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Phytochemical and pharmacological evaluation of prop roots of *Pandanus fascicularis* Lam

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ABSTRACT

Objective: To evaluate the anti-inflammatory and analgesic activities of the ethanol and aqueous extracts of prop roots of *Pandanus fascicularis* (*P. fascicularis*) Lam (pandanaceae). And provide experimental evidence for its traditional use such as rheumatoid arthritis and spasmodic. **Methods:** The anti-inflammatory activity was observed by carrageenan-induced edema of the hind paw of rats. Analgesic activities of prop roots of *P. fascicularis* were determined using acetic acid induced writhing model and tail clip method in mice and rat, respectively. The ethanol fraction was then subjected to chromatographic analysis and a compound has been isolated and characterized by IR, ^{1}H-NMR and mass spectroscopy. **Results:** Edema suppressant effect of ethanol extract was found to be 37.03% inhibition whereas aqueous extract was found to be 63.22% inhibition after 3 h which was nearly equivalent to that of 10 mg/kg of indomethacin (67.81%). Percentage inhibition of writhing compared to control were 63.15%, 54.38%, 14.90% for aspirin, aqueous extract and ethanolic extract, respectively. Both ethanol and aqueous extracts show significant activity against appropriate controls after 60 min of treatment on tail clip method. The structure of the isolated compound is may be characterized as Hepta deca-5-ene-1-ol by analysis it's IR, ^{1}H-NMR and mass spectroscopy data. **Conclusions:** The extracts of prop roots of *P. fascicularis* produce significant analgesic and anti-inflammatory activities, supporting the traditional application of this herb in treating various diseases associated with inflammation and pain.

1. Introduction

The importance of medicinal plants in traditional healthcare practices, providing clues to new areas of research and in biodiversity conservation is now well recognized. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health has been widely observed. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair[1,2]. Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases.

Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics or non-narcotics (NSAIDS) and present well known side and toxic effects[3,4]. On the contrary herbal medicines with good absorption, less toxicity and easy availability have been used since long[5]. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper and effective drugs[6]. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drug[7].

Pandanus fascicularis (*P. fascicularis*) Lam (syn. *P. odoratissimus*) commonly referred to as screw pines are palm-like evergreen trees or shrubs belong to the genus *Pandanus*, order Pandanales, class Liliopsida, and division Mangoliophyta. *Pandanus* comprises 500-600 species and is distributed mainly in subtropical and tropical regions. *P. fascicularis* is native to South Asia and has a significant

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presence particularly in mangrove swamps[8]. Individual plants can reach a height of 20 meters supported by aerial roots. Patients and medical practitioners believe the root and rhizome to be effective against diabetes. The decoction of the *P. odorus* root and rhizome has been traditionally used in treating diabetic patients without much specific evidence and also this plant used for rheumatism and spasmodic. The present study was deals with evaluation of anti inflammatory and analgesic activities of the ethanol and aqueous extracts of prop roots of *P. fascicularis* and isolation and structural elucidation of ethanol extract.

2. Materials and methods

2.1. Chemicals

Indomethacin was obtained from (Micro labs, Bangalore), pentazocin and aspirin were the kind gifts by APL research centre (Hyderabad, India). Acetic acid was purchased from Merck (Mumbai, India). Carrageenan was obtained from Sigma-Aldrich Pvt. Ltd (New Delhi, India). All other reagents were of analytical grade.

2.2. Preparation of extracts

Prop roots of *P. fascicularis* were dried in shade and powdered. The aqueous extract was prepared by cold maceration. The powder was soaked in equal amount of distilled water and stirred intermittently and then left overnight. The macerated pulp was then filtered through a coarse sieve and the filtrate was dried at reduced pressure in the rotor evaporator (Buchi Rotavapor R-114) and finally freeze dried. Ethanolic extract was prepared by extracted with ethanol (95% v/v) in a soxhlet apparatus. The extract was evaporated to dryness under vacuum and dried in vacuum desiccators.

2.3. Preliminary phytochemical screening

The presence of various phytochemical constituents in the extract was determined using standard screening tests[9]. Male albino rats (150-175 g) of Wistar strain and albino mice (25-30 g) were obtained from the Perundurai Medical College, Perundurai. Before and during the experiment rats were housed in polypropylene cages lined with husk in standard environmental conditions (temperature (25±2) ℃, relative humidity 55%±10% and 12: 12 light: dark cycle). The rats were fed on a standard pellet diet ad libium and had free access to water. The experiments were performed after approval of the protocol by the Institutional Animal Ethics committee (IAEC) and were carried out in accordance with the current guidelines for the care of laboratory animals.

2.4. Acute toxicity studies

The animals were randomly divided into three groups (n=6). A control group having carboxymethylcellulose 10 mL/kg by oral route was compared with single dose (5 g/kg; po.) of aqueous and ethanolic extracts of *P. fascicularis*. Access to food and water, toxic symptoms and the general behavior of mice were observed continuously for 1 h after the treatment, intermittently for 4 h, and thereafter over a period of 24 h. The mice were further observed for up to 14 d following treatment for any signs of toxicity and mortality[10].

2.5. Anti-inflammatory activity

Twenty four albino rat each weighing 150-175 g were used in the experiment. The animals were divided into four groups (Ⅰ-Ⅳ) each of six animals: group (Ⅰ) received of the negative control (caroxymethylcellulose), group (Ⅱ) received of positive control (indomethacin 10 mg/kg) and group Ⅲ and Ⅳ received the aqueous and ethanolic extracts at 250 mg/kg body weight, respectively. The anti-inflammatory activity was evaluated by the carrageenan-induced paw edema test in rats. The percentage of the anti-inflammatory effect of ethanol and aqueous extracts were calculated by using Formula 1.

$$\text{Percentage activity} = \frac{C-T}{C} \times 100 \qquad \text{(Formula 1)}$$

(T: Increase paw volume after ethanol and aqueous extract was administered; C: Increase paw volume of control group)

2.6. Analgesic activity

2.6.1. Writhing method

Twenty four albino mice each weighing 25-30 g were used in the experiment. The animals were divided into four groups (Ⅰ-Ⅳ) each of six animals: group (Ⅰ) received of the negative control (caroxymethyl cellulose), group (Ⅱ) received of positive control received pentazocine 5 mg/kg and group Ⅲ and Ⅳ received the aqueous and ethanolic extracts at 250 mg/kg body weight, respectively. Thirty minutes after treatment, the mice were given an intraperitoneal (ip) injection of 3% v/v acetic acid in a volume of 2 mL/kg to induce the characteristic writhing. The number of writhes occurring between 5 and 15 min after acetic acid injection was recorded. The response of the extracts treated animals were compared with that of control[11]. The percentage analgesic activity was calculated from Formula 2.

$$\text{Percentage protection} = \frac{Nc-Nt}{Nc} \times 100 \qquad \text{(Formula 2)}$$

(Nc is the average number of stretches of the control group; Nt is the average number of stretches of the test drug group)

2.6.2. Tail clip method

Twenty four albino rat each weighing 150-175 g were used in the experiment. The selected animals were divided into four groups (Ⅰ-Ⅳ) each of six groups: group (Ⅰ) received of the negative control (caroxymethyl cellulose), group (Ⅱ

) received of positive control received pentazocine 5 mg/kg and group Ⅲ and Ⅳ received the aqueous and ethanolic extracts at 250 mg/kg body weight, respectively. A metal artery clip was applied to the root of the mouse tail to induce pain. A sensitivity test was carried out and animals that did not attempt to dislodge the clip within 15 seconds were discarded. Analgesic activity was evaluated 0, 60, 120, 180 min after oral administration of the extracts and controls. A tail clip was applied and a positive analgesic response was indicated if there was no attempt to dislodge the clip within 5 s in any of the four consecutive trials after a time period of 2 min. The mean value was evaluated[12].

2.7. Fractionation, isolation, purification and characterization of compounds from the ethanol extract

Chromatographic techniques (thin layer chromatography, column chromatography) were used for the isolation of compounds from the fractions. The column chromatographic technique most commonly used for the separation of compounds into several fractions according to the affinity or solvating capacity of the compounds to the solvent used. The study involves in fractionation and isolation of compounds from pharmacologically active ethanol extract. The structure of the compounds were tried to establish by spectroscopic methods.

2.7.1. Study design

In order to carry out column chromatography, a solvent system was established by developing TLC technique. The silica gel (100-200 mesh size) slurry was made with the solvent system established earlier. The slurry was poured time to time into the column very carefully and the silica gel was allowed to settle down to from a uniform packing. Then the stop-cock of the column was opened and the excess of solvent over the column head was allowed to run. The dry crude ethanol extract was mixed with small amount of silica gel in a mortar to get a free flowing powder. The powdered sample was then applied carefully on the top of the prepared column and successfully eluted with solvent/solvent system. Elutes were collected in a number of conical flasks marked from fractions 1-100. Elutes were spotted successfully on TLC plate and the flasks having similar spots were combined together.

2.7.2. Analysis of fraction F2

The fraction F2 containing 20-36 conical flasks having similar spots on TLC plate were combined. Then the fractions were subjected to TLC by using petroleum ether: benzene (50: 50) as a solvent system. The expected bands were separated off and eluted with petroleum ether 100%, petroleum ether: benzene (50: 50), yield of 150 mg obtained. The compound was obtained as dull white sticky amorphous waxy solid. The fraction was characterized by spectroscopy techniques like Perkin-Elmer Vector 22 model FT-IR Spectrophotometer (Nujol),1H-NMR spectra were recorded in a BRUKER DPX-200 MHz using TMS as internal standard and GC-Mass spectrometer spectra was recorded in SHIMADZU QP 50000.

2.8. Statistical analysis

Data were presented as mean ± SEM. Statistical differences between the control and treated groups were tested by one-way ANOVA followed by Student's two-tailed unpaired *t*-test. The differences were considered to be significant at $P<0.05$.

3. Results

3.1. Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of carbohydrates, proteins, aminoacids, saponins, tannins, phenolic compounds, alkalodies and flavonoids.

3.2. Acute oral toxicity

The extracts of *P. fascicularis* were safe up to a dose of 5 g/kg (po.) body weight. Over the study duration of 14 d, there were no deaths recorded in the groups of mice given aqueous and ethanolic extracts of *P. fascicularis*. During the observation period, *P. fascicularis* administration did not induce any variations in the general appearance or toxic signs in the animals.

3.3. Effects of P. fascicularis on carrageenan-induced paw edema in rats

The effect of ethanol and aqueous extract of *P. fascicularis* on carrageenan induced edema in rats is shown in Table 1. Edema suppressant effect of ethanol extract was found to be 37.03% inhibition whereas aqueous extract was found to be 63.22% inhibition after 3h which was nearly equivalent to that of 10 mg/kg of indomethacin (67.81%). The edema suppressant effect was significant in the dose of 250 mg/kg of ethanol ($P<0.05$) and aqueous extracts ($P<0.01$). *P. fascicularis* showed inhibitory effect on carrageenan induced paw edema thus, exhibiting anti-inflammatory effect against acute inflammation.

3.4. Effects of P. fascicularis on acetic acid-induced writhing reflex in mice

The analgesic activity of *P. fascicularis* was evaluated by using the writhing test. Oral administration both aqueous ($P<0.01$) and ethanolic ($P<0.05$) extracts of *P. fascicularis* (250 mg/kg), 30 min before the acid injection, produced a significant inhibition of acetic acid-induced abdominal constrictions in mice (Table 1). Aspirin (300 mg/kg), a standard NSAID used as positive control, also produced significant inhibition of acetic acid-induced writhing response ($P<0.01$). Percentage inhibition of writhing

Table 1

Effect of ethanol and aqueous extracts of *P. fascicularis* Lam, on carrageenan induced rat paw edema and acetic acid induced writhing in mice.

Sl. no	Design of treatment	Dose (mg/kg)	Increase in paw edema at the end of 3 h	No. of writhing for 5 min
1	Control	–	0.87±0.03	57.00±2.24
2	Indomethacin	10	0.28±0.01**	–
3	Aspirin	300	–	21.00±1.12**
4	Ethanolic extract	250	0.60±0.01*	48.50±2.10*
5	Aqueous extract	250	0.32±0.02**	26.00±1.76**

*P<0.05, **P<0.01 compared to control (n=6); Students t-test.

Table 2

Effect of ethanol and aqueous extracts of *P. fascicularis* Lam, on rat by tail clip method.

Sl. no	Design of treatment	Dose (mg/kg)	Pain reaction			
			0 min	60 min	120 min	180 min
1	Control	–	3.00±0.10	3.20±0.16	3.50±0.18	3.50±0.13
2	Pentazocine	5	4.00±0.24	14.00±1.74*	9.50±1.42*	7.90±0.84*
3	Ethanolic extract	250	3.20±0.12	8.50±0.84*	8.00±0.74	6.40±1.10**
4	Aqueous extract	250	5.00±0.16	13.50±1.24*	9.00±1.17	7.40±0.62*

*P<0.05, **P<0.01 compared to control (n=6); Students t-test.

compared to control were 63.15%, 54.38%, 14.9% for aspirin, aqueous extract and ethanolic extract, respectively.

3.5. Effects of P. fascicularis on tail clip method in rat

The analgesic effect of ethanol and aqueous extracts of *P. fascicularis* was evidently effective by giving the dose of 250 mg/kg body weight and their activity exhibited was roughly equivalent to the standard drug of pentazocine 5 mg/kg (Table 2). Both ethanol and aqueous extracts show significant activity (P<0.01) against appropriate controls after 60 min of treatment. Between these two extracts, aqueous extracts shows maximum activity when compared to ethanol extract.

3.6. Isolation and identification of the active compound

The ethanol extract has been selected for isolation of the available active constituents, which has the polarity in between the acetone and aqueous. The purity of isolated compound was checked by TLC using different solvent system. R_f value of the isolated compound 0.62 (benzene: methanol = 90: 10). The isolated compound was characterized by its physical, chemical as well as spectrometric analysis. It is dull white sticky amorphous waxy crystalline compound soluble in pet ether, benzene and chloroform. The melting point is 58-60 ℃ (uncorrected). IR data of the isolated compound shows the intense peaks at the following frequency: 3 784 cm^{-1}, 3 340 cm^{-1}, 2 919 cm^{-1}, 2 852 cm^{-1}, 1 667 cm^{-1}, 1 621 cm^{-1}, 1 457cm^{-1}, 1 374 cm^{-1}, 1 052 cm^{-1}, 923 cm^{-1}, 852 cm^{-1} and 722 cm^{-1}. The peaks at 2 919 cm^{-1}, 282 cm^{-1} and 1 457 cm^{-1} show the paraffinic nature of the compound. Peak at 3 340 cm^{-1} indicates the presence of hydroxyl group and peaks at 1 667 cm^{-1} shows the presence of double bond. Band at 722 cm^{-1} shows its long chain nature.

^{1}H-NMR Spectrum of the isolated compound shows the δ values at the following ppm: 0. 87, 1.25, 2.01, 2.52, 3.37, 3.75 and 4.44. The spectrum shows δ value 2.01 for terminal methyl group, δ : 3.3 for six protons due to three methylene groups directly attached of the OH group resonated at δ : 2.25 as a broad singlet. All other methylene protons resonated at δ : 1.25 and it also indicates the presence of hydroxyl protons. This spectrum also indicates the presence of long chain aliphatic nature of the compound.

Mass spectrum of the compound shows the following fragmentation pattern: 256 (M^+), 236, 201, 183, 167, 153, 137, 153, 97, 83, 69, 61 and 43. The mass spectrum showed it to be a straight chain compound (Base peak at m/z 43). The values indicate the presence of methylene groups and long chain nature of the compound. Formation of fragments at m/z 83 and 167 confirm the position of double bond at C-5.

On the basis of the above data the isolated compound may be characterized as Hepta deca-5-ene-1-ol. Structure: $H_3C-(CH_2)_{10}CH=CH(CH_2)_3CH_2OH$. Molecular formula: $C_{17}H_{34}O$. Molecular weight: 254.

4. Discussion

The anti-inflammatory and analgesic effects of the ethanol and aqueous extracts of *P. fascicularis* were investigated in this study. Inflammation has different phases the first phase is caused by an increase in vascular permeability, the second one by infiltrate of leucocytes and the third one by granuloma formation. We determined anti-inflammatory activity by using inhibition of carrageenan- induced inflammation which is one of the most feasible methods to screen anti-inflammatory agents. The development of carrageenan-induced edema is bi-phasic the first phase is attributed to the release of histamine, serotonin and kinins and the second phase is related to the release of prostaglandins and bradykinins[13].

We observed that aqueous extract of *P. fascicularis* at the given dose possess significant inhibition against carrageenan-induced paw edema in rats whereas ethanol

extract exhibited feeble effect in the treatment of acute inflammation and pain. This response tendency of the extract in carrageenan-induced paw edema revealed good peripheral anti-inflammatory properties of the extract. The anti inflammatory effect of aqueous extract may be due to the presence of flavonoids. Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase and reported to produce anti-inflammatory effects. Since, prostaglandins are also involved in the pain perception, inhibition of their synthesis might be the possible reason for the analgesic activity of the aqueous extract.

The peripheral analgesic effect was tested by acetic acid induced writhing test in mice. The reference drug used was aspirin, as it offers relief from inflammatory pain, by inhibiting the formation of pain mediators in the peripheral tissues, where prostaglandins and bradykinins are said to play a significant role in the pain process. Acetic acid-induced writhing is a standard test for pain sensitivity to opiates as well as to non-opiate analgesic. The associated nociceptive response is believed to involve the release of endogenous substance such as bradykinin and prostanoids, among others, which stimulate the nociceptive endings. Ethanol and aqueous extracts of *P. fascicularis* have shown good analgesic activity in this model and have produced a significant decrease in the writhing counts.

The tail-clip tests are useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level[14]. The significant increase in pain threshold produced by *P. fascicularis* in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems. The analgesic effect produced by the extract may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leucotrienes, and other endogenous substances that are key players in inflammation and pain.

The compound was isolated from the fraction F2 using column chromatography. The structure of the compound may be considered from IR, ^{1}H-NMR and mass spectroscopy data as Hepta deca -5-ene-1-ol.

Therefore, it is concluded that *P. fascicularis* extracts are capable of inhibiting inflammatory reactions as well as pain. Both ethanol and aqueous extracts showed good analgesic and anti-inflammatory effect. The results provided experimental evidence for its traditional use in treating various diseases associated with inflammation and pain. The mechanism involved is not determined and elucidated in the present study and is therefore the likely focus of subsequent research.

Conflict of interest statement

We declare that we have no conflict of interest.

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