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IN-VITRO AND *IN-VIVO* EFFICACY OF ROOT EXTRACT OF *LAWSONIA INERMIS* AGAINST INFLAMMATORY PARADIGM

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ABSTRACT: The study was carried out to evaluate anti-inflammatory activity of alcoholic extract of roots of *Lawsonia Inermis* (LILAC). *In-vitro* anti-inflammatory activity was evaluated by Cyclooxygenase inhibitory assay wherein the LIALC showed a dose-dependent inhibition of both the enzymes with IC_{50} values of 58.2 µg/ml for COX-1 and 7.38 µg/ml for COX-2. In RBC membrane stabilization activity, LILAC at the concentration of 100 µg/ml and 200µg/ml exhibited membrane stabilizing activity. Acute oral toxicity of extract was determined by OECD 423 guidelines wherein mortality was not observed when the extract was administered orally up to 2000 mg/kg. Carrageenan-induced paw edema model evaluated *in-vivo* anti-inflammatory activity; Paw edema was induced by injecting 0.1 ml of 1% w/v carrageenan into the subplantar tissues of the left hind paw of each rat. LILAC at all the three doses, 200, 300 and 400 mg/kg, showed maximum inhibition of 31.8, 40.9 and 50.8% respectively at 180 min. In Cotton pellet-induced granuloma model, Sterile, weighed, cotton pellets (10 ± 1 mg) were implanted subcutaneously in the groin regions of the rats under light anesthesia, the dry weight of cotton pellet was significantly reduced by 36.4, 46.4 and 50.5% at 200, 300 and 400 mg/kg of LILAC respectively. The research findings substantiate the promising anti-inflammatory activity of alcoholic extract of roots of *Lawsonia Inermis* in all the tested *in-vitro* and *in-vivo* models.

INTRODUCTION: In recent years, patients have been at risks of serious side effects with nonsteroidal anti-inflammatory drugs (NSAIDs). The NSAIDs do not significantly improve the long-term disease outcome. Furthermore, long-term treatment with NSAIDs may result in serious side effects, such as gastrointestinal ulcerogenicity and renal morbidity. Besides, the current anti-inflammatory drugs conventionally used to treat patients with associated anti-inflammatory diseases have various adverse side effects. Hence, the discovery of new anti-inflammatory drugs from natural sources with lower and fewer side effects is demanding [1]. Inflammation is an adaptive response of body tissues to external challenge or cellular injury. It is generally thought that inflammation is a beneficial host response defense system, but it can become harmful if dysregulated [2].

Cyclooxygenase also abbreviated as COX is a prostaglandin-endoperoxide synthase enzyme involved in the metabolism of arachidonic acid (AA) and synthesis of prostanoid including potent proinflammatory prostaglandins (PGE_2, PGF_2a). In mammalian cells, COX exists in at least two isoforms COX-1 and COX-2. COX-1 is expressed constitutively in almost all cell types, including platelets and those present in stomach, kidney,

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vascular endothelium, forebrain and uterine epithelium and is regulated as a house keeping enzyme for various physiological functions, whereas COX-2 is inducible and expressed during tissue damage or inflammation in response to proinflammatory cytokines such as IL-1b, interferon gamma, and TNF-a. A crucial proinflammatory role played by the COX has made this enzyme an attractive target for the development of novel anti-inflammatory agents [3].

According to WHO, approximately 70-80% population of developing countries acquire their primary pharmaceutical care from medicinal plants [4]. Medicinal plants have been used for centuries to treat many diseases, and contain bioactive compounds with potentially curative effects. They maintain the health and vitality of individuals and also cure diseases, including inflammation without causing toxicity [5]. Several plants like *Cassia fistula*, *Phyllanthus emblic*, *Lantana camera*, and plant products like Curcumin, Bromelain, Epigallocatechin-3 have been reported for their potent anti-inflammatory activity [6]. In this context, the present study was designed to evaluate the anti-inflammatory activity of roots of *Lawsonia Inermis*.

MATERIALS AND METHODS:

Collection and Preparation of Extracts: The plant material (Roots of *Lawsonia inermis*) was collected from Sri Rangapatnam taluk of Mysuru district, Karnataka, India. The plant was authenticated by Dr. M. N. Naganandani, Assistant Professor, Department of Pharmacognosy, JSS College of Pharmacy Mysuru (voucher specimen no. LI P.COL1).The roots were shade dried for 20 days and then powdered. The Soxhlet extraction method was used for extraction using 90% alcohol as the solvent. The extract so obtained was thick and syrupy with a characteristic odor. The further extract was filtered, concentrated using rotary flash evaporator and dried under vacuum.

In vitro Anti-Inflammatory Activity:

Cyclooxygenase (COX) Inhibitory Assay: [7] COX-1 was isolated from the microsomes obtained from ram seminal vesicles. The solubilized microsomal fraction was passed through DE-52 column and the active fractions pooled were used for the inhibitory assays Human recombinant COX-2 expressed in *Spodoptera frugiperda* (Sf9) cells by baculovirus expression system was employed for the inhibitory assay. The assay mixture contained Tris-HCl buffer (100 mM, pH 8.0), hematin (15 µM), EDTA (3 µM), enzyme (100 µg COX-1 or COX-2) and the LILAC.

The mixture was pre-incubated at 25 °C for 15 min, and then the reaction was initiated by the addition of arachidonic acid and TMPD, in a total volume of 1 ml. The enzyme activity was determined by estimating the velocity of TMPD oxidation for the first 25 seconds of the reaction by following the increase in absorbance at 603 nm. A low rate of non-enzymatic oxidation observed in the absence of COX-1 and COX-2 was subtracted from the experimental value while calculating the percent inhibition

RBC Membrane Stabilization Activity: [8]

Preparation of Erythrocyte Suspension: Blood was withdrawn from anesthetized rats from retro-orbital puncture and collected into the heparinized tubes. The blood was washed three times with 0.9% saline. The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (pH 7.4) which contained in 11 ml of distilled water: $NaH_2PO_4 \cdot 2H_2O$, 0.26 g; Na_2HPO_4, 1.15 g; NaCl, 9 g (10 mM sodium phosphate buffer).

Heat-Induced Haemolysis: Isotonic buffer (5 ml) containing 50, 100, 200 µg/ml of LIALC or vehicle was added to centrifuge tubes. Erythrocyte suspension (30 µl) was added to each tube to make a reaction mixture and mixed gently by inversion. One pair of the tubes were incubated at 54 °C for 20 min in a water bath. The other pair was maintained at 0 - 5 °C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 rpm, and the absorbance (O.D.) of the supernatant was measured at 540 nm using UV spectrophotometer. Diclofenac (50 µg/ml) was used as a reference standard.

Percentage inhibition of hemolysis = 100 × [1- (OD2-OD1 / OD3 – OD1)]

Where,
OD1- test sample unheated;
OD2- test sample heated
OD3- control sample heated

***In-vivo* Anti-Inflammatory Activity:**

Animals: Albino Wistar rats of either sex weighing 150-200 g were used for the experiments. They were procured from a registered breeder. The animal care and handling were carried out in accordance to guidelines issued by the Institutional Animal Ethics Committee, JSS College of Pharmacy, Mysore, Karnataka. Animals were acclimatized to the experimental room for one week before the experiment. Animals were maintained under controlled conditions of temperature (25 ± 1 ºC) and humidity (35 - 50%). The rats were fed on standard food pellets and water *ad libitum*. The studies conducted were approved by the Institutional Animal Ethics Committee (approval no. 050/2010) JSS College of Pharmacy, Mysuru.

Selection of Test Dose and Preparation of Test Samples: Acute oral toxicity of extract was determined by OECD 423 guidelines. In acute oral toxicity study, no animal mortality was observed when the extract was administered orally with 2000 mg/kg. So in the present study, LIALC was tested at a dose range of 50, 100, 200, 300 and 400 mg/kg body weight. The selected dose of standard diclofenac was 5 mg/kg based on previous reports [9]. LILAC and the Standard drug (Diclofenac) were administered orally in the form of suspension. Suspension was prepared in 0.25% w/v Carboxymethyl cellulose (CMC). Suspension of the test and standard was freshly prepared just before the administration.

Carrageenan-Induced Paw Oedema: [10] The rats were divided into seven groups of six each. Group, I treated with 0.25% CMC serve as a control. Group II treated with carrageenan and Diclofenac 5 mg/kg serve as a standard. Group III treated with carrageenan and alcoholic extract of roots of *Lawsonia Inermis* 50 mg/kg. Group IV treated with carrageenan and LIALC 100 mg/kg. Group V treated with carrageenan and LIALC 200 mg/kg. Group VI treated with carrageenan and LIALC 300 mg/kg. Group VII treated with carrageenan and LIALC 400 mg/kg. Paw edema was induced by injecting 0.1 ml of 1% w/v carrageenan into the subplantar tissues of the left hind paw of each rat. The LILAC, Diclofenac, and CMC were given orally to the experiment, standard and control groups respectively.

The extracts were administered orally 30 min before carrageenan administration. The paw volume was measured at intervals of 60, 120, 180 and 240 min by the mercury displacement method using a Plethysmograph. Percentage inhibition of edema was calculated by the following formula.

$$\text{Percentage inhibition of oedema} = [(V_c - V_t) / (V_c)] \times 100$$

Where, V_c and V_t represent an increase in paw volume in control and drug-treated animals respectively.

Cotton Pellet-Induced Granuloma: [11] The rats were divided into six groups of six each. Group, I treated with 0.25% CMC serve as a control. Group II treated with Diclofenac 5mg/kg serve as a standard. Group III treated with LILAC 5 mg/kg. Group IV treated with LILAC 200 mg/kg. Group V treated with LILAC 300 mg/kg. Group VI treated with LILAC 400 mg/kg. Under light anesthesia, sterile weighed, cotton pellets (10 ± 1 mg) were implanted subcutaneously in the groin regions of the rats. The LILAC, diclofenac, and CMC were administered once daily orally for seven consecutive days from the day of cotton pellet implantation.

The animals were anesthetized on the 8th day, and cotton pellets were removed surgically and made free from fat and extraneous tissues. The wet weights of granuloma were estimated, and then pellets were dried overnight at 60 ºC in a hot-air oven. The final dry weight of the pellet was subtracted from the weight of the cotton pellet before implantation. The increment in the dry weight of the pellets was taken as a measure of granuloma formation. The following formula calculated percentage inhibition of granuloma formation.

$$\text{Percentage inhibition of granuloma formation} = [(D_c - D_t) / D_c] \times 100$$

Where D_c and D_t represent the weight of the dry pellet in control and drug-treated group respectively.

Statistical Analysis: All values expressed as mean ± standard error of the mean (S.E.M.) of the indicated number of experiments/animals. Statistical analysis was performed using GraphPad Prism trial version 5.02 by one-way ANOVA followed by post hoc Tukey's multiple comparison tests. A value of $p<0.05$ was considered as significant.

RESULTS:

Cox Inhibitory Assay: LILAC was tested against COX-1 and COX-2 by TMPD assay. LILAC showed dose-dependent inhibition of both the enzymes with IC_{50} values of 58.2 µg/ml for COX-1 and 7.38 µg/ml for COX-2. These results indicate that LIALC has more specificity towards COX-2 than COX-1.

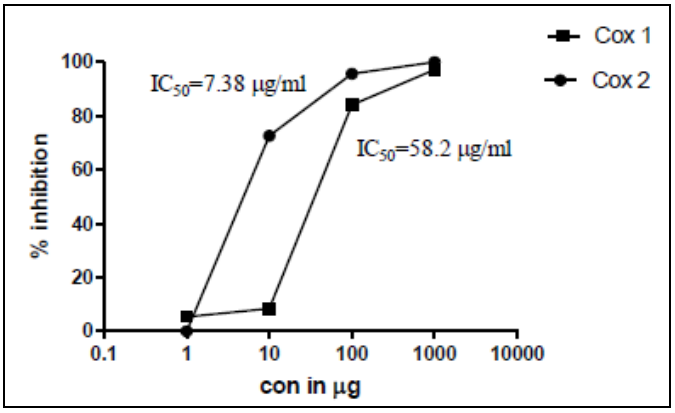

FIG. 1: COX ASSAY

RBC Membrane Stabilization Activity: In membrane stabilizing, activity LIALC was tested in the concentration range of 50, 100, 200 µg/ml. Among the tested concentration LIALC at the concentration of 200 µg/ml exhibited membrane stabilizing activity which was comparable with that of the standard Diclofenac

TABLE 1: EFFECT OF LILAC ON RBC MEMBRANE STABILIZATION ACTIVITY

Group	Absorbance of the Heated sample	Absorbance of the unheated Sample	% Inhibition of hemolysis
Control	0.48 ±0.012	0.056 ±0.0029	-----
Diclofenac 50 µg/ml	0.18 ±0.004[a]	0.075 ±0.003	74.07
LIALC 100 µg/ml	0.35 ±0.019[a,b]	0.092 ±0.002	33.5
LIALC 200 µg/ml	0.25 ±0.021[a]	0.082 ±0.003	57.7

All values are expressed as Mean ± SEM; Experiments performed in Triplicates, [a] p<0.05 compared to control, [b] p<0.05 compared to standard. All data were analyzed by one way ANOVA followed by post hoc Tukey's multiple comparison tests.

Carrageenan-Induced Paw Oedema: Carrageenan injection resulted in a significant increase in paw volume at all the tested intervals, *i.e.* 60, 120, 180 and 240 min. Standard Diclofenac significantly reduced the paw volume at all the tested intervals when compared with control. LILAC at doses of 300 and 400 mg/kg significantly reduced the paw volume at all the tested intervals when compared with the control. The efficacy of LIALC at 400 mg/kg was comparable to that of diclofenac. Diclofenac showed a maximum reduction at 120 min interval (56.8%). LILAC at all the three doses, *i.e.* 200, 300 and 400 mg/kg showed maximum inhibition of 31.8, 40.9 and 50.8 % at 180 min.

TABLE 2: EFFECT OF LILAC IN CARRAGEENAN INDUCED PAW OEDEMA IN RATS

Treatment group	Paw volume in mm				
	60 min	120 min	180 min	240 min	300 min
Control	77.33 ± 3.27	90.16 ± 1.00	98.65 ± 3.06	104.98 ± 2.50	110.16 ± 2.99
Diclofenac 5 mg/kg	37.19 ± 2.08[a]	38.94 ± 1.96a	44.29 ± 2.78[a]	49.86±3.15[a]	53.86±1.66[a]
LIALC 50 mg/kg	74.54 ± 2.62	84. 32 ± 2.45	90.23 ± 3.42	95 ± 1.43	97 ± 2.65
LIALC 100 mg/kg	70.43 ± 3.76	81.67± 3.65	89.43 ± 2.83	92.36 ± 2.46 [a]	94 ± 2.73
LIALC 200mg/kg	64.4 ± 2.97	65.71 ± 1.49[a,b]	67.26 ± 1.64[a,b]	73.62 ± 2.07a,b	77.93 ± 2.53a,b
LIALC 300 mg/kg	55.53 ± 1.55[a,b]	62.84 ± 1.70[a,b]	58.25 ± 2.87[a,b]	64.48 ± 2.59a,b	67.09 ± 1.04a,b
LIALC 400 mg/kg	41.29 ± 2.82[a]	45.00 ± 2.30[a]	49.27 ± 1.14[a]	55.53 ± 2.37a	58.82 ±0 .90a

All values are expressed as Mean ± SEM of 6 animals, [a] p<0.05 compared to control, [b]p<0.05 compared to standard. All data were analyzed by one way ANOVA followed by post hoc Tukey's multiple comparison tests

Cotton Pellet-Induced Granuloma: In this model LILAC and diclofenac significantly (p<0.05) reduced the dry weights of cotton pellet, an index of granuloma formation. The percentage inhibition of granuloma formation by diclofenac was 61.6%. A significant reduction in the dry weight of cotton pellet was observed at all the tested doses of LILAC. The percentage inhibition was 36.4, 46.4 and 50.5% for 200, 300 and 400 mg/kg of LILAC respectively. The efficacy of LIALC at 400 mg/kg was comparable to that of standard Diclofenac.

TABLE 3: EFFECT OF LILAC ON COTTON PELLET-INDUCED GRANULOMA

Group	The weight of the dry pellet in mg	% inhibition of granuloma formation
Control	79.3 ± 3.5	—
Standard Diclofenac 5 mg/kg	30.4 ± 1.8[a]	61.6
LIALC 200 mg/kg	50.2 ± 2.3[a,b]	36.4
LIALC 300 mg/kg	45.7 ± 1.6[a,b]	46.4
LIALC 400 mg/kg	39.2 ± 2.6[a]	50.5

All values are expressed as Mean ± SEM of 6 animals, [a] p<0.05 compared to control, [b] p<0.05 compared to standard. All data were analyzed by one way ANOVA followed by post hoc Tukey's multiple comparison tests

DISCUSSION: In the present study anti-inflammatory activity of LIALC was assessed by employing *in-vitro* models like COX inhibitory assay, membrane stabilizing activity and *in-vivo* models such as carrageenan-induced paw edema, cotton pellet-induced granuloma. Anti-inflammatory activity of most of the plants is attributed to their action on multiple pathways of inflammation. So, to partially support the mechanism of action of LILAC *in-vitro* studies such as COX inhibitory assay and membrane stabilizing activity was carried out.

COX is a membrane-bound enzyme responsible for the oxidation (cyclooxygenase activity) of arachidonic acid to prostaglandin G_2 (PGG_2) and the subsequent reduction (Peroxidase activity) of PGG_2 to prostaglandin H_2 (PGH_2) which serves as mediators of inflammation. COX has been shown to be expressed in at least two different isoforms, a constitutively expressed form COX-1, and an inducible form, COX-2 [12]. LILAC showed inhibition of both isoforms of COX, *i.e.* COX-2 and COX-1 with an IC_{50} of 7.38 µg/ml and 58.2 µg/ml respectively. The IC_{50} of LIALC for COX-2 enzyme was 8 fold less than the IC_{50} of LIALC on COX-1. Since LIALC has shown selective inhibition of COX-2, it might be devoid of various adverse effects associated with conventional anti-inflammatory drugs.

Membrane stabilizing activity is known to be a biochemical index of anti-inflammatory activity. The vitality of cells depends on the integrity of their membranes [13]. Exposure of red blood cell to injurious substances such as hypotonic medium or heat results in lysis of its membrane accompanied by hemolysis and oxidation of hemoglobin. Also, the erythrocyte membrane is analogous to the lysosomal membrane, and its stabilization implies that the extract may as well stabilize lysosomal membranes.

Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon release [14]. LILAC exhibited a significant membrane stabilization activity at all the tested doses.

The presence of edema is one of the prime signs of inflammation [15]. It has been documented that carrageenan-induced rat paw edema is a suitable *in-vivo* model to predict the value of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation [16]. Also, this method is frequently used to assess the anti-oedematous effect of natural products [17]. Carrageenan-induced paw edema represents an inflammatory reaction in two different phases [18]. The initial phase, which occurs between 0 and 2.5 h after the injection of the phlogistic agent, has been attributed to the action of mediators such as histamine, serotonin and bradykinin on vascular permeability [19]. The edema volume reaches its maximum approximately at 3 h of post-treatment and then begins to decline. The late phase, which is also a complement-dependent reaction has been shown to be a result of overproduction of prostaglandins in tissues and may continue until 5 h post-carrageenan injection [19]. LILAC at the dose of 200, 300 and 400 mg/kg effectively inhibited the carrageenan-induced increase in paw volume during the early phase (1-3 h after carrageenan injection) of inflammation and also a weak inhibitory effect at a later phase.

The maximum inhibition of edema was shown by LIALC at the dose of 400 mg/kg at the 3 h interval. The suppression of the first phase of inflammation by the extract may be due to inhibition of the release and activity of the early mediators involved in carrageenan-induced paw inflammation, while the suppression of the later phase might be due to inhibition of prostaglandin synthesis.

Cotton pellet method is widely used to evaluate the transudative and proliferative phases of inflammation. It involves proliferation of macro-phages, neutrophils, and fibroblasts, which are basic sources of granuloma formation [20]. The wet weight of the cotton pellets correlates with transude whereas the dry weight of the pellets correlates with the amount of the granulomatous tissue. Hence, the decrease in the weight of granuloma indicates the ability of the LILAC in reducing the synthesis of proteins, collagen and macrophages infiltration. Administration of LILAC markedly inhibited the granuloma formation as evident by the decrease in dry weight of the cotton pellet. LILAC significantly inhibited the granuloma formation at

all the tested doses. The efficacy of LIALC at the dose of 400 mg/kg was comparable to that of standard diclofenac.

CONCLUSION: The present work was designed with an objective of exploring the possible anti-inflammatory activity of LILAC. The research findings of LILAC substantiate the promising *in-vitro* and *in-vivo* anti-inflammatory activity in all the tested models. The anti-inflammatory activity of LIALC might be due to its action on multiple pathways of inflammation. These include membrane stabilization activity, inhibition of release/activity of inflammatory mediators such as histamine, serotonin, and bradykinin.

However, further studies are warranted to establish the mechanism of LIALC responsible for its anti-inflammatory effect along with evaluation of LILAC against chronic inflammatory disorders.

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CONFLICT OF INTEREST: All authors declare no conflict of interest.

REFERENCES:

1. Anwesa B, Subir KB, Nishith KP and Rabi RC: Anti-inflammatory, anti-lipid peroxidative, antioxidant and membrane stabilizing activities of hydroalcoholic extract of *T. chebula* fruits. Pharm Bio 2013; 51(12): 1515-1520.
2. Chao F, Huizi J and Lehao W: an exploration of traditional Chinese medicinal plants with anti-inflammatory activities. Evidence-Based Complementary and Alternative Medicine 2017; 13(1): 1-10.
3. Shaikh RU: Evaluation of anti-inflammatory activity of selected medicinal plants used in Indian traditional medication system *in-vitro* as well as *in-vivo*. Journal of Traditional and Complementary Medicine 2016; 6(4): 355-361.
4. Syed MS: A possible anti-inflammatory mechanism of ethyl acetate extracts of *Teucrium stocksianum* Bioss. BMC Compl & Alt Med 2015; 15(1): 299-305.
5. Veronica JS, Antonio NC, Manuel JE and Brenda AS: Anti-inflammatory, free radical scavenging and alpha-glucosidase inhibitory activities of *Hamelia patens* and its chemical constituents. Pharmaceutical Biology 2016; 54(9): 1822-1830.
6. Kushagra N, Mukesh KS, Dhansay D, Verma VK and Tripathi KD: Anti-inflammatory activity and chemo profile of plants used in traditional medicine. J Chem Pharm Res 2010; 2(5): 122-130.
7. Abdellatif KRA, Fadaly WAA, Waleed AM and Gehan M: Synthesis, cyclooxygenase inhibition, anti-inflammatory evaluation and ulcerogenic liability of new 1,5-diarylpyrazole derivatives. Journal of Enzyme Inhibition and Medicinal Chemistry 2016; 31(3): 54-60.
8. Mohammed AS, Rafiqul MD, Tahmida KC, Abdur R, Sahab MD, Sahiba FK and Shahid MD: Investigation of *in-vivo* analgesic, anti-inflammatory, *In-vitro* membrane stabilizing and thrombolytic activity of *Atylosia scarbaeoides* and *Crotalaria spectabilis* leaves. Journal of Pharmacology and Toxicology 2017; 12(3): 120-128.
9. Rajendran M, Palanivelu S and Panchanadam S: Synergistic effect of *Kalpa amruthaa* on antiarthritic and anti-inflammatory properties. Its Mechanism of Action. Inflammation 2008; 31(6): 391-398.
10. Zabihi M, Hajhashemi V, Minaiyan M and Talebi A: Evaluation of the central and peripheral effects of doxepin on carrageenan-induced inflammatory paw edema in rat. Res Pharma Sci 2017; 12(4): 337-345.
11. Kumar R, Gupta YK and Singh S: Anti-inflammatory and anti-granuloma activity of *Berberis aristata* DC. in experimental models of inflammation. Indian J Pharmacol 2016; 48(2): 155-61.
12. Hamida J, Bassem K, Hanen N, Mouna C, Mourad J, Abdelkarim BA, Yassine BA and Nacim Z: Anti-inflammatory and antiproliferative activities of the wild edible cruciferous: *Diplotaxis simplex*. Pharmaceutical Biology 2016; 54(10): 2111-8.
13. Ferrali M, Signorni C, Ciccoli L and Comporti M: Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and isouramil. Biochemical Journal 1992; 285: 295-301.
14. Jorge CR, Angel JM, Pablo AE and Maira RS: Antioxidant and anti-inflammatory activities of phenolic compounds isolated from *Melipona beecheii* honey. Food and Agricultural Immunology 2017; 28(6): 1424-1437.
15. Sur T, Pandit S, Battacharyya D, Kumar AC, Lakshmi MS, Chatttopadhyay D and Mandal SC: Studies on the anti-inflammatory activity of *Betula alnoides* bark. Phytotherapy Research 2002; 16(7): 669-671.
16. Morebise O, Fafunso MA, Makinde JM, Olajide OA and Awe EO: Anti-inflammatory and analgesic property of leaves of *Gongronema latifolium*. Phyto Res 2002; 16 (1): S75-S77.
17. Tatiya AU, Saluja AK, Kalaskar MG, Surana SJ and Patil PH: Evaluation of analgesic and anti-inflammatory activity of *Bridelia retusa* (Spreng) bark. Journal of Traditional and Complementary Medicine 2017; 7(4): 441-451.
18. Garcia MD, Fernandez MA, Alvarez A and Saenz M: Antinociceptive and anti-inflammatory effect of the aqueous extract from leaves of *Pimenta racemosa* var. ozua (Myrtaceae). Jou of Ethnopharmacology 2004; 91 (1): 69-73.
19. Kumar T and Jain V: Antinociceptive and anti-inflammatory activities of *B. retusa* methanolic fruit extract in experimental animals. The Scientific World Journal 2014; 13(1): 1-30.
20. Antonisamy P, Dhanasekaran M, Kim HR, Jo SG, Agastian P, and Kwon KB: Anti-inflammatory and analgesic activity of ononitol monohydrate isolated from *Cassia tora* L. in animal models. Saudi Journal of Biological Sciences 2017; 24(8): 1933-1938.

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