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Extraction, isolation and evaluation of the anti-inflammatory activity of the bioactive component present in the bark of *Delonix regia*

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ABSTRACT

The present study deals with the study of anti-inflammatory activity of the extract obtained from the bark of *Delonix regia*. Soxhlet extraction of dried powder of bark of the tree was done with petroleum ether and methanol. Phytochemical test was carried out and was found that the methanol extract contained alkaloid and flavanoids. The crude extract obtained was then subjected to column chromatography and different fractions eluted were then monitored by TLC. The IR spectral analysis revealed the presence of amide and alkyl halide group in the isolated fraction. In-vitro Anti-inflammatory test was carried out by testing protein denaturation while *in-vivo* Anti-inflammatory activity was studied by Carrageenan induced paw-edema model. The in-vitro study showed that the crude extract was having more potential against inflammation than the isolated one while in-vivo study concludes that 400 mg/kg gave more significant result than 200 mg/kg.

Key word: *Anti-inflammatory, Delonix regia, Column chromatography, Paw edema model, In-vivo, In-vitro etc.*

Introduction

Inflammation is essentially, activation of our body's immune mechanism. Inflammation may occur due to different reasons for example it occurs when the part of body get injured, it may be due to acute bronchitis, soar throat, dermatitis etc. To heal the injured part, our body releases neutrophils which are white blood cells containing enzymes. This innate immunity of living beings is not specific to pathogens and sometimes it happens that our autoimmune system may even attack healthy tissues mistaking it for a pathogen. Severe inflammation may lead to unrepairable diseases like atherosclerosis, cancers, rheumatoid arthritis, periodontitis etc. The anti-inflammatory drugs available in market increase the risk of heart attack and may also lead to stomach ulcers. Thus there is a dire need of effective medicines without side effects. Now days we are reverting back to our ancestral heritage of curing, using medicinal plants. The crude extract obtained from such plants are natural and without any adverse side effects (Voravuthikunchai and Limsuwan, 2006; Kumaresan *et al.*, 2011).

The work presented in this article deals with the usage of bark of *Delonix regia* as an anti-inflammatory medicine. *Delonix regia* is a flowering plant of family Fabaceae having fern like leaves. Much work has been done on the flower and leaf extracts of *Delonix regia* (Sharma and Arora, 2015) and these extracts have been used to evaluate antioxidant (Ramakrishnan *et al*., 2018), antidiabetic (Rahman *et al.*, 2011), antidiarrheal, and hepatoprotective activity (Magdy *et al.*, 2011) of its flower and leaf extract.

Many plants are being used as anti-inflammatory medicine (Sreejith *et al.*, 2010; Garg, and Paliwal, 2011) though limited studies have been carried out on the anti inflammatory property of *Delonix regia*. The present study aims to explore the anti inflammatory activity of the methanolic extract of the bark of *Delonix regia*.

Materials and Method

A. Collection of plants: The bark of *Delonix regia* was scraped out and dried in shade. The bark was then powdered into fine powder.

B. Preparation of extract: 100g each of the powdered plant sample was subjected to soxhlet extraction technique using petroleum ether and methanol respectively (Zahid, 2011). The two liquid extracts obtained one with petroleum ether and other with methanol were then stored in airtight bottles in refrigerator.

Standard phytochemical tests were carried out with both the extracts and the methanolic extract was subjected to column chromatography. 20 samples were isolated which were again confirmed by Thin Layer Chromatography. The fractions F2, F8, F10, F12, F15 and F18 showed single spots

C. Acute oral toxicity test: This test was performed according to OECD 423 guidelines. The albino rats were administered with the standard dose upto 2000 mg/kg. The adverse effect was checked after 48 hours of oral dose.

D. In-vitro analysis: This was carried out by protein denatuaration process (Leelaprakash and Dass, 2011). One of the main causes of inflammation is protein denaturation as on denaturation protein losses its biological functions. The reaction was carried out between test extracts and 1% aqueous solution of bovine albumin. The sample was incubated at 37 °C for 20 min and then it was heated to 51 °C for 20 min. The sample was then cooled and absorbance was measured at 660nm. Then percentage inhibition was calculated as:

Percentage inhibition = (Abs Control –Abs Sample) × 100/ Abs control

E. *In-vivo* analysis: In-vivo analysis was done by Carrageenan paw edema model (Vijayasanthi *et al.*, 2015) as it is the most established model used for evaluating anti-inflammatory drugs. For this study four groups of albino rats were made and in each group four rats were taken. First group were injected with 0.1 mL of 1% Carrageenan solution to the left hind paw. Second group of rats were orally administered with 400 mg/kg of the isolated extract F15 before one hour before the carrageenan injection. While the other two groups were administered with 200 mg/kg and 400mg/kg of the crude extract respectively. The paw volume was then measured at time 0, 1, 3, 5 and 24 hour after the injection of carrageenan. The difference between the volumes of two paws was determined and the percentage inhibition of inflammation was calculated in comparison to control.

Results and Discussion

A. Phytochemical Test

The phytochemical tests carried out with the crude extract in petroleum ether revealed the presence of fats and oil while the methanolic extract showed the presence of carbohydrate, alkaloid, saponin, flavonoids etc (Table 1).

The column separation was done and different fractions were collected and further analyzed using

Table 1. Showing the tests of Phytochemicals in Petroleum ether and Methanol extract

S. No.	Phytochemical Test	Petroleum Ether Extract	Methanol Extract
1.	Carbohydrate	–	+
2.	Protein	–	–
3.	Glycoside	–	–
4.	Alkaloid	–	+
5.	Saponin	–	+
6.	Tannin and Phenolic	–	+
7.	Steroids and Triterpenoids	—	–
8.	Fats and Oils	+	–
9.	Flavonoids	–	+

thin layer chromatography. The fractions showing single spots were studied under infrared spectrophotometer. The IR spectra of the fraction F15 has been depicted in Figure 1. The figure clearly shows a large broad peak at 3330 cm^{-1} and a short peak at 1636 cm^{-1}could be possibly due to N-H stretching and C=O stretching respectively of amide group. While a very short peak obtained at 2139 cm^{-1}could be possibly due to C≡C stretching thus showing the presence of alkynes. The peak at 594 cm^{-1} showed the presence of alkyl halide.

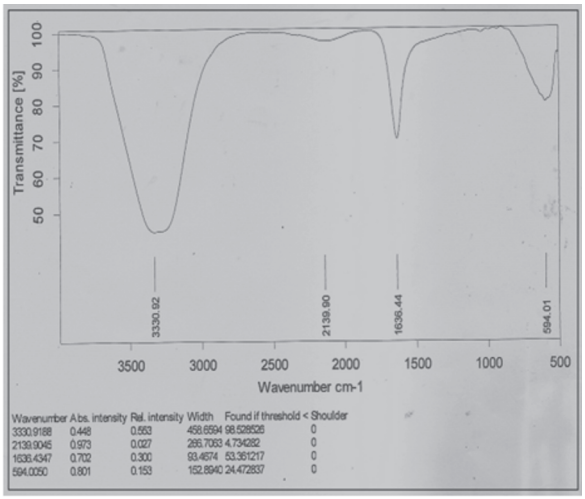

Fig. 1. IR spectra of the Isolated Component F15

B. Acute Oral Toxicity

Acute oral toxicity was performed according to OECD 423 guideline. Standard dose limit was 2000 mg/kg. The adverse effect was checked after 48 hours of oral dose, which revealed that up to 2000 mg/kg NOAEL (Not Observed Adverse Effect Limit) was observed.

C. *In-vitro* Anti-inflammatory Analysis

The protein denaturation test revealed that the crude extract was potentially active against inflammation where as isolated component was less significant. This is because the IC50 obtained for crude extract was 130.7 µg/mL and that of isolated component was 2350.23 µg/mL. Figure 2 clearly depicts the difference in percentage inhibition of the crude and isolated component.

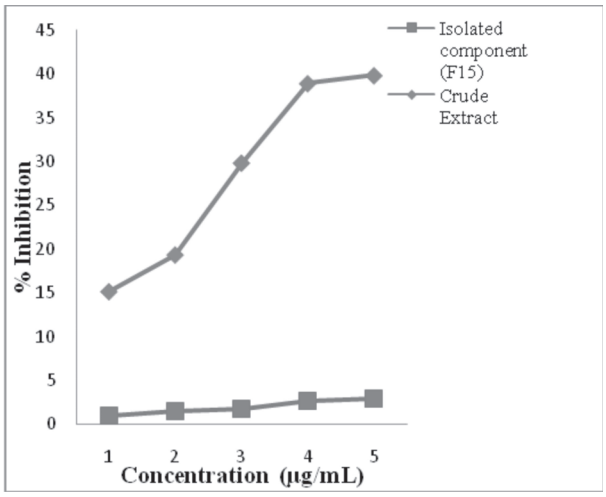

Fig. 2. Graph showing percentage inhibition with increase in concentration of Crude and Isolated extract

D. *In-vivo* Anti-inflammatory activity: *In vivo* activity was performed using carrageenan induced paw edema model. The isolated extract did not show much effect but the crude extract showed significant result ($p<0.001$) with dose of (Table 2) 400mg/kg.

Conclusion

The present research clearly shows that methanol acts as a suitable solvent for the extraction of alkaloids and flavonoids. The *in-vitro* study by protein denaturation technique gave better results with the crude extract than isolated one which shows that the different components present in the extract synergistically act to enhance the anti-inflammatory activity. Moreover the *in-vivo* study confirms its sig-

Table 2. Showing difference in paw thickness of rats of control and different doses of crude extract in Mean & SD form (All data presented in Mean ± SD, (N=4). *P<0.001 as compared to control group. #P<0.05 as compared to control group)

Dose	0 hour	1hour.	3hour.	5hour.	24hour.
Control	0.20±0.21	3.19±0.19	3.24±0.23	3.29±0.13	3.31±0.20
200mg/kg	0.37±0.08	3.15±0.21	3.32±0.19	2.51±0.28#	3.01±0.19
400mg/kg	0.33±0.03	2.19±0.31*	2.14±0.31*	2.10±0.19*	1.93±0.15*

nificant potential as anti-inflammatory at dosage level of 400mg/kg.

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