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## **Evaluation of Free Radical Scavenging, Anti-inflammatory and Analgesic Potential of *Luffa echinata* Seed Extract**

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The different parts of *Luffa echinata* "Bindaal" plant are traditionally claimed and scientifically proved to be used in Liver disorders, jaundice, poisoning and migraine as antioxidant etc. but seeds part of this plant has not been explored yet. The present study was designed to investigate the free radical scavenging, anti-inflammatory and analgesic potential of methanolic extract of *Luffa echinata* seeds (MELE). Free radical scavenging potential of MELE was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl method). The extract showed significant ( $p < 0.05$ ) free radical scavenging activity in dose dependent manner as compared with Ascorbic acid. The maximum free radical scavenging activity of MELE was found to be 82.34% at concentration of  $150 \mu\text{g mL}^{-1}$  by the DPPH free radical scavenging method. Antioxidant effect was also investigated qualitatively using 1,1-diphenyl-2-picrylhydrazyl. The extract was further evaluated for its anti-inflammatory activity by using carrageenan-induced paw oedema in rats. Analgesic activity was evaluated by tail immersion and Hot plate methods in mice. Anti-inflammatory and analgesic activities were evaluated at dose level 50, 150 and  $200 \text{ mg kg}^{-1}$ . The extract showed significant decrease in paw volume (60.57% reduction) and analgesic effect shown at the dose level of  $200 \text{ mg kg}^{-1}$  as compared to the reference drug diclofenac sodium. The MELE may be useful as a natural antioxidant in the treatment of inflammation and pain.

**Key words:** *Luffa echinata*, anti-inflammatory, analgesic, DPPH, antioxidant

## INTRODUCTION

Plants have a key role in the human health care. About 80% of the world population rely on the use of traditional medicine which is predominantly based on plant materials (Kumar *et al.*, 2011). India has the long history of using plants as medicines in the world. There are estimated to be around 25,000 effective plant-based formulations, used in folk medicine and known to rural communities in India (Verma and Singh, 2008). Various phytoconstituents are obtained from various parts of plant like bark, leaves, flowers, roots, fruits, seeds, etc. may contain the active phytoconstituents. Secondary products from plants have maximum medicinal effects (Meena and Patni, 2008).

The constituents obtained from plants have many pharmacological activities like anti-oxidant, anti-diabetes, antibacterial, antiviral and anti-ulcer (Farhan *et al.*, 2012) activities. Antioxidant activity is a fundamental as well as important property for human life.

Generation of free radicals in living body is a normal phenomenon. But in some cases the body's defence mechanism is not able to remove the generated free radicals. The accumulation of excess free radicals produced oxidative stress in the body. In oxidation process the oxygen molecule get reduced and form intermediates called reactive oxygen species (ROS) such as hydrogen peroxide radicals ( $H_2O_2^*$ ), hydroxyl radicals ( $OH^*$ ), nitric oxide radical ( $NO^*$ ), peroxy radical ( $ROO^*$ ) and superoxide radicals ( $O^{2-*}$ ) are some of the example of intermediates that are formed during the oxidizing process (Ganie *et al.*, 2010). These free radicals attack biological molecules, such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury associated with aging, atherosclerosis, carcinogenesis and may lead to the development of chronic diseases related to the cardio and cerebrovascular systems (Ramesh *et al.*, 2011). The inflammatory process is also associated with free radical damage.

Antioxidants are very important for inhibiting or scavenging of radicals that are produced in the body, thus providing protection to humans against infections and degenerative diseases. Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), Propyl gallate (PG), Butylated Hydroquinone (THQ) and Tertiary Butylated Hydroxy Quinone (TVHQ) are commonly used synthetic antioxidants. But these antioxidants have side effects such as liver damage and carcinogenesis. Therefore, there is a need for isolation and characterization of natural antioxidant with less or no side effects, in order to replace synthetic antioxidants (Hamid *et al.*, 2010; Uma *et al.*, 2011).

Plant families like Asteraceae, Liliaceae, Apocynaceae, Solanaceae, Caesalpiniaceae, Rutaceae, Piperaceae, Sapotaceae and Cucurbitaceae are used for their medicinal importance. Cucurbitaceae family consists of *gourds* (bottle gourd, wax gourd), *Cucumber*, *Chayote*, various squashes (including pumpkins), luffas and melons (including watermelons). It consists of 130 genera and over 800 species (Samy and Gopalakrishnakone, 2007). Cucurbits is a major source of secondary metabolites. Cucurbits are among the most important plant families supplying humans with edible products and useful fibers (Dhiman *et al.*, 2012). From literature survey it is noticed that few plants of this family possess ribosome inactivating proteins (such as MAP30, Luffin A and B) and terpenes with immunomodulatory, antiretroviral, anti-HIV activities besides other pharmacological actions like antidiabetic, antihyperlipidemic, anticancer and free radical scavenging activity (Rahman, 2003).

Fruits of cucurbits are very useful for human health such as in blood purification, removal of constipation and for digestion and energy supplement. Seeds, root and fruit parts of some cucurbits are reported to possess purgatives, emetics and anthelmintic properties due to the secondary metabolite which possess cucurbitacin content (Gill *et al.*, 2010).

Many plants show significant antioxidant activity like *Acacia catechu*, *Allium cepa*, *Allium sativum*, *Aloe vera*, *Curcuma longa*, *Luffa cylindrica*, *L. acutangula*, *Luffa echinata* (Yadav *et al.*, 2004). *Luffa echinata* popularly known, as 'Bindal' in Hindi and Devdali' in Sanskrit is a slender herb belonging to the Cucurbitaceae, grows widely in Gujrat, Bihar, Rajasthan region of India. It is a spreading tendril climber. Leaves are simple alternate, serrated and pubescent. Flowers are white, red or yellow, axillary and unisexual. Fruits are spiny, slightly oblong and yellowish, contain many bitter tasting seeds (Vankar *et al.*, 2006). Echinatin, Saponins, gypsogenin, amariin, cucurbitacin-B and E, saponin, sitosterol, echinatin-A and B, oleanolic acid and betacin-B, elaterin glucoside, graviobioside-B, sitosterol glucoside has been reported to be isolated from the plant. The 50% ethanolic extract of this plant used to potentiate phenobarbitone induced hypnosis in mice, hypoglycaemic action in rat, Saponins present in *Luffa echinata* fruit has been reported to produce fall in blood pressure in cats and dog and it is beneficial in jaundice and have curative effect in chronic bronchitis and lung complaints (Khalaf *et al.*, 2008). It is reported that the methanolic seed extract of this plant also prevents  $H_2O_2$ -induced oxidative stress (Sharma *et al.*, 2010).

This plant has other therapeutic activities such as in intestinal colic, in viral hepatitis, skin-diseases, epilepsy, sinusitis, diabetes, haemorrhoids, oedema, cough, urinary retention, fever and using as a purgative and emetic drug in various diseases. The juice of fresh leaves is also consumed as a blood purifier. Fine powder of mature fruits with 'Bael' (*Aegle marmelos*) leaves and betle (*Piper betle*) leaves is given for dog bite for 21 days once a week (Ajmal, 2010). Several of these properties have been attributed to the presence of flavonoids (Ahmed *et al.*, 2001). Flavonoids have also been found to possess various biological properties such as hepatoprotective, anti-thrombotic, antiviral activities and these actions have been co-related with their ability to scavenge oxygen and oxygen generated free radical (Kumar *et al.*, 2000). The seeds have been used traditionally in Ayurveda for the treatment of various disorders such as peptic ulcer, vermifuge and cure to diabetes.

At present, no known scientific study on an anti-inflammatory, free radical scavenging and analgesic activity of the plant seed extract have been reported in the literature so far. Therefore, this study is aimed at exploring the plant (seeds) *Luffa echinata* for their therapeutic potential actions, by evaluate the free radical scavenging, analgesic and anti-inflammatory activity on seeds of *Luffa echinata*.

## MATERIALS AND METHODS

**Plant material:** The seeds were purchased from the Khari Baoli (spice market), Delhi (India) in October 2011. The seeds were cleaned, dust particles were removed and the healthy looking seeds were selected for identification and authentication. The seeds were authenticated from CSKHPKV, Palampur (H.P.) on 9/11/11. The seeds were dried and carefully powdered in a grinder at room temperature and were kept in a properly protected container.

**Drugs and chemicals:** Ascorbic acid and carrageenan were obtained Central Drug House, Ascorbic acid, ethyl acetate and methanol AR, Toluene LR was obtained from SD fine chem Limited Mumbai. Silica Gel 60-120, potassium dihydrogen phosphate, sodium hydroxide, silica gel G were obtained from E-Merck Ltd., Mumbai. Chloroform obtained from Loba Chemie Pvt. Ltd. Mumbai. 1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemical Co.

**Experimental animals:** The study was carried out on Wistar albino rats (170-200 g) and Swiss albino mice

(25-30 g) of either sex as reported in the literature. These animals were obtained from Punjab Agriculture University (PAU), Ludhiana, Punjab. The mice and rats were fed with standard laboratory diet and kept under environmental conditions, (temperature 25-28°C and 12 h light/dark cycle) (Agrahari *et al.*, 2010). Five animals in each group were used in all sets of experiments. The experimental protocol was duly approved by institutional Animal Ethics Committee (IAEC) and care of animals was carried out as per the guidelines of committee for the purpose of Control and Supervision of Experiments on animals (CPCSEA), Ministry of Environment and Forest, Government of India (Muthumani *et al.*, 2010).

**Extraction:** The powdered seeds of *Luffa echinata* plant (1 kg) were extracted using methanol as solvent system, for 72 h using cold maceration. The solvent was filtered off and a residue collected was macerated again with the fresh solvent (Chaudhary *et al.*, 2010). Both solvents were combined and filtered with Whatman filter paper and concentrated under reduced pressure on a rotary evaporator (Hedolph) at 40°C to obtain the crude extract. The crude extract obtained was diluted with distilled water and partitioned successively with hexane. The aqueous layer was separated and concentrated on water bath. The yield of crude (MELE) methanolic extract of *Luffa echinata* was calculated and further used for investigation (Kumar *et al.*, 2000).

**Phytochemical screening:** Phytochemical screening of extract was carried out for various constituent such as: flavonoids, tannins, alkaloids, steroids, terpenoids, carbohydrates, anthraquinone glycosides, coumarin glycosides, proteins, saponins, carbohydrates by using standard procedure.

The alkaloids were tested using various reagents such as Dragendorff's reagent (potassium bismuth iodide solution), Hager's reagent (picric acid solution) and Mayer's reagent (potassium iodide). The sterols were tested by Liebermann-Burchard's Test (red/pink/purple/violet color confirms the triterpenoids). The Ferric chloride test was used for tannins (blue green colour confirms the tannins), Molisch test for carbohydrates, ninhydrin reagent test for proteins and amino acids. The flavonoids were tested by concentrated nitric acid (crimson or magenta color with concentrated nitric acid) (Hameed *et al.*, 2011; Farhan *et al.*, 2012).

**Quantitative free radical scavenging activity on DPPH radical:** The antioxidant activity of *Luffa echinata* plant extract was measured by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay (Sannigrahi *et al.*, 2010;

Subhan *et al.*, 2008). About 1 mL (50-150  $\mu\text{g mL}^{-1}$ ) solution of extract was added to 1.5 mL of freshly prepared methanol solution of DPPH (0.05 mM). The tubes were then incubated at room temperature for 30 min in dark and the absorbance was taken by a spectrophotometer (Shimadzu UV-1700 Pharma spec) at 517 nm against a blank solution. A blank without DPPH was used to remove the influence of the colour of the samples. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. A methanol solution of DPPH was used as negative control. Ascorbic acid was used as reference drugs. Each reading was performed in triplicate.

Percentage inhibition was calculated by using the following equation:

$$\text{Percentage inhibition} = \frac{A_0 - A_s}{A_0} \times 100$$

Where:

$A_0$  = Absorbance of the negative control,

$A_s$  = Absorbance of the sample/ standard.

**Analgesic activity:** Analgesic effect of plant extract was assessed by the Eddy's hot plate and Tail immersion method in Swiss albino mice. Mice of either sex were divided into 5 groups, with six animals in each group:

**Group 1:** Disease group with no treatment or normal saline

**Group 2:** Group which received the standard drug, served as positive control (5 mg  $\text{kg}^{-1}$ )

**Group 3:** Methanol extract at dose of 50 mg  $\text{kg}^{-1}$  p.o.

**Group 4:** Methanol extract at dose of 100 mg  $\text{kg}^{-1}$  p.o.

**Group 5:** Methanol extract at dose of 200 mg  $\text{kg}^{-1}$  p.o.

**Eddy's hot plate method:** The analgesic activity of the given drug was determined by the basal reaction time. The rats were placed on the analgesimeter maintained at 55°C. The response produced by the animal like tail withdrawn due to radiant heat by placing the tip (1-2 cm) of the tail on the radiant heat source are noted which is calculated as the basal reaction. Time duration for the basal reaction response were calculated for standard as well as test dose treated animals were divided into 5 groups with six animals in each groups:

**Group 1:** Control group or disease group

**Group 2:** Diclofenac sodium as positive control.

**Group 3:** Methanolic extract at dose of 50 mg  $\text{kg}^{-1}$  p.o.

**Group 4:** Methanolic extract at dose of 100 mg  $\text{kg}^{-1}$  p.o.

**Group 5:** Methanolic extract at dose of 200 mg  $\text{kg}^{-1}$  p.o.

The animals were individually placed on the hot plate maintained at 55°C, one hour after their respective treatments, until the time of either licking or jumping occurs. This time was recorded using a stop-watch. The latency was recorded before and after 60, 120 and 180 min following oral or subcutaneous administration of the standard or the test compound (Saraswathi *et al.*, 2011).

**Tail immersion method:** The procedure is based upon the principle that the drug selectively prolonged the reaction time of a typical tail withdrawal reflex in mice. Mice were divided into five groups and were held in position in a suitable restrainer with the tail extending out. The 3-4 cm area of the tail was marked and immersed in the water bath thermo-statistically maintained at 51°C. The withdrawal time of the tail from hot water (in sec) was noted as the reaction time or tail flick latency. The maximum cut off time for immersion was 180 sec to avoid the injury of the tissues of tail. 0.2 mL of 0.9% NaCl solution was administered to control animals. The initial reading was taken immediately before administration of test and standard drugs and then 60, 120, 180 min after the administration (Ahmad *et al.*, 1992).

#### **Anti-inflammatory activity**

**Carrageenan-induced rat paw oedema:** The animals were divided into five groups of six animals each and were fasted for a period of 24 h prior to the study:

**Group 1: Disease control:** Carrageenan (1%)

**Group 2: Standard:** Carrageenan+diclofenac sodium (10 mg  $\text{kg}^{-1}$ , p.o.)

**Group 3: Test group, MELE 50:** Carrageenan+methanol extract (50 mg  $\text{kg}^{-1}$ , p.o.)

**Group 4: Test group, MELE 100:** Carrageenan+methanol extract (100 mg  $\text{kg}^{-1}$ , p.o.)

**Group 5: Test group, MELE 200:** Carrageenan+methanol extract (200 mg  $\text{kg}^{-1}$ , p.o.)

Acute inflammation was induced by injecting 0.1 mL of 1% (w/v) solutions of carrageenan in saline medium, underneath the planter region of the right hind paw of the rats (carrageenan was prepared as 1% w/v solution in 0.9% w/v NaCl and inject 0.1 mL). The vehicle, extracts and the standard drugs were administered orally 60 min prior to the carrageenan injection (phlogistic agent). The paw volume was measured with plethysmograph at 60, 120 and 180 min after carrageenan injection (Sharma *et al.*, 2010). The percentage of inhibition of oedema was calculated using formula:

$$\text{Percentage inhibition of oedema} = \frac{V_c - V_t}{V_c} \times 100$$

Where:

$V_t$  = Paw volume in test group animals

$V_c$  = Paw volume in control group animals

**Statistical analysis B:** The results are expressed as Mean±SEM of six animals in each group. The data were evaluated by one-way ANOVA followed by Tukey's multiple comparison tests. The p-values <0.05 were considered statistically significant.

## RESULTS

**Preliminary phytochemical investigation:** The preliminary phytochemical investigation of methanol extract of *Luffa echinata* plant's seeds shows the presence of alkaloids, Triterpenoids, glycosides, flavonoids compounds (Table 1).

**1,1-diphenyl-2-picrylhydrazyl radical scavenging activity study:** The methanol extract of *Luffa echinata* seeds showed concentration dependent scavenging activity. Free radical scavenging effect shown by the plant extract was 53.09 and 68.40% at 50 and 100  $\mu\text{g mL}^{-1}$ , respectively. The highest radical scavenging activity of methanol extract was found to be 82.35% at concentration 150  $\mu\text{g mL}^{-1}$  in case of quantitative analysis. The results are shown in Table 2.

**Analgesic activity study:** Analgesic studies of plant methanol extract were determined by tail immersion and eddy's hot plate method. The extract at doses of 50, 100

and 200  $\text{mg kg}^{-1}$  showed reaction time  $7.47 \pm 0.05$ ,  $7.75 \pm 0.08$  and  $8.31 \pm 0.10$  sec, respectively by hot plate method after 3 h. By using Tail immersion method, at same doses, the extract showed the reaction time of  $10.13 \pm 0.20$ ,  $11.70 \pm 0.70$  and  $15.17 \pm 0.20$  sec, respectively after 3 h. All these observed values were significant ( $p < 0.05$ ) in comparison to control and standard (Table 3, 4). Maximum analgesic effect was observed at 180 min interval.

**Anti-inflammatory activity study:** The methanol extract of plant seeds was evaluated for anti-inflammatory activity in acute and chronic experimental animal models and the results are summarized in Table 5. The result obtained indicates that anti-oedematous effect of orally administered MELE on carrageenan induced paw oedema in rats, showing a dose dependent anti-inflammatory activity. At 50  $\mu\text{g mL}^{-1}$  dose, MELE caused a reduction in paw oedema 41.09%, 3 h after the sub plantar injection of carrageenan. The extract at the test doses of 100 and 200  $\text{mg kg}^{-1}$  b.wt. reduced the oedema induced by carrageenan by 56.46% and 60.57%, respectively at 3 h. Whereas the standard drug showed 70.16% of inhibition as compared to the control group or disease group. All the observed values were significant ( $p < 0.05$ ) in comparison with control and standard drug.

## DISCUSSION

In the present study the methanol extract of *Luffa echinata* seeds was evaluated for its *in vitro* antioxidant activity followed by *in vivo* analgesic and anti-inflammatory activities. In preliminary phytochemical screening, triterpenoids and flavonoids were observed to be higher concentration (Musa *et al.*, 2009). The observed antioxidant activity may be due to the presence of any of these compounds. *In vitro* antioxidant activity was done by DPPH method and the reduction capability of 1,1-diphenyl-2-picrylhydrazyl was determined by the decrease in its absorbance at 517 nm induced by antioxidants.

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing. Antioxidant may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent disease.

It is earlier reported that carrageenan induced inflammation is useful for the detection of orally active anti-inflammatory agents (Di Rosa *et al.*, 1971). The MELE showed dose dependent inhibitory activity over a period of 3 h. In first sixty minutes there was a peak inflammation condition, after that there was decline in inflammation

Table 1: Phytochemical screening of methanolic extract of *Luffa echinata*

Chemical constituents	Result
Resins	-
Tannins	-
Saponin	-
Alkaloids	+
Steroids	-
Flavonoids	++
Glycosides	-
Triterpenoids	+++
Carbohydrates	-
Protein and amino acid	-

-: Absent, +: Minor presence, ++: High presence, +++: Very high presence

Table 2: Percentage inhibition of DPPH radical by methanol extract of *Luffa echinata* and ascorbic acid

Concentration of extract ( $\mu\text{g mL}^{-1}$ )	Scavenging of DPPH (%)	
	Extract	Ascorbic acid
50	53.09±0.69	62.59±0.84
100	68.40±0.84	74.41±0.67
150	82.35±0.51	89.69±0.48

Values are Mean±SEM of triplicate experiments

Table 3: Analgesic activity by Eddy's hot plate method

Group	Treatment	Dose (mg kg <sup>-1</sup> )	Reaction time (sec)			
			0 min	60 min	120 min	180 min
1	Control	0.5 mL distilled water	3.300±0.08	3.233±0.19	3.403±0.062	3.397±0.07
3	Diclofenac	10	3.300±0.08 <sup>a</sup>	5.770±0.06 <sup>a</sup>	7.453±0.120 <sup>a</sup>	9.450±0.28 <sup>a</sup>
3	MELE	50	3.300±0.17 <sup>ab</sup>	4.80±0.025 <sup>ab</sup>	6.490±0.017 <sup>ab</sup>	7.473±0.058 <sup>ab</sup>
4	MELE	100	3.300±0.08 <sup>ab</sup>	4.91±0.033 <sup>ab</sup>	6.830±0.052 <sup>ab</sup>	7.750±0.086 <sup>ab</sup>
5	MELE	200	3.300±0.08 <sup>ab</sup>	6.83±0.052 <sup>ab</sup>	6.900±0.011 <sup>ab</sup>	8.310±0.101 <sup>ab</sup>

Values are Mean±SEM of 6 animals in each group, Values with different superscripts are significantly different from normal control at p<0.05, MELE: Methanolic extract of *Luffa echinata*

Table 4: Analgesic activity study of MELE seeds by tail immersion method

Group	Treatment	Dose (mg kg <sup>-1</sup> )	Tail immersion latency (sec)		
			60 min	120 min	180 min
1	Control	CMC (1%)	3.20±0.057	3.44±0.020	3.55±0.028
2	Diclofenac	10	12.23±0.080 <sup>a</sup>	16.43±0.02 <sup>a</sup>	17.10±0.05 <sup>a</sup>
3	MELE	50	6.233±0.120 <sup>ab</sup>	8.20±0.152 <sup>ab</sup>	10.13±0.202 <sup>ab</sup>
4	MELE	100	7.233±0.145 <sup>ab</sup>	10.50±0.513 <sup>ab</sup>	11.70±0.709 <sup>ab</sup>
5	MELE	200	10.07±0.088 <sup>ab</sup>	11.93±0.425 <sup>ab</sup>	15.17±0.202 <sup>ab</sup>

Values are Mean±SEM of 6 animals in each group, Values with different superscripts are significantly different from normal control at p<0.05, MELE: Methanolic extract of *Luffa echinata*, CMC: Carboxymethyl cellulose

Table 5: Effect of MELE seeds on carrageenan induced paw oedema in rats

Groups	Treatment	Dose (mg kg <sup>-1</sup> )	Mean paw volume (mL)			Inhibition (%)
			60 min	120 min	180 min	
1	Control	1% CMC	0.530±0.007	0.625±0.007 <sup>a</sup>	0.657±0.010	-
2	Diclofenac	10	0.380±0.004 <sup>a</sup>	0.336±0.011 <sup>a</sup>	0.209±0.007 <sup>a</sup>	70.16
3	MELE	50	0.456±0.012 <sup>ab</sup>	0.411±0.007 <sup>ab</sup>	0.387±0.006 <sup>ab</sup>	41.09
4	MELE	100	0.431±0.005 <sup>ab</sup>	0.391±0.004 <sup>ab</sup>	0.286±0.008 <sup>ab</sup>	56.46
5	MELE	200	0.424±0.002 <sup>ab</sup>	0.379±0.004 <sup>ab</sup>	0.259±0.009 <sup>ab</sup>	60.57

Values are Mean±SEM of 6 animals in each group, Values with different superscripts are significantly different from normal control at p<0.05, MELE: Methanolic extract of *Luffa echinata*, CMC: Carboxymethyl cellulose

process at 50, 100 and 200 mg kg<sup>-1</sup> dose level. Carrageenan induced inflammation is believed to be biphasic, the early phase (1-2 h) is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings, the late phase is sustained by prostaglandins released and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Sannigrahi *et al.*, 2010). Present results revealed that administration of methanolic extract inhibited the oedema during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation. The inhibitory effect of the extract 200 mg kg<sup>-1</sup> on carrageenan induced inflammation over period of 3 h is similar to the effect of most non-steroidal anti-inflammatory drugs. This suggests that it acts in later phase probably involving arachidonic acid metabolites which produce oedema dependent on neutrophils mobilization (Musa *et al.*, 2009). The extract showed little effect at 50 mg kg<sup>-1</sup>, maximum effect was recorded at 200 and at 100 mg kg<sup>-1</sup> dose seed extract shown lesser effect in comparison to 200 mg kg<sup>-1</sup> and standard drug and greater effect than 50 mg kg<sup>-1</sup> dose. At 3 h maximum

inhibition, 60.57% at 200 mg kg<sup>-1</sup> dose level was observed in carrageenan induced paw oedema after which the activity began to decrease. The observed inflammatory activity by MELE at 50 and 100 mg kg<sup>-1</sup> dose level were lesser in comparison to standard drug (diclofenac sodium) whereas 200 mg kg<sup>-1</sup> dose level was very near to standard drug.

The methanolic extract of *Luffa echinata* seeds also showed significant analgesic action at all three dose levels i.e. 50, 100 and 200 mg kg<sup>-1</sup> as compared to standard drug. The duration as well as the intensity of analgesia was dose dependent. Analgesia occurs by thermal stimuli, chemical stimuli and physical stimuli which produces pain in two phases. The first phase or neurogenic phase, is due to the release of substance P and it is followed by a second phase or inflammatory phase, which is characterized by the release of serotonin, histamine, bradykinin and prostaglandins. Active substances against this pain model may interfere with one of these mediator systems or may act on the Central Nervous System (CNS) by blocking the pain influx transmission. Tail immersion and Hot plate methods were carried out to evaluate the analgesic potential of seed extract which show dose dependent results.

The methanol extracts of plant seeds dose dependently, significantly and selectively prolonged the reaction time of the typical tail withdrawal reflex in mice (Saraswathi *et al.*, 2011). Diclofenac sodium, a well known NSAID inhibits only the 2nd phase of this pain model, while central analgesics inhibit both phases. Methanolic extract of *Luffa echinata* seeds significantly inhibited both phases, which suggests that, it possesses central analgesic activity, but could possess peripheral activity since the effect was higher in the 2nd phase.

In the hot plate test, a central model that has a selectivity for opioid-derived analgesics, intraperitoneal treatment with plant seeds extract showed a potent antinociceptive effect on the acute noxious thermal stimulation and confirming the central activity of this extract. It would therefore, be effective in the management of chronic pain. Further investigations need to be carried out to test the seed extract in specific disease related cell or animal models.

This study has discovered the potential for antioxidant activity and also justified scientifically the traditional use of plant in anti-inflammatory and analgesic conditions. However, the exact bioactive compounds and their mechanism involved in analgesic and anti-inflammatory activities of the MELE are still to be confirmed by other pharmacodynamics studies. Phytochemical investigation is also proposed in order to isolate the active fraction and eventually the pure compound.

### CONCLUSION

The extract of *Luffa echinata* seeds was found to have free radical scavenging potential and this may be one of the probable reasons for the reduction in carrageenan-induced paw oedema in rats along with decrease in analgesia in mice model. Thus, it may be used as a natural antioxidant which can be employed as analgesic, anti-inflammatory agent. It might be helpful to develop a new and economic antioxidant compound in future. It may be useful to cure or in the treatment of various diseases like atherosclerosis, heart failure, liver dysfunction, neurodegenerative disorders, cancer and diabetes mellitus due to presence of significant antioxidant potential. Furthermore, the *in vivo* antioxidant activity of methanolic extract of *Luffa echinata* seeds needs to be assessed prior to clinical use.

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