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UNVEILING THE THERAPEUTIC POTENTIAL OF *Plumeria pudica*: A COMPREHENSIVE PHARMACOLOGICAL AND PHARMACOGNOSTIC INVESTIGATION

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ABSTRACT
This study provides a comprehensive pharmacological and pharmacognostic evaluation of *Plumeria pudica*, focusing on its antibacterial, anti-inflammatory, and antioxidant properties, which may support its traditional medicinal applications. Antimicrobial activity was assessed through *in vitro* tests, which demonstrated inhibitory activity against microorganisms such as *Candida albicans, Escherichia coli, and Staphylococcus aureus*. The plant's antioxidant potential was demonstrated in scavenging assays using hydrogen peroxide and nitric oxide, with notable IC50 values suggesting a strong free radical neutralizing capacity. These findings suggest that *P. pudica* possesses potent pharmacological properties, meriting further research for potential therapeutic applications in managing infections, inflammation, and oxidative stress-related diseases. The antimicrobial inhibition by the test drug EEPP was 35±0.15, 27±0.13, 28±0.16, and 30±0.16 as compared to standard ciprofloxacin. The hydrogen and nitrogen scavenging activity of the I/C-50 values were 17.5 and 19.3 µg/ml for the ethanolic extract of *Plumeria pudica*, whereas they were 6.7 and 8.0 µg/ml for ascorbic acid. The percentage of inhibition of paw edema for histamine, serotonin, and formalin-induced edema was 64.44%, 55.29%, and 55%, respectively, which indicates a positive anti-inflammatory action of the test drug compared to the standard indomethacin, where the P value is less than 0.05.
Keywords: *Plumeria pudica*, Antioxidant Activity, Pharmacognostic Profile, Pharmacological Study.
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INTRODUCTION

Plant medicines from traditional wisdom have greatly benefited China, India, and Africa. These three countries have lots of contributions to plant-based medicine rather than contemporary medicine.[1] Traditional medicine in India is a rich and diverse field, encompassing various systems that have been practiced for centuries.[2] Ayurveda is a 5,000-year-old system emphasizing holistic health, using herbal remedies, yoga, and lifestyle modifications.[3] Herbal therapy offers a promising adjunctive approach to conventional treatments for inflammatory diseases. Further studies are needed to understand their mechanisms and unlock their full therapeutic potential.[4] Natural ingredients have less risk of chemical interactions and are more cost-effective than pharmaceuticals.[5] The present work attempts to prove the traditional use of *Plumeria pudica* (*P. Pudica*) using scientific data. Native to tropical America, *P. Pudica* (family: Apocynaceae) can be found from southern Mexico to northern South America. The species is most concentrated in India.[6] It is mainly used as a garden and park plant and is useful in temples and cemeteries in many parts of India.[7] Several medicinal activities have been reported on the *P. Pudica* plant. Commonly used to treat chronic diarrhea and dysentery is the protein component of water-soluble *P. Pudica* (LPPp), which has been investigated in several models, including diarrhea produced by castor oil, cholera toxin, or prostaglandin E2 (PGE2).[8] The Protein ingredient that is soluble in water. When P. Pudica was tested at various doses, it considerably reduced intestinal transit, intestinal fluid buildup, intestinal motility, and diarrheal stools. LPPp has stopped the changes in glutathione and malondialdehyde. Proteinases and proteinase inhibitors found in *Plumeria pudica* latex have been linked

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to LPPp's anti-inflammatory properties and have lowered the degree of rodent diarrhea brought on by castor oil and cholera toxin.[9] *P. pudica* leaf extracts contain bioactive substances that have broad-spectrum antibacterial properties.[10] The *P. Pudica* plant has been included in historical literature for its laxative, anti-allergic, and carminative properties. It can cure leprosy and ascites and is also known to have cytotoxic, ulcer protective, inflammation suppressive, anti-microbial, and diuretic abilities[11] and [12]*P. Pudica* is extracted from its leaves (ethanol and chloroform). The ethanolic extract leaf extract comprises Proteins, lipids, carbohydrates, alkaloids, flavonoids, glycosides, phenols/tannins, steroids, and terpenoids, which are likewise observed in the chloroform extract leaves. This indicates that the polar ethanolic extract has more efficacy than the non-polar chloroform extract at extracting a greater quantity of secondary metabolites from the plant sample [13]The study analyzed ethanol and chloroform extracts from *P. pudica*, revealing the presence of proteins, lipids, steroids, alkaloids, flavonoids, glycosides, terpenoids, and tannins/phenols.[14] According to the study, more secondary metabolites may be extracted from the plant material by the polar ethanolic extract than by the non-polar chloroform extract.

EXPERIMENTAL

Materials and Methods

Analytical quality chemicals and reagents, such as acetone, methanol, ethanol, hydrochloric acid, ferric chloride, Mayer's reagent, Fehling solutions A and B, chloroform, sulfuric acid, ammonia, glacial acetic acid, Sodium nitroprusside, hydrogen peroxide, ascorbic acid, DPPH, DMSO, Hydrogen phosphates of sodium and disodium, sulfanilamide, and BM reagent were among the compounds and reagents utilized Finar Scientific Chemicals, Mumbai, Maharashtra.

Collection, Taxonomic Identification, and Authentication of Plant Specimens

P. pudica plant material was collected from Araku Valley, Visakhapatnam, India, in January 2022 and authenticated through the Botanical Survey of India, Hyderabad (voucher specimen: BSI/DRC/2021-22/Tech/434). The plant's leaves and aerial components were thoroughly cleaned using water. Leaves of the plant were harvested, fragmented, and shade-dried. The dried material was subsequently pulverized using a grinder. These were prepared for cold maceration by using ethanol as a solvent. The mixture was intermittently shaken and stirred over 7 days to facilitate solvent penetration. After 7 days, the mixture was filtered through a Buchner funnel or filter paper to separate the soluble extract from the insoluble plant material. The filtered liquid extract was transferred at a temperature between 40-50°C through a rotary evaporator (rotovap) with a vacuum pressure of 100-200 mbar and a rotation speed of 100-150 rpm. The solvent (ethanol) was evaporated, leaving a concentrated, dried extract.

Preparation of Plant Extracts

A sufficient quantity of *P. pudica* leaves was shade-dried to create a fine powder. The best extractive value was the extraction solvent criterion.[15] The study involved extracting powdered plant material and ethanol through cold maceration. Here, 1.5 kg of powdered leaves of *P. pudica* and ethanol were taken, and this process continued for 48–72 hours. After that, their extractive values were contrasted. The extract was dried with a ZGSIT rotary evaporator (model R-1001-VN, China). A preliminary screening was carried out on several extracts to determine which phytoconstituents were present.

Pharmacognostic Study of Macroscopic and Microscopic Characteristics of Leaves

The leaves, flowers, and roots of the *P. pudica* were taken to evaluate their macroscopic characteristics. A Nikon D-5600 digital camera was used to capture macro images of various features. These macroscopic analyses followed Evans' (2009) pharmacognosy textbook instructions.[16] For microscopic inspection, the materials were dissolved in formaldehyde, acetic acid, and ethanol (FAA solution for 48 hours. Stem, rachis, petiole, and leaf sections were cut transversely, stained with safranin, and mounted for microscopic analysis. Microscopic analysis was performed using a trinocular microscope (Axiolab 5) with brightfield illumination, and images were captured with a Zeiss Axiocam 208 digital camera. Quantitative microscopy assessed leaf parameters, including vein islets, palisade ratio, epidermal cells, vein terminations, stomata, and stomatal index, using rectangular leaf segments.[17] The specimens were cleared in chloral hydrate solution and mounted on slides for examination under a trinocular microscope (Olympus BX43) with brightfield illumination, equipped with a digital camera and drawing tube. To find

any adulteration in the crude 152 medication samples, the powder's properties were examined. Plant material was prepared for microscopic examination by applying a glycerol drop (50%) to a glass slide, following pretreatment with saturated chloral hydrate. Images were captured using a Nikon ECLIPSE E200 digital camera attached to a trinocular microscope under brightfield illumination. Data was recorded, and physicochemical properties (extractive values and ash content) were evaluated per Indian Pharmacopoeia standards.[18]

Antimicrobial Activity Screening

The test microorganisms, including *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans*, were obtained from MTCC, IMTECH, Chandigarh, and maintained on nutrient agar slants at 4°C. The antimicrobial activity of *P.* pudica's ethanolic extract was assessed using tube dilution, disc diffusion, and cup plate assays. The extract was dissolved in DMSO to prepare concentrations of 50, 100, 150, and 200 mg/mL. Ciprofloxacin and fluconazole's antibacterial and antifungal activity was evaluated by comparing their inhibition zones to reference standards of 50 µg/mL to evaluate the antibacterial and antifungal activity. This study aims to establish the microbial potency of extracts on different tested microorganisms as well as determine how much test material is required.

***In-vitro* Antioxidant Activity**

Hydrogen peroxide and nitric oxide were used in a variety of scavenging assay techniques to evaluate *P. pudica*'s antioxidant capacity. Plant extract solutions were prepared at concentrations of 10-50 µg/mL using distilled water. To ensure accuracy and consistency, experiments were conducted in triplicate. Additionally, within the same concentration range, all studies were conducted again using normal ascorbic acid in place of the plant extract (test). A Shimadzu UV-1900i UV-Vis double-beam spectrophotometer was used to measure absorbances.

Percentage Activity involving scavenging=(Ab-At)/Ab×100

Thus, the percentage of scavenging is determined by utilizing the formula, Where: At symbolizes the combination of solutions' absorbance (test or standards). Ab stands for the absorbance of the blank. A comparison between the concentration (X-axis) and the scavenging activity percentage (Y-axis) was made. Additionally, by extrapolating the resulting graph, in order to attain a 50% scavenging effect, the plant extract's half inhibitory concentration (IC50) was calculated.

Hydrogen Peroxide Scavenging Assay

Gülçin İ's 2005 methodology[18] was used in an H2O2 scavenging experiment, where hydrogen peroxide was dissolved in a 7.4 pH phosphate buffer solution. Using a Shimadzu UV-Vis 1700, a hydrogen peroxide solution with a pH of 7.4 was made, and its concentration was determined spectrophotometrically at 230 nm. A 10 to 50 µg/ml hydrogen peroxide solution was combined with the plant extract in distilled water. A comparative analysis of Hydrogen peroxide absorption at 230 nm was measured. In a phosphate buffer control without hydrogen peroxide, after 10 minutes.

The Test for Scavenging Nitric Oxide Radicals

The Sreejayan *et al.* (1997) approach assessed the nitric oxide scavenging activity.[19] The test tubes were composed of 1 ml of a 25 mM sodium nitroprusside mixture supplemented using 4 milliliters of different test sample concentrations, which were incubated for three hours at 370˚C. The test tubes contained 1 ml of a 25 mM sodium nitroprusside solution and 4 ml of different test samples, which were incubated for three hours at 370˚C. The absorbance of the chromophore, created when nitrite was diazotized with sulphanilamide and coupled by ethylene diamine dihydrochloride naphthyl, was spectrophotometrically analyzed at 570 nm.

***In-vivo* Analgesics and Anti-Inflammatory Activities**

Animals Require Ethical Consideration

The Institutional Animal Ethics Committee in New Delhi, India, authorized the proposed study's standard protocols by CPCSEA guidelines. The Institutional Animal Ethics Committee, New Delhi, India (Approval Number: CUTM/IAEC-11/CPCSEA), authorized standard methods for the *in vivo* studies in the proposed study. The National Institute of Science Education and Research, Bhubaneswar, India,

provided male Swiss albino rats weighing 200-250 grams for the study. After seven days of acclimatization to the experimental setting, the animals were divided into groups and placed in cages with eight animals each. A typical pellet diet, unrestricted water availability, and upkeep. The environment was maintained at 25 ± 2°C with a 12-hour light-dark cycle, and animals were fasted for 12 hours before testing.

Treatment Groups and Protocol Details

Male mice were randomly assigned to six groups, with six animals in each group. Distilled water was administered orally to the I Group (control) and II (disease control) at a dose of 10 mL/kg. In addition, animals in groups IV, V, VI, and III were given. The ethanolic extract of *P. pudica* (100, 300, and Oral dose of 500 mg/kg, test drug *EEPP*) and indomethacin (Oral dose of 10 mg/kg with conventional medication), diclofenac (20mg/kg), respectively. After the experiment was over, the animals were put back in the animal house so they could be utilized again after a period of elimination.

Acute Oral Toxicity Study

The guidelines set by the OECD were followed in conducting the study on acute toxicity. The extract was given to the animals orally in a succession of dosages, and they were closely watched for any behavioral or autonomic alterations within 12 hours following the dose, as well as for mortality within 24 hours. The endpoint dose was established at 2000 mg/kg because there was no fatality within 24 hours. Three dose levels were selected for further evaluation: 100 mg/kg (low), 200-270 mg/kg (intermediate), and 400 mg/kg (high).

Test for Acetic Acid-Induced Writhing Using a Modified Protocol

With a few minor modifications, the techniques outlined by Minaiyan *et al.* (2024) were used to perform the acetic acid-induced writhing test.[20] The medications and solvent therapy were the same as in the previous study, and five groups of six mice were randomized at random. Mice were administered 1% acetic acid (10 mL/kg) intraperitoneally 30 minutes after treatment. Then, each animal was placed in a clear cage so that their writhing could be seen. One writhing refers to a contraction of the abdominal wall and a hind-limb stretch. Animals were observed for 5-30 minutes after acetic acid administration. The following formula was utilized to calculate the percentage of inhibition of writhing:

$$\text{Percentage of writhing inhibition}=[1-WT/WC]\times 100$$

Where WC and WT show, correspondingly, the average number of writings from the test group and the control group.

Assessment of Edema Induced by Serotonin and Histamine in the Rat Paw

This quantity of paw edema was measured using the Khan *et al.* (2022)[21] technique. The study involved treating six rats with different concentrations of *Plumeria pudica*, distilled water, and indomethacin. After a one-hour oral treatment, 0.1 ml of newly generated solutions containing 0.001 mg/mL of histamine or serotonin were subplantarly injected into rats' right hind feet to elicit paw edema. After receiving an injection of histamine or serotonin, the thickness of the paw was measured every 60 minutes for five hours:

Percentage of inhibition=100×(1-Vt/Vc). The mean edema in the control group is represented by Vc, while Vt represents the edema in the group undergoing extract or conventional treatment. Histamine (0.1% w/v) or serotonin (0.2% w/v) injection into the right hind paw induced edema in rats. Paw thickness was measured at 0, 1, 2, 3, and 4 hours after histamine or serotonin administration.

Nociceptive Response to Formalin Test

The antinociceptive efficacy was ascertained by applying the formalin test procedure.[22] Rats were administered oral doses of indomethacin or EEPP (100, 300, 500 mg/kg) 1 hour before formalin injection into the right hind paw. Paw-licking duration was measured during early (0-5 minutes) and late (15-40 minutes) phases. The licking period was monitored for 30 minutes after the formalin injection. There was a notable decrease in the overall amount of pain time in comparison to the control group, the test groups demonstrated antinociceptive efficacy. The majority of pain research is done with animal models in

experimental assays, which can help us understand the physiopathology of the disease and enhance the search for new medications. The formalin nociception test is one of the most effective ways to predict results when studying acute pain in rodents. The test monitors pain-related reactions to a subcutaneous injection of an inflammatory agent in the hind paw, specifically a 2.5% formalin solution. Following the injection, two distinct licking/biting episodes occur, followed by a quiescent interval. Significantly, the underlying mechanisms and duration of these stages differ. As a result, the initial acute phase (phase I), which is routinely recorded for five minutes after formalin injection, shows acute peripheral pain, most likely caused by nociceptors activating directly through TRPA1 channels. In contrast, phase II hypersensitivity is caused by central nociceptive sensitization and continuing inflammatory input. It begins five to fifteen minutes after the quiescent period and lasts about fifteen to thirty minutes on average. Here, we go into depth on how to do a valid and repeatable formalin test on mice. The formalin test is an experimental test used to determine the nocifensive behavior of mice. Thus, the mice's response (i.e., licking and flinching) to a subcutaneous injection of formalin, commonly in the plantar hind paw, is measured by injecting 50 µl of 4. The dorsal surface received an injection of 5% formalin of a rat's or a cat's forepaw. Since then, the formalin test has been widely used to assess nociception and inflammation-related reactions, with adjustments made based on particular study aims. Mice and rats are frequently used due to their natural grooming characteristics (forepaw licking). Rodents' rear paws are frequently injected with 2.5% formalin to generate nociceptive reflexes, such as paw "flinching" and licking, that last 45 to 90 minutes.

RESULTS AND DISCUSSION

Macroscopic Description

During the macroscopical observation, we observed that the shrub of *Plumeria pudica* is about tall with strong woody stems and branches. Leaves are basic. They can occur individually (one at a time) or alternately (one on each side of the stem), but they typically occur in pairs and very rarely in whorls. The core of the white blossoms is yellow. The fibrous roots are present. The flowers are white with a jasmine odor, a characteristic taste, and a length of about 3 cm with a cobra's hood in shape. The leaves are green in color with a characteristic odorand size of about Length 18cm with a Width of 2.3 cm, and the shape is Violaceous. The stems are green in color, cylindrical, woody at the bottom, and tender at the top, having a characteristic odor.

Microscopic Analysis and Description

Microscopic Evaluation of the Leaf

The leaf's transverse section displays a dorsiventral leaf, while both the upper and lower epidermis have stomata. The midrib region displays the vascular bundle. Loosely arranged spongy parenchyma can be seen. A patch of collenchyma is present (Fig.-1).

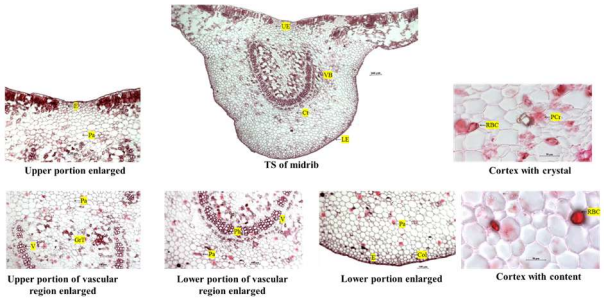

Fig.-1: Transverse Section (TS) of P. pudica Leaf

Microscopic Evaluation of the Stem

Histological analysis of the stem can be explained as the outer single-layered epidermis is present. Inner cortex: a thin layer of cortex is present, with 1-3 celled thick cambium, and thickening can be observed. Where a large pit is present (Fig.-2).

Powdered Microscopy

The Presence of crystals with the Presence of parenchymal cells is found in powder (Fig.-3).

Extraction and Phytoconstituent Screening

The solvent ethanol used in the extraction process, the % of yield was 19.5 %. A first screening procedure was performed on the aforementioned extracts to determine whether phytoconstituents were present or not. The screening outcomes of the steroids, triterpenoids, tannins, alkaloids, phenols, and flavonoids were discovered by the phytochemical examination of *EEPP*. (Brain and Turner, 1975; Harborne, 1992; Evans, 2009).[18]

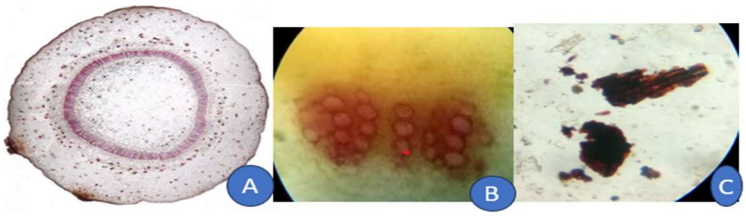

Fig.-2: (A) TS of *P. pudica* stem: Powdered Microscopy (B) Presence of Crystals, (C) Presence of Parenchymal Cells

Assessment of Antimicrobial Potency

Comparable to the standard, the extract demonstrated strong antibacterial efficacy against a range of infections. The ethanolic extract showed significant dose-dependent antibacterial activity ($p<0.001$). At 200 mg/mL, the extract showed inhibition zones of 35±0.17 mm against *B. subtilis*, 27±0.15 mm against *E. coli*, 28±0.15 mm against *B. pumilus*, and 30±0.17 mm against *P. aeruginosa* (Fig.-3 and Table-1). Additionally, *C. albicans* was susceptible to the extract at this concentration.

Table-1: shows that the Ethanolic Extract of *P. pudica* Exhibited Antimicrobial Activity, as evidenced by Growth Inhibition Zones (mm)

Name of the organism	Standard (Ciprofloxacin 50µg/ml)	T_1 (50mg of *EEPP*)	T_2 (100mg of *EEPP*)	T_3 (150mg of *EEPP*)	T_4 (200mg of *EEPP*)	Control
B. pumilus	50±0.24	25±0.18	30±0.12	33±0.43	35±0.17	-
B. subtilis	50±0.24	22±0.15	24±0.16	26±0.23	27±0.15	-
E. coli	50±0.23	24±0.19	25±0.17	26±0.18	28±0.15	-
P. aeruginosa	50±0.22	25±0.41	26±0.13	28±0.15	30±0.17	-

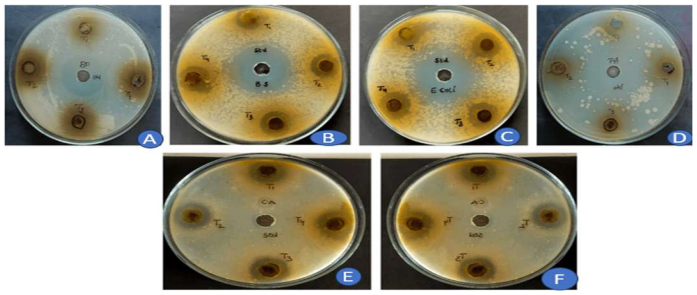

Fig.-3: Antimicrobial Activity of P. Pudica Ethanolic Extract Against Various Microorganisms

Evaluation of Antioxidant Activity in P. pudica Ethanolic Extract

Hydrogen Peroxide Scavenging Assay

Using standard ascorbic acid as a reference, the ethanolic extract of *P. pudica* demonstrated remarkable antioxidant potential in the in vitro testing procedures. In the H2O2 radical and nitric oxide (Fig.-4) scavenging experiments, the extract's IC50 values were found to be 6.7µg/ml for ascorbic acid and 19.13 µg/ml for *EEPP,* respectively, indicating the test drugs have notable scavenging activity of H2O2 free radicals.

Assay for Nitric Oxide Scavenging

P. pudica's ethanolic extract exhibited strong antioxidant properties, comparable to ascorbic acid. In Nitric oxide radical and nitric oxide (Fig.-5) scavenging experiments, the extract's IC50 values were found to be 17.5µg/ml of test drugs *EEPP* and 8.00 µg/ml of standard Ascorbic acid, respectively, indicating the test drugs have notable scavenging activity of NO free radicals.

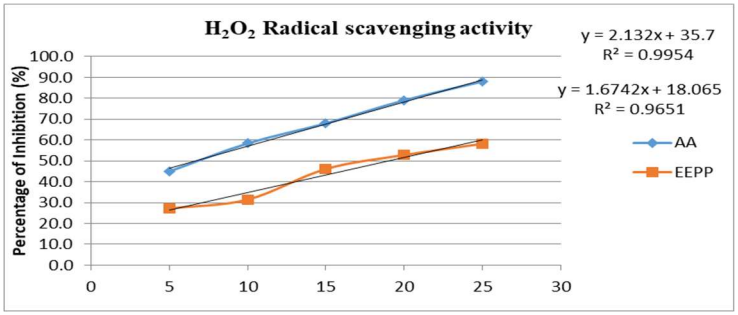

Fig.-4: Comparison of H_2O_2-Induced Antioxidant Activity between the Test Ethanolic Extract of *plumeria pudica* (*EEPP*) and the Standard Ascorbic Acid

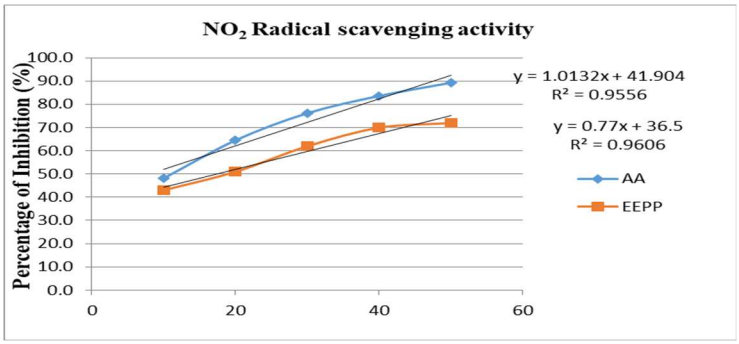

Fig.-5: Comparison of NO-Induced Antioxidant Activity Between the Test Ethanolic Extract of *plumeria pudica* (*EEPP*) and the Standard Ascorbic Acid

Anti-Nociceptive and Anti-Inflammatory Properties of *P. pudica* Ethanolic Extract

This study demonstrated that the ethanolic extract of P. pudica (*EEPP*) exhibits in vivo anti-inflammatory activity through various methods, including acetic acid-induced writhing test (Table-2), serotonin and histamine-induced rat paw edema (Tables-3 and 4), and formalin-induced nociception test (Table-5).

Table-2: Assessment of the Effects of *P. pudica* Ethanolic Extract and Indomethacin's Impact on Intraperitoneal Acetic Acid-Induced Nociception in Mice (The Data Indicated Significant Differences at $p < 0.05$ in a Column with Distinct Superscripts)

Treatment Groups	Average Writhes	
EEPP (100mg/kg)	60	16.67
EEPP (300mg/kg)	55	21.43
EEPP (500mg/kg)	49	30.00
Indomethacin (5mg/kg)	38	45.71
Control (Distilled water)	70	-

Table-3: Evaluation of the Anti-Edematous Effects of *P. pudica* Ethanolic Extract and Indomethacin's Effect on Serotonin-Induced Paw Edema in Rats was Statistically Significant ($P < 0.05$), with Distinct Differences Indicated by Varying Superscripts

Treatment Groups	30 minutes		60 minutes		90 minutes		120 minutes	
EEPP (100mg/kg)	145±5.21	27.50	137±5.19	28.65	122±5.20	31.46	114±5.21	32.94
EEPP (300mg/kg)	125±4.27	37.50	114±4.26	40.63	101±4.27	43.26	89±4.26	47.65
EEPP (500mg/kg)	111±4.61	44.50	100±4.60	47.92	89±4.61	50.00	76±4.60	55.29
Indomethacin (5mg/kg)	80±6.12	60.00	69±6.09	64.06	58±6.08	67.42	52±6.10	69.41
Control (Distilled water)	200	-	192	-	178	-	170	-

Table-4: Paw Volume (Cm) Changes Following Administration of *P. pudica* Ethanol Extract or Indomethacin in the Rat Model of Histamine-Induced Paw Edema (Significant variations ($p < 0.05$) of Superscripts are shown by Columns with Different Symbols)

Treatment Groups	30 minutes		60 minutes		90 minutes		120 minutes	
EEPP (100mg/kg)	152±4.69	49.33	144±4.91	50.34	135±4.86	51.79	125±4.69	53.70
EEPP (300mg/kg)	132±5.12	56.00	125±5.09	56.90	118±5.02	57.86	109±4.97	59.63
EEPP (500mg/kg)	114±5.21	62.00	109±4.95	62.41	103±4.78	63.21	96±4.66	64.44

Indomethacin (5mg/kg)	80±3.18	73.33	75±2.98	74.14	70±2.69	75.00	65±2.51	75.93
Control (Distilled water)	300±5.16	-	290	-	280	-	270	-

Table-5: Formalin-induced Nociception Test (Significant differences at $p < 0.05$ are indicated by Columns with Distinct Superscripts)

Group N=6	Nociceptive Response (number of licks and flinches)			
Treatment Groups	Initial phase (0-5 minutes)		Delayed phase (15-45 minutes)	
Distilled water(ml.) control	39±2.1	-	40±1.92	-
EEPP (100mg/kg)	32±2.12	18.00	22±2.91	45
EEPP (300mg/kg)	27±2.09	30.77	20±1.86	50
EEPP (500mg/kg)	25±2.13	35.90	18±1.86	55
Diclofenac (20mg/kg)	22±2.15	43.59	12±1.78	70

In comparison to different in vivo anti-inflammatory models, all treatment dosages exhibit dose-dependent inhibitory efficacy that is not statistically different from the standard. The in vivo study results showed the plant's dose-dependent anti-inflammatory and analgesic qualities. The acetic acid-induced writhing test showed a notable inhibition rate. At the same time, the *EEPP* in the standard group also possesses the exact percentage of inhibition. In comparison to the common drug indomethacin, the plant extract shows significant inhibition results when given at 300 and 500 mg/kg dosages in high concentrations. All oral treatment dosages provide an adequate inhibitory impact against various anti-inflammatory models.

To determine the pharmacognostic properties of the plant *P. pudica*, the current study examined the plant under both macroscopic and microscopic examination. The cup-plate test Tube Dilution Technique confirms the antimicrobial efficacy of the substance against both bacteria and fungi. It also clarifies *P. pudica's* ethanolic plant extract's anti-inflammatory properties, which may stem from this plant's antioxidant capacity. Triterpenoids, steroids, tannins, phenols, and flavonoids may have contributed to this outcome in the extract.[23] Pathogens are a wide range of organisms that can seriously injure their hosts, including bacteria, fungi, viruses, and protozoa. Pathogens have coexisted with human populations throughout history and contributed to several epidemics. One of the most catastrophic epidemics in human history was the Black Death, which swept through Europe in the fourteenth century, killing a staggering one-third of the population. The USA, Japan, and the former Soviet Union have all researched the use of pathogens as biological warfare agents.[24] White Frangipani, or the Apocynaceae family, includes *P. pudica,* a plant species endemic to Panama, Colombia, and Venezuela. Many people are aware of the aesthetic and spiritual aspects of this plant. It is also well-recognized for being used as a medicine to treat digestive tract infections and ailments.[25] Leprosy and ascites can be effectively treated with it due to its anti-inflammatory, antipyretic, antioxidant, anti-allergic, anti-ulcer, antibacterial, anti-tumor, cytotoxic, and diuretic effects.[26] *Plumeria pudica* has been shown to possess antibacterial[27], anticancer[28], and antioxidant[29] properties via its numerous species. According to Devprakash *et al.* (2012)[30], Steroids, alkaloids, glycosides, terpenoid-reducing sugars, saponins, tannins, carbonyls, and flavonoids have all been found in an extract from *Plumeria* species *Plumeria alba* flower extract has cytotoxic activity and may be a useful source of antioxidants, according to research by Rahman *et al.* (2014).[31] The flavone glycoside compounds found in *Plumeria rubra L*. exhibit antioxidant and hypolipidemic properties.[32] The study's in vivo anti-inflammatory findings indicated that assessment also demonstrated that treatment with *P. pudica* ethanolic extract satisfactorily inhibited inflammation. The plant extract's analgesic qualities were shown in the mice's acetic acid-induced writhing test model, indicating that it may have peripherally mediated analgesic effects. In the context of anti-inflammatory studies, the extract dramatically decreased the edema in the hind paws of the mice. The well-known analgesic and anti-inflammatory qualities of phytochemicals, including tannins, flavonoids, and steroids, account for *P. pudica*'s potent analgesic and anti-inflammatory characteristics.[33] According to these results, *P. pudica* and all of its phytoconstituents represent a potentially effective novel therapeutic alternative with powerful anti-inflammatory, antibacterial, and free radical-scavenging properties.[34] Additionally, the start of several diseases is predominantly caused by free radicals, also known as reactive oxygen species, which are primarily responsible for the onset of several diseases, including diabetes[35], cardiac issues[36], and several related

repercussions. Flavonoids, alkaloids, and glycosides were identified in the plant's phytochemical profile, which is recognized for its potent antioxidant properties. This suggests that *P. pudica* may have important properties like anti-oxidant, anti-inflammatory, and anti-diabetic properties, underscoring the need for more research.

CONCLUSION

The current study possesses scientific evidence for the traditional medicinal applications of *P. pudica*, revealing its broad spectrum because of the existence of phytochemicals such as steroids, tannins, and flavonoids that support antibacterial, anti-inflammatory, and antioxidant activity. Results from in vitro and in vivo investigations showed that P. pudica has strong antioxidant activity in addition to inhibiting harmful microbes, which may lessen oxidative cell damage. The anti-inflammatory and analgesic effects observed in animal models further emphasize its potential as a natural remedy for inflammation and pain relief. Given these findings, the plant *P. pudica* shows promise for the discovery of new therapeutic compounds. Future studies should focus on isolating and characterizing its active compounds and exploring their mechanisms of action for broader pharmaceutical applications.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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