) and vehicle were administered orally before 60 min. The increase in reaction time against control group was calculated.

Statistical analysis

Values from *in vivo* anti-inflammatory and analgesic activity are mean ± SD for six animals. Analysis was performed using one-way analysis of variance (ANOVA) and Tukey's post hoc multiple comparison tests (software "Prism 5.0") was applied for determining the statistical significance between different groups. The results were judged significant if *P<0.05, **P<0.01 and ***P<0.001.

RESULTS

The plant extracts did not exhibit any mortality up to the dose level of 2000 mg/kg. So, the extracts are safe for long term administration. The results of phytochemical screening were presented in Table 2.

In vitro 5-LOX inhibition

To assess the relative efficacy of different plant extracts, the concentration of the extract required to produce 50% inhibition of LOX was determined. The IC_{50} values of *S. alternifolium* chloroform root extract, *S. alternifolium* methanolic root extract and standard drug, Zileutin against 5-LOX inhibition were found to be 8.83, 73.48 and 4.36 µg/ml, respectively. The data is presented in the Table 1. This clearly indicates that *S. alternifolium* chloroform root extract has shown 5-LOX inhibition almost on par with the standard drug, Zileutin does. Based on this result, *in vivo* anti-inflammatory studies were carried out (Table 3).

Effect of plant extracts on rat paw edema

Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins, whereas the second phase is related to the release of prostaglandin and slow reacting substances which peak at 3 h. The results are presented in Figure 1. The chloroform and methanol extracts of *S. alternifolium* root at the dose level of 250 and 500 mg/kg decreased the oedema significantly (P<0.001) at 1.5, 2, 3rd and 4th h after administration of the extract. When compared to the control group, the effect was almost comparable with standard drug diclofenac sodium at 2nd, 3rd and 4th h after administration (Figure 1). *S. alternifolium* chloroform root extract exhibited high degree of anti-inflammatory activity in comparison to the *S.*

Table 2. Phytochemical screening of *Syzygium alternifolium* methanol and chloroform extracts.

Photochemical constituent	Methanolic extract	Chloroform extract
Flavonoids	+++	+++
Alkaloids	++	+++
Glycosides	++	++
Steroids	++	+
Phenols	++	+++
Terpenoid	+	+
Saponins	+	+
Resins	-	-
Tannins	+	+
Cardiac glycosides	+	++

– = Absent; + = present; ++ = moderately present; +++ = appreciable amount.

Table 3. *In-vitro* 5-LOX inhibition of both *Syzygium alternifolium* chloroform root extract (SAC) and *Syzygium alternifolium* methanolic root extract (SAM).

5-LOX inhibition activity	
Plant extract	**IC_{50} (μg/ml)**
Syzygium alternifolium chloroform root extract	8.83
Syzygium alternifolium methanolic root extract	73.48
Zileutin	4.36

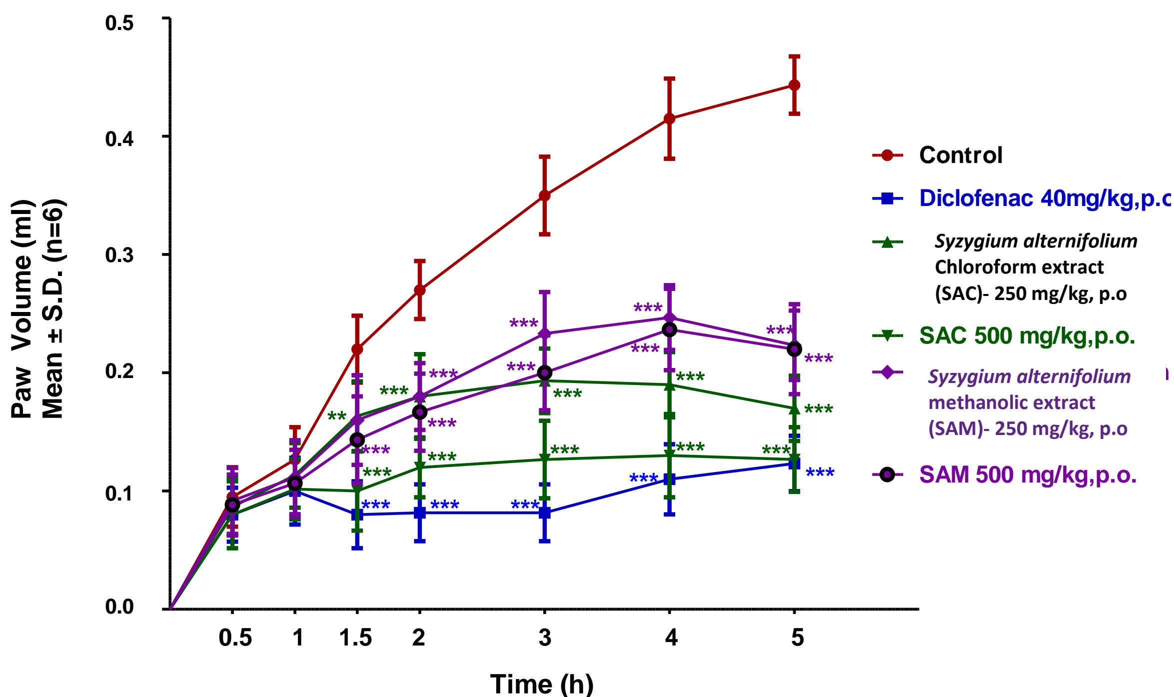

Figure 1. Effect of both *Syzygium alternifolium* chloroform root extract (SAC) and *S. alternifolium* methanolic root extract (SAM) on rat paw edema of Wistar rats. **P<0.01; ***P<0.001 vs. Control group analyzed by two way ANOVA followed by Bonferroni 't' test.

Table 4. Effects of both *Syzygium alternifolium* chloroform root extract (SAC) and *S. alternifolium* methanolic root extract (SAM) against TPA-induced ear edema in mice as measurement of swelling thickness and weight measurement of edema.

Test group	Dose (mg/ear)	Swelling thickness (μM) ± S.E.M	Weight Edema (mg) ± S.E.M
Control Group	0.5	262.34 ± 21.8	20.52 ± 1.5
Syzygium alternifolium chloroform extract (SAC)	0.5	120.25 ± 16.2***	11.12 ± 2.1***
Syzygium alternifolium methanolic extract (SAM)	0.5	155.80 ± 13..2***	14.73 ± 0.8***
Diclofenac Sodium	0.5	22.35 ± 0.5***	7.84 ± 1.1***

Data is expressed as mean ± SEM (n = 6), ***P<0.001 significant compared to Control.

alternifolium methanolic extract root extract.

Effect of plant extracts on mouse ear edema

Similar pattern of activity was followed for both the measurements of ear swelling thickness and weight of ear edema. Although both *S. alternifolium* chloroform root extract and *S. alternifolium* methanolic root extract have significantly shown the anti-inflammatory effect in mouse ear model, however, the former has exhibited more degree of anti-inflammatory activity in comparison to latter (Table 4).

Effect of plant extracts on acetic acid induced writhes

The results are presented in Figure 2. Although both *S. alternifolium* chloroform root extract and *S. alternifolium* methanolic root extract have significantly reduced the number of writhes, the former, however, exhibited more degree of anti-inflammatory activity in comparison to latter (Figure 2).

Effect of plant extracts on hot plate induced analgesia

The standard drug, tramadol 10 mg/kg p.o. has shown significant analgesic activity at the intervals of 1 h (*P<0.05), 2 h (***P<0.001) and 3 h (***P<0.001), whereas both *S. alternifolium* chloroform root extract and *S. alternifolium* methanolic root extract did not shown significant analgesic activity at two dose levels of 500 and 1000mg/kg in mice (Table 5).

DISCUSSION

Based on the *in vitro* data, it is clear that *S. alternifolium* chloroform root extract might possess potent active principles which inhibits 5-LOX enzyme since the IC_{50} value of *S. alternifolium* chloroform root extract is as low as IC_{50} value of Zileutin. This provides scientific evidence for the anti-inflammatory potential of *S. alternifolium* plant extract. The root extracts of this plant deserves further evaluation of anti-inflammatory effects in whole animal models of inflammation, asthma and chronic obstructive pulmonary disease for which 5-LOX is a good target. In continuation to this, plant extracts were evaluated for *in vivo* biological activities in two inflammatory models and two pain models. The most widely used primary test to screen new anti-inflammatory agents measures the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent. Carrageenan induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 to 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Winter et al., 1962; Brito and Antonio, 1998).

S. alternifolium chloroform root extract has shown more significant anti-inflammatory effect in two inflammatory models (carragenan induced paw edema and TPA induced ear edema) than *S. alternifolium* methanolic root extract. Notable in this study is the strong correlation between *in vitro* and *in vivo* anti-inflammatory data. High potency of 5-LOX inhibitory activity of this *S. alternifolium* chloroform root extract and high efficacy of this plant extract in the *in vivo* models heightened the importance of this plant extract for further evaluations to isolate the active principles that are contributing to the observed anti-inflammatory activity. Studies are under progress in our laboratory to isolate the compounds and to study the biological activities of the same. However, it is speculated that the probable mechanism of anti-inflammatory action of *S. alternifolium* may be due to its influence on the cycloxygenase pathway since it is interfering with prostaglandins biosynthesis as evidenced by the maximum anti-inflammatory activity at the end of the third hour

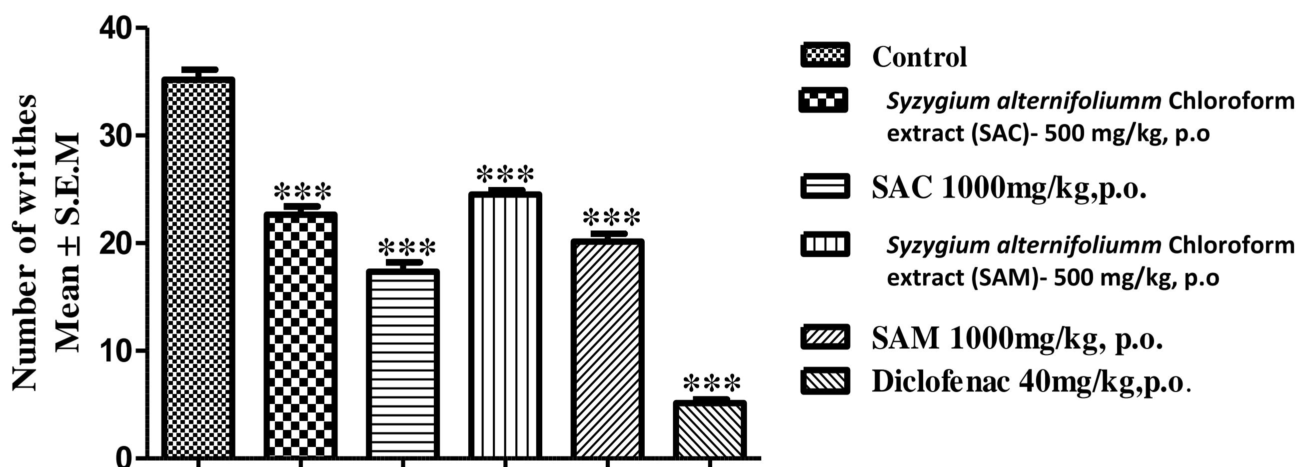

Figure 2. Effect of both *Syzygium alternifolium* chloroform root extract (SAC) and *Syzygium alternifolium* methanolic root extract (SAM) on acetic acid induced writhes in albino mice. ***p<0.001 vs. Control group by one way ANOVA followed by Dunnett's multiple comparison test.

Table 5. Effects of both *Syzygium alternifolium* chloroform root extract (SAC) and *S. alternifolium* methanolic root extract (SAM) on algesia produced by Hot plate method in mice.

Name of the group	Reaction time (s)				
	1 h	2 h	3 h	4h	5h
Control group	16.3 ± 0.64	17.2 ± 0.98	17.6 ± 1.56	16.2 ± 1.22	18.1 ± 1.46
Diclofenac Sodium (40 mg/kg)	19.54 ± 0.5*	23.36 ± 0.84***	26.5 ± 0.96***	22.5 ± 0.96***	20.5 ± 1.26
Syzygium alternifolium chloroform extract (SAC)- 500 mg/kg, p.o.	16.16 ± 1.71	18.50 ± 0.80	17.12 ± 1.24	18.42 ± 1.24	18.63 ± 0.85
SAC- 1000 mg/kg p.o.	17.91 ± 1.02	17.08 ± 1.35	18.25 ± 1.85	17.32 ± 0.74	18.74 ± 1.57
Syzygium alternifolium methanolic extract (SAM) 500 mg/kg, p.o.	17.85 ± 1.52	18.45 ± 1.28	19.12 ± 2.84	18.12 ± 1.35	19.82 ± 0.95
SAM-1000 mg/kg p.o.	18.84 ± 1.34	18.50 ± 0.86	17.07 ± 1.29	18.76 ± 1.55	17.41 ± 1.37

Each value is mean ± S.E.M (n=6), *Denotes significance difference when compared to control values at *P<0.05, ***P<0.001.

after the challenge with carageenan. *S. alternifolium* exhibited potent analgesic activity at the dose levels of 500 and 1000 mg/kg in acetic acid induced writhing test but plant extracts did not exhibit the analgesic activity in hot plate method. Moreover, in the hotplate method, both the plant extracts have not shown significant analgesic activity on hot plate analgesiometer; this clearly indicates that plant extracts possibly act through the peripheral mechanisms but not via the central mechanisms. Extracts have demonstrated to have analgesic effect in acetic acid induced writhes. This result provides evidence that plant extracts might possess active principles which produce peripheral analgesic effect without mediating spinal and supra-spinal actions.

Conclusion

Our findings clearly indicates that *S. alternifolium* chloroform root extract has shown 5-LOX inhibition almost at par with the standard drug, Zileutin does. In *in vivo* anti-inflammatory studies, *S. alternifolium* chloroform root extract has exhibited more degree of anti-inflammatory activity in comparison to the *S. alternifolium* methanolic root extract. Based on the correlation between both *in vitro* data and *in vivo* data, it was

concluded that *S. alternifolium* chloroform root extract has exhibited more degree of efficacy in comparison to the *S. alternifolium* methanolic extract.

ACKNOWLEDGEMENTS

We are grateful to the Department of Arya College of Pharmacy, Vaagdevi College of Pharmacy for providing research facilities for *in vivo* pharmacological studies, and Laila Impex Pharma Ltd for providing the research facilities for *in vitro* studies in the present investigation.

REFERENCES

Brito Arms, Antonio MA (1998). Oral anti-inflammatory and anti-ulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia (*Turneraceae). J. Ethnopharmacol. 61:215-228.

De Young LM, Kheifets JB, Ballaron SJ, Young JM (1989). Edema and cell infiltration in the phorbol ester treated mouse ear are temporally separate and can be differently modulated by pharmacologic agents. Agents Activity 26:335-341.

Ecobichon DJ (1997). The basis of toxicology testing. RC press, New York: pp. 43-86.

Harborne JB (1973). Phytochemical methods, A Guide to Modern Techniques of plant analysis, Chapman and Hall, London, Ltd: pp. 49-188.

Kasetti RB, Rajasekhar MD, Kondeti VK, Fatima SS, Kumar EG, Swapna S, Ramesh B, Rao CA (2010). Antihyperglycemic and antihyperlipidemic activities of methanol: water (4:1) fraction isolated from aqueous extract of *Syzygium alternifolium* seeds in streptozotocin induced diabetic rats. Food. Chem. Toxicol. 48(4):1078-1084.

Reddenna P, Whelan J, Maddipati KR, Reddy (1990). Purification of arachidonate 5-LOX from potato tubers. Methods. Enzymol. 187:268-277.

Thammanna, Rao KN, Nagaraju N (1990). Medicinal plants of Tirumala. Published by MVS Prasad, TTD, Tirupathi, India.

Ulusu NN, Ercil D, Sakar MK, Tezcan EE (2002). Abietic acid inhibits lipooxygenase activity. Phytother. Res. 16:88-90.

Winter CA, Risley EA, Nuss W (1962). Carrageenan induced edema in hind paw of rats as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 111:544-547.