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PHYTOPHARMACOLOGICAL EVALUATION OF *PERGULARIA DAEMIA* AS AN ANTI-INFLAMMATORY AGENT

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ABSTRACT

The whole-plant, *Pergularia daemia* (Family: Asclepediaceae), extract (50% alcohol) was investigated for phytochemical, physico-chemical parameters and its anti-inflammatory activity. Preliminary organic analysis revealed the presence of alkaloids, flavonoid, steroid, triterpenoid and phenolic compounds in the extract. Physiochemical studies revealed that total ash is 13.62%, acid insoluble ash is 1%, alcohol soluble extractive value is 17.6%, water soluble extractive value is 30.4% and loss on drying at 105°C is 10.6%. The anti-inflammatory activity was evaluated using carrageenan-induced paw edema (acute inflammation) and chronic models like; cotton pellet granuloma and carrageenan air pouch granuloma. Oral administration of the extract (50 and 100 mg/kg) exhibited significant anti-inflammatory activity in acute and chronic models ($p < 0.01$) of inflammation. In conclusion, present investigation established specific identities that will be useful in identification and authentication of the raw drug and pharmacological evidences to support the folklore claim that P. daemia is used as anti-inflammatory agent.

Key words: *Pergularia daemia*, physicochemical, carrageenan, cotton-pallet

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INTRODUCTION

In ethanomedicinal practices the traditional healer use *Pergularia daemia* (Asclepiadaceae) as anthelmintic, laxative, antipyretic and expectorant, and is also used to treat infantile diarrhoea and malarial intermittent fevers and possesses stomachic, laxative and diuretic properties, useful in cough, biliousness and sore eyes[1-3]. Plant has been documented for antidiabetic[4], wound healing[5], hepatoprotective activity[6], antibacterial[7], anti-urolithiatic, diuretic[8] and antifertility[9]. Phytochemical review revealed the presence of triterpenes, saponins cardenolides and alkaloids[10] while Anajanyulu et al. (1998)[11] reported the presence of triterpenes and steroidal compound. Sathish et al. (1998)[10] reported the anti-inflammatory, anti-pyretic and analgesic activities of the plant. In addition plant was also documented for uterine stimulatory activity[12] and insecticide activity[13]. Therefore the present study had been done to document its physicochemical parameters, which will be utilized by the pharmaceutical industries for the authentication and quality control of this drug and to assess anti-inflammatory activity of *Pergularia daemia* in different animal models to verify or controvert the claims made in the tradition medicine.

MATERIAL AND METHODS

The plant material was bought from Botanical Source of India, Jodhpur (No. 12/2007). The dried powdered plant material was extracted with 50% alcohol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure to obtain semi solid mass. The dose of extract selected on the basis of our previous work[8]. Physico-chemical constants such as percentage of total ash, water soluble ash, water and alcohol soluble extractives and loss on drying (LOD) were calculated as per the Indian Pharmacopoeia. Preliminary phytochemical tests were done as per the standard methods[14].

Animals

Albino Wistar rats of the either sex (200 - 250 g) were used for the present study. Animals were housed in groups of five under standard laboratory conditions of temperature (25 ± 20 C) and 12/12 hr light/dark cycle. They were provided with standard pellets and tap water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Carrageenan-induced rat pedal inflammation:

Rats were divided in four groups of 6 animals each. Group A: saline control; Group B: 10 mg/kg indomethacin; Group C: extract – 50 mg/kg; Group D: extract – 100 mg/kg. One hour after the oral administration of drugs, acute paw oedema was induced by injecting 0.1 ml of 1%

carrageenan in 0.9% saline. Paw volume was measured with the help of plethysmometer by mercury displacement method at 0 and 3 hours. The percentage inhibition of paw oedema in treated groups was then calculated by using the formula:

Percentage inhibition = (1 – Vt/Vc) X 100; Where Vt = is the oedema volume in the drug treated; Vc = is the oedema volume in the control group

Cotton pellet granuloma

The granulomas were developed by the method described by Naik et al. (1980)[15] Rats were divided into four groups of 6 animals each. Group A: saline control; Group B: 10 mg/kg indomethacin; Group C: extract – 50 mg/kg; Group D: extract – 100 mg/kg. Following one hour of oral administration of drugs, sterile cotton pellets weighing 10 mg were implanted subcutaneously in both the axillae of rats under ether anesthesia. Drugs were given daily for 10 days. On the 11th day, rats were sacrificed and the cotton pellets with the surrounding granulomas were resected out and their wet and dry weights were recorded.

Carrageenan air pouch granulomas

The inflammation was produced in rats by the method described by Hambleton and Miller (1989)[16]. The dorsal subcutaneous space was injected with 20 ml of air to create a pouch, which was reinflated on the fourth day with 10 ml of air. On the seventh day, rats were divided into twelve groups of 6 animals each. Group A: saline control; Group B: 20 mg/kg prednisolone; Group C: extract- 50 mg/kg; Group D: extract – 100 mg/kg. Drugs were administered orally and, 1 hour later, 1 ml of 1% carrageenan suspended in normal saline was injected into the pouch. The drug treatment was given daily for four days and 24 hours after the last dose the rats were sacrificed. Five millilitres of ice cold normal saline containing 0.1% EDTA was injected into the pouch and the exudate was collected in a graduated tube and its volume was measured. The granulomas were also resected and their wet and dry weights were recorded. Mean difference from control in the fluid volume, wet and dry weights were calculated.

RESULTS AND DISCUSSION

Result of preliminary phytochemical analysis conducted on 50% alcohol extract of *Pergularia daemia* showed presence of flavonoids, steroids, alkaloids, glycosides, sugars and triterpenes (Table 1).

The physical constant evaluation of the drugs is a vital parameter in detecting adulteration of drugs. The ash value was determined in three different forms i.e. total ash, acid insoluble ash and water soluble ash (Table 2). The total ash is essential in the assessment of purity of drugs like the

Table 1: Phytochemical Screening

Test	50% Ethanolic extract
Alkaloids	+
Steroids	+
Flavonoids	+
Tannins	+
Terpenoids	+
Carbohydrate	+
Glycoside	+

Table 2: Physico-chemical parameter of plant

Sr. No.	Quality Parameters	% w/w
I	Ash values:	
	Total ash	13.62 %
	Acid insoluble ash	1%
	Water soluble ash	6.2%
II	Extractive value:	
	Ethanol soluble	17.6%
	Water soluble	30.4%
III	Loss on drying	10.6%

presence or absence of foreign inorganic matter such as silica or metallic salts and minerals or earthy material attached to the plant material. Water soluble ash is the water soluble portion of the total ash indicating the presence of sugar, acids and inorganic compounds and alcohol soluble extractive values indicated the presence of polar constituents. Acid insoluble ash measures the amount of silica present, especially as sand[17]. The total ash of crude powder of *P. daemia* was 13.62%, acid insoluble ash was 1% and water soluble ash was 6.2%. Less amounts of these three parameters prove that the inorganic matter, sugar and silica was less in *P. daemia.* For the determination of exhausted or adulterated drugs extractive values were also determined. The extractive value of crude powder was in water (30.4%) and in alcohol (17.6%). The moisture content of dry powder of whole plant *P. daemia* was 10.6% which is not much high, hence it would put off bacteria, fungi or yeast growth.

The Carrageenan induced paw edema is characterized by biphasic event through contribution of special inflammatory mediators. During the first phase (the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin participate, while during second phase (3 – 4 h after carrageenan injection), Kinin and prostaglandins are involved[17]. Present study results revealed that administration of hydroalcoholic extract at 50 and 100 mg/kg, p.o. and standard drug indomethacin at 10 mg/kg, p.o. inhibited the edema preparatory from the first hour and

throughout all phases of inflammation dose dependently (P < 0.01), which is probably inhibition of different chemical mediators of inflammation (Table 3).

Table 3: Effects of ethanolic extract and various fractions of *Pergularia daemia* on rat paw oedema induced by carrageenan (Acute inflammation)

Treatment	Dose (mg/kg)	Time, mean paw volume ± SEM (% inhibition)				
		0 hr	1/2 hr	1 hr	2 hr	3 hr
Control	0	0.25 ± 0.02	0.42 ± 0.01	0.56 ± 0.02	0.64 ± 0.04	0.73 ± 0.02
Indomethacin	10	0.24 ± 0.03	0.34 ± 0.03 (19.05)	0.30 ± 0.01* (46.43)	0.27 ± 0.02* (57.81)	0.22 ± 0.02* (69.86)
Ethanolic extract	50	0.23 ± 0.02	0.38 ± 0.03 (9.52)	0.40 ± 0.03* (28.57)	0.31 ± 0.01* (51.56)	0.24 ± 0.03* (69.86)
	100	0.22 ± 0.01	0.35 ± 0.02 (16.66)	0.34 ± 0.01* (39.28)	0.29 ± 0.02* (54.69)	0.21 ± 0.01* (71.23)

n = 6, each value represents mean ± SEM. *P < 0.01 as compared with the control group (One-way ANOVA followed by Dunnett's test)

Further, the effect of extract was evaluated on the proliferative phase of inflammation in cotton pellet granuloma and air pouch granuloma models. The cotton-pellet granuloma is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transuda, the dry weight of the pellet correlates with the amount of granulumatous tissues[19]. Extract at doses 50 and 10 mg/kg, when administered orally, produced reduction in the weight of granuloma induced by cotton-pellet. Both the wet and dry weights of granuloma were significantly reduced in extract treated at 100 mg/kg group (72.77 ± 2.83 mg and 24.5 ± 0.35 mg) as compared to control group (134.85 ± 7.15 mg and 46.37 ± 4.31 mg). The effect of extract was comparable with that of indomethacin (wet weight 70.52 ± 1.73 mg and dry weight 21.38 ± 2.84) (Table 4).

Table 4: Effect of hydroalcoholic extract of *P. daemia* on Cotton pellet granuloma (Chronic inflammation)

Treatment	Dose (mg/kg)	Granuloma weight		% Inhibition	
		WET (mg)	DRY (mg)	WET	DRY
Control	0	134.85 ± 7.15	46.37 ± 4.31		
Indomethacin	10	70.52 ± 1.73*	21.38 ± 2.84*	47.70	53.89
Ethanolic extract	50	77.65 ± 2.57*	28.21 ± 0.31*	42.41	39.16
	100	72.77 ± 2.83*	24.5 ± 0.35*	46.04	47.16

n = 6, each value was represented as mean ± SEM. * P < 0.01, when compared to the control group (one-way ANOVA followed by Dunnett's test)

In addition the effect of extract on proliferative phase of inflammation carrageenan-induced air-pouch model was selected in which tissue degradation and fibrosis occurs. During the repair

process of inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels occurs, which are the basic sources of forming a highly vascularized reddish mass, termed granulation tissue[20]. Hydroalcoholic extract of *P. daemia* also exhibited anti-inflammatory activity in carrageenan air pouch model by significantly decreasing the exudation volume and weight of granuloma. The exudates volume in extract (50 mg/kg), extract (100 mg/kg) and prednisolone treated groups of rats was 5.23 ± 0.24, 4.63 ± 0.19 and 4.54 ± 0.42 ml as against the control group (7:78 ± 0.44 ml). The extracts at both the doses also produced a significant reduction in the wet and dry weight of granuloma as compared to control. The effect of extract was more pronounced than that of prednisolone (wet: 40.18 % inhibition; dry: 45.16 % inhibition) as compared to control (Table 5). Further, the 50% alcoholic extract of *P. daemia* had shown to contain alkaloids, glycosides, tannins, flavonoids, sterols and/or triterpenes. Though, the anti-inflammatory effect of *P. daemia* needs to be characterized and the nature of active principle responsible for producing the anti-inflammatory activity remains to be identified.

Table 5: Effect of hydroalcoholic extract of *P. daemia* on Carrageenan air pouch granuloma

Treatment	Dose (mg/kg)	Exudate Volume (ml)	Granuloma weight		% Inhibition	
			WET (g)	DRY (g)	WET	DRY
Control	0	7.78±0.44	3.36±0.21	0.62±0.08		
Prednisolone	20	4.54±0.42**	2.01±0.19**	0.34±0.03**	40.18	45.16
Ethanolic extract	50	5.23±0.24**	2.43±0.20*	0.28±0.02**	27.68	54.84
	100	4.63±0.19**	1.98±0.31**	0.17±0.04**	41.07	72.58

n = 6, each value was represented as mean ± SEM. * P < 0.05, ** P < 0.001, when compared to the control group (one-way ANOVA followed by Dunnett's test)

CONCLUSION

In conclusion, on the basis of study results suggests that the physico-chemical parameters can be useful for the identification of the species which may be helpful to pharmaceutical industries for the quality control of the commercial samples. Further, our results indicate that *P. daemia* possesses potent anti-inflammatory activity which is comparable to standard anti-inflammatory drugs in various models that we have studied and serves as a possible rationale for the use of P. daemia in traditional medicine for inflammation.

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