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# EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF PAJANELIA LONGIFOLIA (WILLD.) K. SCHUM.

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***Abstract***

Oxidative stress and inflammation are key contributors to various chronic diseases, necessitating the exploration of natural compounds with therapeutic potential. This study evaluates the antioxidant and anti-inflammatory activities of *Pajanelia longifolia* extract. Antioxidant potential was assessed using DPPH, ABTS, superoxide radical scavenging, and lipid peroxidation inhibition assays, showing strong free radical scavenging ability. The anti-inflammatory activity was tested in Swiss albino mice using a carrageenan-induced paw edema model, where the extract significantly reduced inflammation in a dose-dependent manner. These findings suggest *P. longifolia* as a promising natural therapeutic agent against oxidative stress and inflammation-related disorders.

***Key words***: *Pajanelia longifolia*, antioxidants, anti-inflammatory, DPPH, ABTS, carrageenan.

## Introduction

Medicinal plants serve as invaluable resources for human health, forming the basis for numerous therapeutic agents (Che and Zhang, 2019). They are integral to Ayurveda and other traditional medicine systems, where they are widely utilized for managing ailments such as colds, fevers, and digestive disorders, as well as chronic conditions like diabetes, arthritis, and cardiovascular diseases. Beyond their role in treatment, medicinal plants contribute significantly to preventive healthcare by enhancing immunity and promoting overall well-being. Recognized by the World Health Organization as a promising source for drug development, these plants contain bioactive compounds including alkaloids, flavonoids, terpenoids, saponins, and phenolics that exhibit diverse health-promoting properties. Notably, their antioxidant and anti-inflammatory effects are crucial in mitigating oxidative stress and chronic inflammation, which are associated with the progression of diseases such as cancer, cardiovascular disorders, diabetes, and neurodegenerative conditions (Prata *et al.*, 2024).

*Pajanelia longifolia*, a member of the Bignoniaceae family, holds significant value in traditional medicine. Its roots and bark have been traditionally used for their antiseptic, anti-diarrheal, and anti-rheumatic properties, with applications in treating fever, wounds, edema, urinary disorders, and respiratory ailments (Saha *et al.*, 2017; Padyana *et al.*, 2011). These ethnobotanical uses highlight its potential for broader medicinal applications.

The present study aims to assess the antioxidant and anti-inflammatory properties of *Pajanelia longifolia* inflorescence extract.

## Materials and Methods

### Collection and extraction of Plant materials

Inflorescences of *Pajanelia longifolia* (Willd.) K. Schum were collected from Chengannur, Alappuzha, Kerala, India. The collected inflorescences were washed with dechlorinated water, shade-dried at room temperature, and then powdered. A total of 20 grams of powdered sample was extracted with 70% methanol for 24 hours, repeated three times. The extracts were filtered, and the solvent was

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evaporated to obtain solidified crude extracts. These crude extracts were stored in clean vials and maintained at 4°C in a refrigerator.

**Animals**

Male Swiss albino mice (25–30 g) were used for the present investigation. The animals were obtained from the Small Animal Breeding Station, Kerala Agricultural University, Mannuthy, Kerala, India. They were housed under standard laboratory conditions (22–28°C, 60–70% relative humidity, and a 12-hour dark/ light cycle). The rats were provided with standard commercial laboratory chow (pellet form, Lipton India) and water ad libitum. All animal experiments were conducted with prior approval from the Institutional Animal Ethics Committee (IAEC) and followed IAEC guidelines.

**In vitro Antioxidant activity of *Pajanelia longifolia* extract DPPH free radical Scavenging activity**

The free radical scavenging activity of the extracts was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, with modifications to the method described by Coruh *et al.* (2007). A 0.05 mg/ml solution of DPPH in methanol was prepared, and extract solutions of varying concentrations were dissolved in methanol. A 0.1 ml portion of each extract solution was added to 1.4 ml of the DPPH solution. The absorbance at 517 nm was measured after 5 minutes of incubation at room temperature. The percentage of DPPH radical scavenging was calculated using the formula:

Percentage of inhibition = (Control OD – Treated OD/ Control OD) x100

**ABTS radical scavenging activity**

The assay was performed by evaluating the interaction of the extract with a stable free radical derived from 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS). The production of the ABTS radical cation was carried out as described by Long and Halliwell (2001), with some modifications. Briefly, a stock solution of ABTS (7 mM) was prepared in water. To this solution, ammonium persulfate (2.45 mM final concentration) was added, allowing for the partial oxidation of ABTS to generate the ABTS radical. The ABTS radical solution was then diluted to achieve an absorbance of 0.75 at 734 nm in phosphate-buffered saline (PBS, pH 7.4). For the assay, 10µl of extract at different concentrations was added to 1 ml of the ABTS radical solution. The absorbance was measured spectrophotometrically after 6 minutes of incubation, using PBS as a reference. The percentage of inhibition was calculated using the formula:

Percentage of inhibition = (Control OD – Treated OD/ Control OD) x100

**Superoxide radical scavenging activity**

The reaction mixture contained 3 mg of KCN dissolved in EDTA (6 µM), riboflavin (2 µM), NBT (50 µM), various concentrations (10–1000 µg/ml) of the extract, and phosphate buffer, with a final volume of 3 ml. The tubes were exposed to uniform illumination with an incandescent lamp for 15 minutes. Absorbance was measured at 530 nm before and after illumination (McCord and Fridovich, 1969). The percentage of superoxide radical inhibition was calculated using the formula:

Percentage of inhibition =(Control OD – Treated OD/ Control OD) x100

**Lipid peroxidation assay**

Lipid peroxidation levels were measured following the method of Ohkawa et al. (1979). Extract (10–1000 µg/ml) was incubated with 0.1 ml of 25% rat liver homogenate containing 30 mMKCl, Tris-HCl buffer (0.04 M, pH 7.0), ascorbic acid (0.06 mM), and ferrous ion (0.16 mM) in a total volume of 0.5 ml for 1 hour. After incubation, 0.4 ml of the reaction mixture was treated with 0.2 ml of SDS (8.1%), 1.5 ml of TBA (0.8%), and 1.5 ml of acetic acid (20%, pH 3.5), and then heated in a boiling water bath

at 100°C for 1 hour. The mixture was cooled, followed by the addition of 5 ml of a pyridine-butanol mixture (15:1), mixed thoroughly, and centrifuged at 3000 rpm for 10 minutes. The absorbance of the clear supernatant was measured at 532 nm against the pyridine-butanol mixture as a blank. The percentage inhibition of lipid peroxidation was calculated as:
Percentage of inhibition =(Control OD – Treated OD/ Control OD) x100

**Anti-inflammatory activity of *Pajanelia longifolia* extract**
The anti-inflammatory activity of *P. longifolia* inflorescence extract was evaluated using a carrageenan-induced acute inflammation model in Swiss albino mice. The extracts were prepared in the required volume using double-distilled water for administration. Male Swiss albino mice (25–30 g) were divided into four groups, each consistingof seven animals. Inflammation was induced by injecting 25 µl of a 1% carrageenan suspension in 0.9% normal saline subplantarly into the right hind paw. Paw volume was measured 1 hour before and at intervals for 5 hours after carrageenan administration using a verniercaliper. The extracts were administered orally at a dosage of 100 mg/kg body weight 1 hour before carrageenan injection. Diclofenac (10 mg/kg) served as the standard reference drug. The percentage inhibition of inflammation was calculated using the following formula (García *et al.*, 1995):

Percent inhibition = [($V_T$- $Vo$) control − ($V_T$– $V_o$) treated group / ($V_T$– $V_o$) control] × 100,

Were, $V_T$ - Paw oedema at various time intervals and $V_o$ - Initial paw oedema

**Experimental groups and dosages**
Group 1: Control, injected with 1% carrageenan (25 µl).

Group 2: Administered with 10 mg/kg Diclofenac + 1% carrageenan injection (25 µl).

Group 3: Administered with *P. longifolia* extract (100 mg/kg body weight) + 1% carrageenan injection (25 µl).

Group 4: Administered with *P. longifolia* extract (250 mg/kg body weight) + 1% carrageenan injection (25 µl).

**Statistical analysis**
All in vitro experiments were performed in triplicates, and the results were expressed as Mean ± SD. In vivo experiments were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. A p-value of less than 0.05 ($P < 0.05$) was considered statistically significant. Statistical analysis was performed using GraphPadInstat 3.00 software (GraphPad Software Inc., San Diego, California, USA).

**Results and Discussion**
In the present study, the antioxidant and anti-inflammatory activities of the 70% methanolic extract of *Pajanelia longifolia* inflorescence were analyzed. The extract yield was determined to be 15.2%.

**Anti-oxidant activity of *Pajanelia longifolia* extract**
The antioxidant potential of *P. longifolia* extract was assessed using DPPH, ABTS, superoxide radical scavenging assays, and lipid peroxidation inhibition. The results were expressed in $IC_{50}$ values, representing the concentration required to neutralize 50% of the free radicals in the reaction mixture. The extract demonstrated varying levels of antioxidant activity across the different assays.

In the DPPH radical scavenging assay, the extract exhibited potent antioxidant activity, effectively neutralizing free radicals and inhibiting chain propagation reactions, with an $IC_{50}$ value of 5.5 µg/ml (Table 1). Similarly, in the ABTS radical scavenging assay, the extract showed an $IC_{50}$ value of 10.5µg/ml (Table 1). The ability of the extract to scavenge superoxide radicals generated by the photoreduction of riboflavin was recorded with an $IC_{50}$ value of 196.33 µg/ml (Table 1).The

lipid peroxidation inhibition assay demonstrated that the extract effectively reduced oxidative damage by inhibiting lipid peroxides induced by $Fe^{2+}$/ascorbate and $Fe^{3+}$/ ADD/ascorbate in rat liver homogenate. The $IC_{50}$ value for lipid peroxidation inhibition was determined to be 150 µg/ml (Table 1).

Oxidative stress is associated with a wide range of diseases, including neurodegenerative disorders, cardiovascular conditions, and the aging process (Ghasanfari et al., 2006). Identifying plant-based antioxidants remains a crucial area of research, as these natural compounds may help mitigate oxidative damage (Lin et al., 1995). While biological systems possess inherent antioxidant defence mechanisms, these systems are often insufficient to completely prevent oxidative stress-related damage (Henmani and Parihar, 1998). Therefore, plant-derived antioxidants provide a safe and effective means of protecting the body from oxidative damage. The results of this study indicate that *P. longifolia* extract exhibits significant antioxidant activity, with radical inhibition increasing in a concentration-dependent manner.

## Anti-inflammatory activity of *Pajanelia longifolia* extract

The anti-inflammatory potential of *P. longifolia* inflorescence extract was evaluated using Swiss albino mice, with Diclofenac (10 mg/kg body weight) serving as the standard reference drug. Inflammation was induced by subplantar injection of carrageenan into the hind paw, leading to swelling and erythema. The inflammatory response showed a time-dependent increase, reaching its peak at the third hour post-injection. The extract was administered at doses of 100 mg/kg and 250 mg/kg body weight. The treated groups exhibited a significant reduction in carrageenan -induced acute inflammation. The extract at 100 mg/kg reduced paw edema by 40.42%, while the higher dose of 250 mg/kg showed an even greater reduction of 53.19% (Table 2). These findings suggest that *P. longifolia* extract possesses notable anti-inflammatory properties, demonstrating a dose-dependent effect in mitigating acute inflammation.

Carrageenan-induced paw edema is a widely used in vivo model to evaluate the anti-edematous effects of natural compounds. This model is known to involve a biphasic in-flammatory response. The initial phase is primarily driven by the release of histamine and serotonin, followed by kinins, which increase vascular permeability and lead to fluid accumu-lation in the tissues, resulting in edema (Winter et al., 1962; Williams & Morley, 1973). This phase is further accompanied by leukocyte migration, with prostaglandins playing a key role in the later stages of inflammation (Castro *et al.*, 1968).

**Table 1.** Antioxidant activity of *P.longifolia* extract
*IC50 Value is the amount of extract needed to inhibit or

| Sl. No. | Antioxidant Assay | *$IC_{50}$ Value (µg/ml) |
|---|---|---|
| 1 | DPPH radical scavenging activity | 5.5 ± 1.2 |
| 2 | ABTS radical scavenging activity | 10.5 ± 2.5 |
| 3 | Superoxide radical scaveng-ing activity | 196.33 ± 5.56 |
| 4 | Lipid peroxidation inhibition activity | 150.00 ± 5.00 |

scavenge 50% of free radicals produced in the reaction mixture.

**Table 2.** Effect of *P.longifolia*extract on carrageenan induced paw edema in SwissAlbino Mice

| Groups | Initial Paw thickness (mm) | Paw thickness on 3rd hour (mm) | Increase in paw thickness (mm) | Percentage of inhibition (%) |
|---|---|---|---|---|
| Control | 0.236 ± 0.005 | 0.33 ± 0.007 | 0.094 | ---- |
| Diclofenac | 0.24 ± 0.007 | 0.28 ± 0.01** | 0.04 | 57.44 |
| P. longifolia100 mg/kg | 0.239 ± 0.01 | 0.295 ± 0.03* | 0.056 | 40.42 |
| P. longifolia250 mg/kg | 0.235 ± 0.011 | 0.279 ± 0.02** | 0.044 | 53.19 |

Values are mean ± SD, for 6 animals in each group. ** $p < 0.01$; *$p < 0.05$, when compared to control

## Conclusion

*Pajanelia longifolia* extract exhibits strong antioxidant and anti-inflammatory properties, effectively scavenging free radicals and reducing inflammation in a dose-dependent manner. These findings suggest its potential as a natural therapeutic agent for oxidative stress and inflammation-related disorders.

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