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Biological screening of some selected Indian medicinal plants for anti-inflammatory activity

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Abstract
Biological screening of medicinal plants have remained a cornerstone in natural product research, serving as a bridge between traditional medicine and modern pharmacological discoveries. This paper explores the methodology of phytochemical screening, highlights important classes of secondary metabolites, and discusses the biological activities associated with medicinal plants including anti-inflammatory. Furthermore, the paper provides a conceptual framework for future research, focusing on integrating traditional knowledge with modern analytical techniques. The study concludes that medicinal plants continue to provide valuable leads for novel therapeutic agents, reinforcing their importance in the advancement of natural drug discovery.

Keywords: Phytochemicals, Medicinal Plants, Biological Activities, Natural Products

Introduction
Medicinal plants have played an indispensable role in healthcare since the dawn of human civilization. Their therapeutic potential has been documented in ancient systems of medicine such as Ayurveda, Unani, Traditional Chinese Medicine, and Indigenous healing practices. With increasing interest in plant-based remedies, pharmacognosy has emerged as a scientific discipline dedicated to the study of medicinal plants, their constituents, and their applications. Phytochemicals, particularly secondary metabolites, are responsible for the biological activities that underpin the medicinal value of plants [1-2]. Unlike primary metabolites, which are essential for growth and reproduction, secondary metabolites provide ecological advantages such as defense against pathogens, predators, and environmental stress. These compounds, when studied and isolated, often yield pharmacologically active molecules with therapeutic relevance [3-4].

Biological activities of medicinal plants
Medicinal plants demonstrate multiple biological activities due to their phytochemical richness. These activities form the basis of their therapeutic use in both traditional and modern medicine [5].

Materials and Methods
Plant material
Fresh plant/plant parts were collected randomly from Sagar region, India The details of the plant/plant parts screened, their families, voucher number, vernacular names and their therapeutic uses are given. Fresh plant materials were washed in tap water, air dried and then homogenized to fine powder and stored in airtight bottles [6].

Extraction
10 g of air-dried powder was taken in 100 ml of petroleum ether in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 h, the supernatant was discarded and petroleum ether was evaporated from the powder. This dry powder was then taken in 100 ml of solvent (methanol or acetone) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 h, the extracts were centrifuged at 5000 g for 10 min, the supernatant was collected, solvents were

evaporated, and the dry extract was stored at 4oC in airtight bottles. The extraction was done at least three times for each plant and the mean values of extractive yields are presented [6-8].

Adenanthera pavonina L. *Alangium salvifolium* (Linn. f.) Wang, *Argemone mexicana* L., *Aristolochia bracteolata* Lam., *Boerhavia diffusa* L, *Cassia occidentalis* L. were screened for acute anti-inflammatory study

Animals

Wistar albino rats of either sex (200-300 g) were used for the anti- inflammatory studies. The animals were obtained from veterinary college Mhow M.P.All the rats were housed in standard plastic rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were kept at the animal house the animals were kept in a group of 6-8 animals per cage and facilitated with standard environmental condition of photoperiod (12:12 h dark: light cycle) and temperature (25 ± 2ºC). They were provided with commercial rat and mice feed (Pranav Agro Industries Ltd., Amruth Brand rat & mice pellet feed) and water given ad libitum. The use of these animals and the study protocols were approved by CPCSEA approved local ethical committee [9-11].

Pharmacological Experiments

Preparation of test drug and standard drug

The test drug (Methanol extract of all the plants) and standard drug (Indomethacin) were prepared as a suspension in distilled water using mortal and pestle [12].

Animal Grouping [13].

The animals were divided in to fourteen groups for acute anti-inflammatory study. Each group consisted of six animals of either sex. The groups were:

- **Group I:** Vehicle treated control (Distilled water)
- **Group II:** Indomethacin (2.5 mg/kg)
- **Group III:** Methanol extract of *Aristolochia bracteolata* (200 mg/kg)
- **Group IV:** Methanol extract of *Aristolochia bracteolata* (400mg/kg)
- **Group V:** Methanol extract of *Argemone mexicana* (200 mg/kg)
- **Group VI:** Methanol extract of *Argemone mexicana* (400 mg/kg)
- **Group VII:** Methanol extract of *Adenanthera pavonina* (200mg/kg)
- **Group VIII:** Methanol extract of *Adenanthera pavonina*(400mg/kg)
- **Group IX:** Methanol extract of *Alangium salvifolium* (200 mg/kg)
- **Group X:** Methanol extract of *Alangium salvifolium* (400 mg/kg)
- **Group XI:** Methanol extract of *Boerhavia diffusa* (200 mg/kg)
- **Group XII:** Methanol extract of *Boerhavia diffusa* (400 mg/kg)
- **Group XIII:** Methanol extract of, *Cassia occidentalis* L. (200 mg/kg)
- **Group XIV:** Methanol extract of, *Cassia occidentalis* L. (400 mg/kg)

Carrageenan induced rat paw edema (Winter *et al*. 1962)

A plethysmograph was used to measure the animals' initial paw volume after they had been grouped (Bhatt *et al*. 1977). The 10 ml glass vessel (25 mm x 65 mm) used in the plethysmograph was fixed with a 2 ml glass syringe using pressure tubing. After adding about 4 ml of mercury to the syringe, the micropipette's mercury level was set to zero. Water filled the gap in the glass jug between the zero mark and the fixed mark. The water's starting level was changed and made equal to zero. Water was precisely up to the tibiotarsal joint on the paw. By releasing the linked syringe's pressure, the glass vessel's elevated water level was brought to the predetermined point. The level where water and mercury interface in the micropipette was recorded as paw volume [14-15].

Subsequently, the animals in each group were given either distilled water, indomethacin (2.5 mg/kg), or a methanolic extract of the plants (200, 400 mg/kg; p.o.). All of the animals received hydration (4 ml/100 g body weight) following the medication delivery. A subplantar injection of freshly made 0.1 ml of 1% carrageenan (Hi-media) in normal saline was used to generate oedema one hour after drug administration. At one, two, and three hours following the carrageenan injection, the paw volume was measured. The formula used to calculate the percentage increase in paw volume was as follows:

$$\text{Increase in paw volume (\%)} = \frac{\text{Final paw volume}}{\text{Initial paw volume Initial paw volume}} \text{x10}$$

Results and Discussion

Carrageenan injection into the rat paw provokes a local, acute inflammatory reaction that is a suitable criterion for evaluation of anti-inflammatory agents (Segura *et al*. 1998). The inflammation consists of two phases, early phase which is related to the production of histamine, 5-hydroxytryptamin, bradykinins and cyclooxygenase products and delayed phase which is linked to neutrophil infiltration, as well as production of arachidonic acid metabolites.The results of the present study showed that the extracts of all the plants showed decrease in paw volume in carrageenan induced inflammation in rats at the dose level of 200 mg/kg and 400 mg/kg as compared to the control group [16-17].

Alangium salvifolium showed significant decrease in paw volume at 1 h (51.9%, P≤ 0.05) and 2 h (47.57%, P≤ 0.01) at 400 mg/kg dose level. *Alangium salvifolium* did not show significant decrease in paw volume at 200 mg/kg dose level.

A. mexicana also did not show any significant decrease in paw volume at 200 mg/kg dose level. However, *A. mexicana* showed significant decrease in paw volume at 1h (58.58%, P≤ 0.01), 2 h (42.43%, P≤ 0.05) and 3 h (42.80, P≤ 0.05) at 400 mg/kg dose level.

Boerhavia diffusa showed significant decrease in paw volume at 1 h (50.63%, P≤ 0.01) and 2 h (48.15%, P≤ 0.05) at 200 mg/kg dose level and also showed decrease at 400 mg/kg (59.24%, P≤ 0.01 at 1 h and 54.46%, P≤ 0.001 at 2 h). It did not show any significant anti-inflammatory activity at 3 h.

Aristolochia bracteolata showed significant decrease in paw volume at 1 h (55.32%, P≤ 0.01), 2 h (48.63%, P≤ 0.01) and 3 h (39.78%, P≤ 0.05) at 200 mg/kg dose level. At 400 mg/kg dose level, it showed significant decrease in paw volume at 1 h (62%, P≤ 0.01) and 2 h (50.82%, P≤ 0.01) only. *Adenanthera pavonina* did not show any significant anti-inflammatory activity at 200 mg/kg dose level *A.*

mexicana. While it showed significant decrease in paw volume at 2 h (27.22%, P≤ 0.05) at 400 mg/kg dose level only.

Cassia occidentalis L. showed significant decrease in paw volume at both the dose levels. It showed significant decrease in paw volume at 1 h (58.54%, P≤ 0.01), 2 h (54.22%, P≤ 0.001) and 3 h (48.71%, P≤ 0.05) at 200 mg/kg dose level, and at 400 mg/kg dose level, the decrease in paw volume at 1 h was (71.61%, P≤ 0.001), 2 h (56.68%, P≤ 0.001) and 3 h (44.3%, P≤ 0.05) at. Anti- inflammatory screening of *Cassia occidentalis* L. was comparable with the standard indomethacin at the 1 h at 400 mg/kg dose level [18-20].

Table 1: Anti-inflammatory screening of six medicinal plants by carrageenan induced rat paw edema

			1h		2h		3h	
Group No.	**Groups**	**Dose**	**% Increase In paw Volume**	**% Inhibition**	**% Increase In paw volume**	**% Inhibition**	**% Increase In paw volume**	**% Inhibition**
I	Control	2.5 mg/kg	25.45 ± 2.88	-	38.98 ± 2.45	-	38.94 ± 5.33	-
II	Indomethacin	200 mg/kg	9.36 ± 1.25***#	67.45↓	13.05 ± 3.17***#	66.53↓	10.67 ± 3.39**#	72.61↓
III	*Alangium salvifolium* (Linn.	400 mg/kg	15.39 ± 4.13#	38.89↓	31.16 ± 5.49#	20.05↓	28.02 ± 3.91#	28.04↓
IV	*Alangium salvifolium* (Linn.	200 mg/kg	12.69 ± 3.69*#	51.90↓	20.44 ± 4.2**#	47.57↓	24.88 ± 3.98#	36.10↓
V	*Adenanthera pavonina* L	400 mg/kg	23.12 ± 2.58#	25.64↓	35.54 ± 4.10#	8.82↓	27.80 ± 4.11#	28.61↓
VI	*Adenanthera pavonina* L	200 mg/kg	10.73 ± 1.44**#	58.58↓	22.44 ± 4.60*#	42.43↓	22.27 ± 2.65*#	42.80↓
VII	*Argemone mexicana* L	400 mg/kg	13.05 ± 2.55**#	50.63↓	20.21 ± 5.66*#	48.15↓	25.67 ± 3.35#	34.09↓
VIII	*Argemone mexicana* L.	200 mg/kg	10.60 ± 2.26**#	59.24↓	17.75 ± 1.90***#	54.46↓	27.74 ± 4.02#	28.76↓
IX	*Aristolochia bracteolata*	400 mg/kg	11.71 ± 2.50**#	55.32↓	20.02 ± 2.38**#	48.63↓	23.45 ± 3.43*#	39.78↓
X	*Aristolochia bracteolata* Lam.	200 mg/kg	10.71 ± 3.05**#	62.00↓	19.17 ± 3.88**#	50.82↓	29.88 ± 4.55#	23.28↓
XI	*Boerhavia diffusa* L.	400 mg/kg	22.70 ± 4.56#	23.38↓	25.88 ± 5.62#	33.60↓	24.12 ± 3.78#	38.06↓
XII	*Boerhavia diffusa* L.	200 mg/kg	21.83 ± 2.86#	19.41↓	28.37 ± 1.90*#	27.22↓	23.80 ± 3.28#	38.87↓
XIII	*Cassia occidentalis L*	200 mg/kg	11.70 ± 2.65**#	58.54↓	17.85 ± 2.32***#	54.22↓	19.97 ± 4.93*#	48.71↓
XIV	*Cassia occidentalis L*	400 mg/kg	8.08 ± 1.59***#	71.61↓	16.89 ± 2.77***#	56.68↓	21.69 ± 3.95*#	44.30↓

Data are expressed as mean ± SEM, P≤ (0.05*, 0.01**, 0.001***), F ≤ 0.05

Anti-inflammatory activity of 6 plants studied was as following manner at 1h: (400 mg/kg) > Adenanthera pavonina L. (400 mg/kg) > Alangium salvifolium (Linn (400 mg/kg) > A. mexicana (400 mg/kg) > Cassia occidentalis L. (200 mg/kg) > Cassia occidentalis L (200 mg/kg) > Aristolochia bracteolata Lam. (400 mg/kg) > A. speciosa (200 mg/kg) > Aristolochia bracteolata Lam. (200 mg/kg) > A. mexicana (200 mg/kg) > Boerhavia diffusa L. (200 mg/kg) > Boerhavia diffusa L. (400 mg/kg).Anti-inflammatory activity of 6 plants studied was as following manner at 2 h: Cassia occidentalis L (400 mg/kg) > Alangium salvifolium (Linn (400 mg/kg) > Cassia occidentalis L (200 mg/kg) > Aristolochia bracteolata Lam. (400 mg/kg) > Aristolochia bracteolata Lam. (200 mg/kg) > Alangium salvifolium (Linn (200 mg/kg) > Adenanthera pavonina L (400 mg/kg) > A. mexicana (400 mg/kg) > Boerhavia diffusa L (200 mg/kg) > Boerhavia diffusa L (400 mg/kg) > Adenanthera pavonina L (200 mg/kg) > A. mexicana (200 mg/kg).

Anti-inflammatory activity of 6 plants studied was as following manner at 3 h: Cassia occidentalis L (200 mg/kg) > Cassia occidentalis L (400 mg/kg) > A. mexicana (400 mg/kg) > Alangium salvifolium (Linn (200 mg/kg) > Adenanthera pavonina (400 mg/kg) > Adenanthera pavonina (200 mg/kg)

> Aristolochia bracteolata Lam (400 mg/kg) > Boerhavia diffusa L (200 mg/kg) > Boerhavia diffusa L (400 mg/kg) > A. mexicana (200 mg/kg) > Aristolochia bracteolata Lam (200 mg/kg) Alangium salvifolium (Linn (400 mg/kg).

Conclusion

According to the study's findings, all of the plants exhibited anti-inflammatory properties when compared to Aristolochia bracteolata, the control group. While Alangium salvifolium Linn, Boerhavia diffusa L, and Cassia occidentalis L shown stronger efficacy at a lower dose (200 mg/kg) than a higher dose (400 mg/kg) at three hours, Lam and A. mexicana demonstrated anti-inflammatory effect in a dose-dependent manner. At 1 and 2 hours, Adenanthera pavonina exhibited greater action at a lower dose of 200 mg/kg than at a higher dose of 400 mg/kg. Methanol extracts of Cassia occidentalis L., Alangium salvifolium (Linn), Adenanthera pavonina, and A. mexicana had greater anti-inflammatory action at 1 h than at 3 h at both dosages. A. mexicana methanol extract displayed more activity at 3 hours compared to1 h in lower dose, while it showed higher activity at 1 h than at 3 h in higher dose. Methanol extract of Adenanthera pavonina showed higher anti-inflammatory activity at 3 h than at 1 h.

Therefore, the early phase of the inflammation caused by carrageenan was significantly inhibited by methanol extracts of Alangium salvifolium (400 mg/kg), A. mexicana (400 mg/kg), Adenanthera pavonina (200 and 400 mg/kg), Boerhavia diffusa (200 and 400 mg/kg), and Cassia occidentalis L (200 and 400 mg/kg). The late phase of the inflammation caused by carrageenan was significantly inhibited by A. mexicana (400 mg/kg), Alangium salvifolium (200 mg/kg), and Cassia occidentalis (200 and 400 mg/kg).

Adenanthera pavonina displayed less anti-inflammatory action than the other five plant extracts, with Cassia occidentalis L.'s methanol extract having the strongest anti-inflammatory activity, followed by A. mexicana and Alangium salvifolium.

Thus, on the bases of availability of plant material and literature search, Cassia occidentalis L (Leaf + stem) was selected for further pharmacognostic, toxicological and pharmacological studies.

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