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Evaluation of anti-inflammatory, antibacterial and acaricidal activities of various leaf extracts of *Pongamia pinnata*

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Abstract
Different leaf extracts of *Pongamia pinnata* i.e. aqueous, alcoholic, acetone and chloroform were investigated for *in-vivo* anti-inflammatory activity and *in-vitro* antibacterial and acaricidal activities at the dose rate of 50 mg/kg and 100 mg/kg. For determining anti-inflammatory activity carrageenan induced paw edema model was used. *In-vitro* antibacterial activity was assessed against clinical isolates of *E.coli* and *S. aureus* at the extract concentrations of 50 and 100 mg/ml. Fully engorged adult *R.* (B.) *annulatus* female ticks were collected from Bhatinda region of Punjab for determining acaricidal activity by AIT method. The results showed dose dependent anti-inflammatory activity with maximum reduction in oedema observed in the alcoholic extract group but no significant *in-vitro* antibacterial and acaricidal activities were recorded in the present study.

Keywords: anti-inflammatory, antibacterial, acaricidal, *Pongamia pinnata*

Introduction
Medicinal plants have been curing various disorders in humans and are considered an integral part of the Indian traditional medicinal system, better known as the Ayurvedic system of medicine (Basu 2002) [1]. Generally, natural drug substances often perform vital and appreciable roles in the modern system of medicine thereby justifying their presence in the prevailing therapeutic arsenal. These serve as extremely useful natural drugs, provide basic compounds which prove to be less toxic and more effective drug molecule (Kan 2006) [2]. Thus antimicrobial research is geared towards the discovery and development of novel antibacterial and antifungal agents.

Pongamia pinnata (L.) Pierre [family: Leguminosae] is a medium –sized glabrous tree popularly known as Karanja in Hindi, Indian beech in English and Sukhchain in Punjabi. It is adaptable tree for tropical and sub-tropical regions which requires excellent drainage and a sunny location. It grows easily from seed. *Pongamia* is a medium size, nitrogen fixing tree native to India, Indonesia, Malaysia, and Myanmar. It has been successfully introduced to humid tropic lowland worldwide and to parts of Australia, China, New Zealand and United States. *Pongamia* trees are legumes, so it helps to replenish soil nitrogen. Historically, this plant has long been used in India and neighbouring regions as a source of traditional medicines, animal fodder, green manure, timber, fish poison and fuel. The *pongamia* growth is estimated at more than 1 million hectares in India. The fruits and sprouts are used in folk remedies for abdominal tumors in India, the seeds for keloid tumors in Sri Lanka and a powder derived from the plant for tumours in Vietnam (Chopade *et al*. 2008) [3]. In ancient India, seeds were used for skin ailments while today the oil from the seeds is used as a liniment for rheumatism. Leaves are active against *Micrococcus*; their juice is used for cold, coughs, diarrhoea, dyspepsia, flatulence, gonorrhoea and leprosy. Roots are used for cleaning gums, teeth and ulcers while bark is used internally for bleeding piles and is said to be an excellent remedy for itch, herpes and pityriasis versicolor. Powdered seeds are valued as a febrifuge, tonic and in bronchitis and whooping cough. So, the present study was conducted to evaluate the anti-inflammatory, antibacterial and acaricidal activities of different leaf extracts of *Pongamia pinnata* in wistar rats.

Material and Methods
Plant material
Based on ethno pharmacological information, leaves were collected from campus of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab and were authenticated by the botanist of Collaborative Ayurveda Research Centre, GADVASU,

Ludhiana. Immediately after collection leaves were washed and dried under sunlight. The dried leaves were finely grounded into powder, weighed and kept for further analysis.

Extraction
Different types of extracts of the plant were prepared using various menstruum viz water, ethanol, acetone and chloroform by maceration technique. 100 grams of powdered leaves was soaked in 1 litre menstruum at room temperature for 48-72 hours and stirred at frequent time intervals. After maceration, the extract was initially filtered using muslin cloth and then re-filtered again using Whatman filter paper No 1. The filtrate obtained was evaporated in oven at a temperature of 40°C. The residue obtained was lyophilized and kept at 4°C in air tight bottles until used. Percent yield for different solvents viz. water, ethanol, acetone and chloroform was found to be 8%, 3.8%, 3% and 2.8% respectively.

Drugs and chemicals
Carboxymethyl cellulose sodium, DMSO, carrageenan, meloxicam and ampicillin were used in experiments.

Animals
The present investigation was conducted on 50 rats aged 3-4 months, weighing180-200 grams, at Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The animals were purchased from small animal colony, GADVASU. The animals were acclimatized to the environment for ten days before starting experiment and kept in cages under standard laboratory conditions of temperature (27 - 30ºC), with a 12-h light cycle. All animals were fed commercial rat pellets procured from Ashirwad industries, Mohali (Punjab). Feed and water were provided ad libitum to the animals. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) vide reference no VMC/13/1786-1806 dated 4/4/13 and was conducted in accordance with ethical committee guidelines. Animals were divided into ten groups of five animals each. Group I served as control, group II as positive control and remaining groups served as test groups**.** Group III and IV were administrated with aqueous extract of concentration 50 mg/kg and 100 mg/kg orally, respectively. Group V and VI were administered with alcoholic extract at concentration of 50 mg/kg and 100 mg/kg, respectively. Similarly group VII and VIII with acetone extract and group IX and X with chloroform extract having concentrations of 50 mg/kg and 100 mg/kg, respectively.

Anti-inflammatory activity
In this experiment all groups were administered with 1% carrageenan subplanter@ 0.1 ml to induce inflammation as per method of Winter *et al* (1962) [4]. In group II meloxicam @ 1 mg/kg orally was given as standard drug. Rest of the groups were treated as described above. Increase in paw volume was noted at time interval of 1 h, 2 h, 3 h, 4 h, 5 h and 6 h respectively.

Antibacterial activity
Different extracts of the plant were evaluated for their antibacterial activity against gram +ve (*Staphylococcus aureus*) and gram –ve (*Escherichia coli*) bacteria. These activities were compared with standard drug (ampicillin) against these microorganisms.

Microbial strains
Strains of two bacteria, namely *Staphylococcus aureus and Escherichia coli* were obtained from the pure clinical isolates maintained in the Department of Microbiology, GADVASU, Ludhiana.

Preparation of the extract for antimicrobial assay
Different concentrations (5% and 10%) of the plant extracts were dissolved in dimethylsulphoxide (DMSO) for antimicrobial study.

Culture Media and Chemicals
Absolute alcohol, Mueller Hinton agar medium (Merck Specialities Private Limited, Mumbai), nutrient broth (Merck Specialities Private Limited, Mumbai), MLA, MSA (HIMEDIA®) were commercially procured.

Well diffusion method assay
The agar well diffusion method (Collins *et al* 1995) [5] was followed to test the antibacterial activity of the different plant extracts. The sterilized media were poured into pre-sterilized petri plates (in triplicate) at a depth of approximately 4 mm (15 ml) and allowed to solidify and the bacterial colonies grown on the selective media were swabbed over the solidified media. Then the wells were cut using a sterile well punch. Different plant extracts dissolved in corresponding solvents (water for aqueous and DMSO for other extracts) at concentrations 5% and 10% were delivered into the wells. DMSO (50 µl), and ampicillin (10μg/ml) were maintained as negative and positive controls, respectively. The plates were incubated at 37º C for 24 h. Following incubation the zones of inhibition were measured and noted down.

Acaricidal activity
Fully engorged adult R. (B.) annulatus female ticks were collected from Bhatinda region. For evaluation of anti-tick activity of the aqueous and ethanolic leaf extracts; adult immersion test (AIT) (Drummond *et al*., 1973; Sharma *et al*., 2012) [6, 7] was adopted. Adult engorged female ticks were washed thoroughly thrice with tap water and kept on filter paper for drying. The ticks were distributed randomly into various groups of ten each and were weighed individually. A dose dependent response study was conducted by exposing adult ticks to various concentrations (0.625, 1.25, 2.5, 5.0 and 10.0%) of aqueous (SLA) and ethanol (SLE) extracts of leaves of *Pongamia pinnata* and in each concentration; ten ticks in duplicate were maintained. All the ticks were immersed for 2 min while control ticks were immersed in distilled water and 10% ethanol for SLA and SLE, respectively and then transferred to the Petri dishes padded with Whatmann filter paper no. 1. After 24 h, the ticks were transferred to glass vials covered with muslin cloth and kept in desiccators placed in incubator maintained at 28 ± 1 °C and 85 ± 5% relative humidity. The effect of the extracts on tick mortality was recorded and ticks which did not oviposit even after 14 days post-treatment were considered as dead (Singh *et al* 2014) [8]. The ticks which survived after exposure in various concentrations were reared subsequently for generating the data on the efficacy of extracts on inhibition of oviposition.

Results and Discussion
Anti-inflammatory activity
The results for the change in paw volume on administration of various leaf extracts of *Pongamia pinnata* on carrageenan induced inflammation in right paw of wistar rats are given in the Table 1. Carrageenan 0.1 ml was injected subplanter to induce inflammation in all the animals. Group I served as

control and group II served as standard. In the standard group meloxicam @ 1 mg/kg was administered orally. In group III, group V, group VII and group IX aqueous, alcoholic, acetone and chloroform extracts were given respectively, @ 50 mg/kg whereas in group IV, group VI, group VIII and group X respectively these extracts were given @ 100 mg/kg orally. All the drugs were administered half an hour before the subplanter injection of carageenan.

In the results of evaluation of anti-inflammatory activity of leaf extracts of *Pongamia pinnata*, after two hours of carageenan injection, the inflammation in standard group was lowest as compared to all the extract treated groups and highest in the control group. All the extract treated groups showed significant anti-inflammatory activity with maximum activity in group VI in which the inflammation started to subside after three hours of treatment and achieved normal paw volume after five hours of the carageenan injection. It is well known that non-steroidal anti-inflammatory drugs like meloxicam mitigate the inflammatory response by inhibiting the formation of chemical mediators at the peripheral target sites where PGs and bradykinin are proposed to play a significant role. Therefore it is likely that the activity demonstrated by the extracts in the present study may be due to inhibition of these mediators as the phyto-chemical screening of the extracts proves the presence of flavonoids which act in the same way as NSAIDS to curb inflammation.

In connivance with the present study, Rao *et al* (2007) [9] also revealed the anti-inflammatory activity of three different type of leaf extracts (petroleum ether, ethylalcoholic and aqueous) of *Pongamia pinnata*. Also Singh *et al* (2013) [10] reported *in-vitro* anti-inflammatory activity of hydroalcoholic extract of *Pongamia pinnata* seed.

Antibacterial activity

The results for the antibacterial activity of different types of leaf extracts of *Pongamia pinnata* against *E.coli* and *S.aureus* are shown in Tables 2 and 3 respectively. The results showed non-significant zones of inhibition when compared to the control, depicting no antibacterial activity of any of the leaf extracts of *Pongamia pinnata* against *E.coli* and *S.aureus*.

In contrast to the results obtained in present study, Arote *et al* 2009 reported maximum antibacterial activity in the petroleum ether extract of *Pongamia pinnata* leaves against *S. aureus, S. epidermidis & P. vulgaris* and mild antibacterial activity against *E. coli*, and *S. typhimurium*.

Acaricidal activity

The results showing the effects of different concentrations of the aqueous & alcoholic leaf extracts of *Pongamia pinnata* on the acaricidal activity against *Rhipicephalus* (B.) *microplus* are shown in Table 4. The results clearly demonstrate that there was no dose dependent effect on the mortality of the ticks by any of the concentrations of the extracts of the plant after 14 days of treatment. Also it was observed that there was no inhibition of oviposition by any of the concentrations of the extracts of the plant revealing no acaricidal activity of the different concentrations of the extracts used in the study.

Conclusion

In this study all extracts showed dose dependent anti-inflammatory activity with maximum activity in the alcoholic extract of *Pongamia pinnata*. Study also revealed no antibacterial activity in the different leaf extracts of plant against clinical isolates of *E.coli* and *Staphylococcus aureus* at the extract concentrations of 50 and 100 mg/ml as there was no significant zone of inhibition in all extracts when compared to the control. No dose dependent mortality of ticks was observed in any of the concentrations of the leaf extracts.

Table 1: Effect of different leaf extracts of *Pongamia pinnata* on carrageenan-induced paw oedema in rats

		Increase in paw volume(ml) in comparison to normal (0 hour) value					
Group	**Dose mg/kg**	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**	**6 hr**
Aqueous	50	0.14 ± 0.005^{e}	0.33 ± 0.005^{d}	0.32 ± 0.008^{c}	0.20 ± 0.007^{c}	0.14 ± 0.006^{b}	0.04 ± 0.005^{d}
Aqueous	100	0.15 ± 0.004^{de}	0.34 ± 0.005^{cd}	0.16 ± 0.004^{f}	0.10 ± 0.009^{d}	0.01 ± 0.004^{d}	0.00 ± 0.00^{e}
Alcoholic	50	0.10 ± 0.01^{f}	0.27 ± 0.004^{e}	0.26 ± 0.003^{de}	0.17 ± 0.005^{c}	0.08 ± 0.003^{c}	0.04 ± 0.007^{d}
Alcoholic	100	0.25 ± 0.01^{c}	0.34 ± 0.005^{cd}	$0.26\pm0.01d^{e}$	0.12 ± 0.02^{d}	0.02 ± 0.005^{d}	0.01 ± 0.005^{e}
Acetone	50	0.28 ± 0.015^{ab}	0.37 ± 0.007^{b}	0.35 ± 0.007^{b}	0.20 ± 0.009^{c}	0.16 ± 0.01^{b}	0.07 ± 0.008^{c}
Acetone	100	0.28 ± 0.016^{ab}	0.35 ± 0.005^{cd}	0.23 ± 0.008^{e}	0.10 ± 0.01^{d}	0.01 ± 0.005^{d}	0.01 ± 0.005^{e}
Chloroform	50	0.26 ± 0.009^{bc}	0.43 ± 0.010^{a}	0.42 ± 0.011^{a}	0.31 ± 0.019^{b}	0.24 ± 0.015^{a}	0.17 ± 0.010^{a}
Chloroform	100	0.30 ± 0.010^{a}	0.36 ± 0.008^{bc}	0.27 ± 0.010^{d}	0.17 ± 0.008^{c}	0.06 ± 0.011^{c}	0.00 ± 0.00^{e}
Standard (Meloxicam)	2	0.18 ± 0.008^{d}	0.26 ± 0.006^{e}	0.15 ± 0.009^{f}	0.09 ± 0.012^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}
Control		0.31 ± 0.006^{a}	0.43 ± 0.008^{a}	0.42 ± 0.010^{a}	0.36 ± 0.007^{a}	0.26 ± 0.006^{a}	0.13 ± 0.010^{b}

Table 2: Effect of different leaf extracts of *Pongamia pinnata* on antibacterial activity against *E.coli* (Measurements on Zone of inhibition (mm)

Group	Dose (concentration)	Zone of inhibition (mm)
DMSO	--	1.66 ± 0.33^{b}
Standard Drug (Ampicillin)	10 ug/ml	18.3 ± 0.33^{a}
Aqueous	50 mg/ml	2 ± 0.30^{b}
	100 mg/ml	2.3 ± 0.33^{b}
Alcoholic	50 mg/ml	1.6 ± 0.33^{b}
	100 mg/ml	2 ± 0.50^{b}
Acetone	50 mg/ml	2 ± 0.30^{b}
	100 mg/ml	2.3 ± 0.33^{b}
Chloroform	50 mg/ml	2.6 ± 0.30^{b}
	100 mg/ml	3 ± 0.50^{b}

Table 3: Effect of different leaf extracts of *Pongamia pinnata* on antibacterial activity against *S.aureus* (Measurements on Zone of inhibition (mm))

Group	Dose (concentration)	Zone of inhibition (mm)
DMSO	--	1.66 ± 0.33^{b}
Standard drug (ampicillin)	10 ug/ml	29.3 ± 0.40^{a}
Aqueous	50 mg/ml	2.3 ± 0.33^{b}
	100 mg/ml	2.3 ± 0.33^{b}
Alcoholic	50 mg/ml	3.3 ± 0.30^{b}
	100 mg/ml	3.2 ± 0.30^{b}
Acetone	50 mg/ml	2.6 ± 0.33^{b}
	100 mg/ml	3 ± 0.33^{b}
Chloroform	50 mg/ml	2.6 ± 0.60^{b}
	100 mg/ml	2.3 ± 0.60^{b}

Table 4: Acaricidal activity of different leaf extracts of *Pongamia pinnata* against *Rhipicephalus* (B.) *microplus*

Extracts	Concentration	Total Number Of Ticks	Number Of Ticks Died	Percent Mortality
Aqueous	0.625	10	0	0
	1.25	10	0	0
	2.5	10	0	0
	5	10	0	0
	10	10	0	0
	Control	10	0	0
Alcoholic	0.625	10	0	0
	1.25	10	0	0
	2.5	10	0	0
	5	10	0	0
	10	10	0	0
	Control	10	0	0

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