

## Biomedical Application of Beach Morning Glory *Ipomoea pes-caprae*

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**Abstract:** *Ipomoea pes-caprae* is a medicinal plant used in many countries for the treatment of several ailments including inflammatory and algesic processes. The present study describes the antimicrobial, hemolytic, analgesic and anti-inflammatory effects of the methanolic extract obtained from flower parts of this plant. The results indicated that the methanolic extract exhibited considerable antinociceptive activity against two classical models of pain in mice. Methanolic extract presented a calculated the value of 5 and 2 mg kg<sup>-1</sup> of body weight, i.p. against tail flick test and also inhibited both phases of pain (tail flick and hot plate). The maximum for paw licking Analgesic Response (AR) was recorded 4 in crude extract of *Ipomoea pes-caprae* flower extract after 45 min of administration and minimum of 1 AR showed 30 and 45 min after administration whereas minimum of 3 AR was recorded in 60 min, respectively. The methanolic extracts exhibited the considerable antimicrobial activity against human bacterial and fungal pathogens. Preliminary phytochemical analysis suggested the presence of steroids, terpenoids, alkaloids and flavonoids. These findings support, at least in part, the popular use of *I. pes-caprae* to treat dolorous processes.

**Key words:** Inflammatory, antimicrobial, hemolytic, antinociceptive, phytochemical, inflammatory

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### INTRODUCTION

Medicinal plants discovered by traditional societies are proving to be an important source of potentially therapeutic drugs (Cox and Balick, 1994). This approach is actually one of several methods that can be applied in selecting plants for pharmacological studies. Regulation of exploitation and exportation is therefore essentials together with international coordination for their conservation to ensure their availability for the future. Nowadays herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimal side effects in clinical experience and relatively low cost (Sheela and Kannan, 2003).

The plants belonging to the genus *Ipomoea* (Convolvulace) consists of more 200 species that widely distributed in tropical and subtropical countries. Some of them are frequently used in folk medicine for the treatment of several diseases (Souza *et al.*, 2000). *Ipomoea pes-caprae* (Convolvulaceae) known as salsa-da-praia, pe-de-cabra or batateira-da-praia in Brazil is used in folk medicine against inflammation and gastrointestinal disorder and as an analgesic agent (Souza *et al.*, 2000; Krogh *et al.*, 1996). *Ipomoea pes-caprae* is a traditional

medicinal plant used in the treatment of headache and various types of inflammation including jellyfish sting dermatitis (Pongprayoon *et al.*, 1991; Bandaranayake, 1998, 2002).

### MATERIALS AND METHODS

**Plant materials:** *Ipomoea pes-caprae* plant leaf was collected from Parangipettai coastal area near Annankovil landing centre during January, 2010. The leaf extracts of *Ipomoea pes-caprae* was obtained through solvent methanol.

**Preparation of extracts:** A 100 g amount of the pulverised dried leaf was continuously extracted with methanol solvent for 24 h immersion. After 24 h the sample was collected and concentrated by using rotary evaporator (Lark VC 100A). The extract was then poured into a weighed flask and further dried in a desiccating chamber to a constant weight. The dried extracts were stored 4°C for further analysis.

**Protein estimation:** Protein concentration was determined by the method of Bradford (1976). The standard protein sample was prepared at 2 mg mL<sup>-1</sup> of BSA. The

assay relies on the binding of the dye Coomassie Blue G250 to the protein molecule measured calorimetrically at 595 nm. Dilutions of protein standards with concentrations of 20, 40, 60, 80 and 100 µg/100 µL were assayed.

**Hemolytic assay:** Crude methanolic extracts of *Ipomoea pes-caprae* was assayed on human, chicken and goat blood followed by the method of Prasad and Venkateshwaran (1997). Samples of chicken and goat blood were obtained from the nearby slaughter house in Parangipettai while the human blood groups were obtained from local hospital using 2.7% Ethylene Diamine Tetra acetic Acid (EDTA) solution as an anticoagulant at 5% of the blood volume and brought to the laboratory. The blood was centrifuged thrice at 5,000 rpm for 5 min. About 1% erythrocyte suspension was prepared for hemolysis study.

**Antimicrobial assay:** Both gram positive and gram negative bacterial (human pathogens) strains were taken for the test. The bacterial strains used for the investigation are shown in Table 1. These organisms were collected from the Rajah Muthaiya Medical College, Annamalai University, Tamil Nadu, India.

**Preparation of the seeded test plates:** Each of the test organisms was transferred to the test tube containing 16 mL previously autoclaved media with the help of the sterilized inoculating loop at 45°C under laminar air flow unit. The test tubes were shaken by rotation to get a uniform suspension of organism. The bacterial suspensions were immediately transferred to the sterile petri-dishes aseptically. The petri-dishes were rotated several times to assure homogeneous distribution of the test organisms. The medium was poured into petri dishes in such a way as to give a uniform layer of depth of approximately 4 mm. After the medium become cooled to room temperature, it was stored in a refrigerator (4°C).

**Preparation of the discs:** The agar disc diffusion techniques involved placing sterile paper discs (Whitman No. 1 filter paper) of 5 mm diameter impregnated with different crude extracts and dried in a hot air oven at 60°C on agar plates seeded with the test organism. Three types

of discs were used for antimicrobial screening; sample discs, standard discs and blank discs. The sample disc was prepared by applying sample solution of the desired concentration on the sterile filter paper discs (6 mm in diameter) with the help of a micropipette in an aseptic condition. Similarly blank discs and other discs were prepared to serve as negative control and test sample, respectively. In this investigation aqueous was used as blank. These discs were left for few minutes in aseptic condition under UV light for complete sterilisation.

**Antibacterial activity:** Sample soaked discs, standard disc and negative control disc were placed gently on the solidified agar plates, freshly seeded with the test organisms with the help of a sterile forceps to assure complete contact with medium surface. The special arrangement of the disc was such that the discs were not closer than 15 mm to the edge of the plate and far enough apart to prevent overlapping the zones of inhibition. The plates were then inverted and kept in refrigeration for about 24 h at 4°C. This was sufficient time for the material to diffuse into a considerable area of the medium. The normal saline and solvents were used as positive and negative controls, respectively. The whole set-up was incubated as 37°C for 18 h after (which diameter of zones of inhibition were measured).

**Antifungal activity:** Antifungal activity was determined against fungi followed by the method (Bauer *et al.*, 1966). The stock culture was maintained in Glucose Peptone Yeast and Sucrose (GPYS) medium. Fungal inoculum (0.2 mL) of 48 h old culture was distributed uniformly on to the surface of agar plates containing GPYS medium with the help of a sterile cotton swab. Culture medium was prepared by adding dextrose (20 g L<sup>-1</sup>), peptone (10 g L<sup>-1</sup>) and agar (25 g L<sup>-1</sup>) in distilled water and was sterilised in an autoclave at a pressure of 151 b in<sup>-2</sup> and a temperature of 120°C. At the time of inoculation, the disc impregnated with plant extract (100 µg g<sup>-1</sup> disc of 10 mm diameter) was placed. The inhibition zone was measured after incubation period of 48 h.

**Analgesic activity**

**Tail-flick method:** Analgesic activity was measured according to the method described by D'Amour and Smith (1941) using tail flick analgesia meter (INCO, India) with a variable 150 W, 25 V lamp as the heat source. During the testing period the mice were restrained in a plastic tube to which they had been previously adapted twice (10 min) a day for 3 days. The tail flick latency was recorded as the time onset of stimulation to the withdrawal of the tail from a light beam. The beam of the light was focused on some spot at about 6 cm from the tip of the tail of each animal. The crude extract was dissolved

Table 1: Bacterial strains

Bacterial pathogens	Fungal pathogens
<i>E. coli</i>	<i>Aspergillus niger</i>
<i>Salmonella typhi</i>	<i>Candida albicans</i>
<i>Vibrio cholera</i>	<i>Mucor</i> sp.
<i>Salmonella paratyphi</i>	<i>Penicillium</i> sp.
<i>Staphylococcus aureus</i>	<i>Rhizopus</i> sp.
<i>Klebsiella pneumonia</i>	<i>Aspergillus flavus</i>

in DMSO at the dose of 5 mg kg<sup>-1</sup> of body weight and then injected i.p. to mice. Mice without administration of any toxin or known painkiller were used as control while those injected i.p. with paracetamol (Crocin® at 0.25 mL/170±2 g) will serve as reference standards. The mice were tested 30 min of after injection. Analgesic activity was expressed as a ratio between the difference in reaction time of envenomated mice and control since analgesic potential was proportional to the difference in tail flick latency between the toxin and control.

**Hotplate method using mice:** Hot plate method was described by Eddy and Leimbach (1953), Tornos *et al.* (1999). The animals were individually placed on a hotplate and maintained for 55°C. Reaction of animals such as paw licking or jump response was taken as an end point. Extracts were injected at dose level of 5 mg kg<sup>-1</sup> of body weight was injected i.p. to mice (20±2 g).

## RESULTS AND DISCUSSION

The amount of protein content in *Ipomoea pes-caprae* leaf extract was showed 16.64 µg g<sup>-1</sup>. The hemolytic assay, leaf crude extract of *Ipomoea pes-caprae* was pronounced hemolytic activity on chicken and goat erythrocytes showed in Fig. 1 and 2. The chicken erythrocytes, the haemolytic activity were 156 HU and goat erythrocytes, the haemolytic activity were 312 HU. The maximum haemolytic unit was recorded in goat erythrocytes and minimum was noticed in chicken erythrocytes.

**Antibacterial activity:** Antibacterial activity of *Ipomoea pes-caprae* was measured as the radius of the zone of inhibition around the disc (Fig. 3). In methanolic extract the maximum inhibition zone formation showed 20 mm against *E. coli* and minimum inhibition zone 4 mm was found in *S. paratyphi*. In acetone the maximum inhibitor zone was found in *E. coli* and *V. cholera* (15 mm) and minimum zone of inhibition was found in both *Salmonella typhi* and *Staphylococcus aureus*. With respect to chloroform the maximum zone of inhibition was found in *Staphylococcus aureus* (7 mm) and minimum 2 mm showed *E. coli*. As regards the n-butanol extracts the maximum zone was shown in *E. coli* (14 mm) and minimum zone was shown in *S. paratyphi* (8 mm).

**Antifungal activity:** Antifungal activity of *Ipomoea pes-caprae* was measured as the radius of the zone of inhibition around the disc (Fig. 4). In methanolic extract the maximum inhibition zone formation showed 3 mm against *C. albicans* and minimum inhibition zone 1 mm was found in *Rhizopis* sp. and *A. flavus*. In acetone the maximum inhibitor zone was found in *A. flavus* (6 mm) and minimum zone of inhibition was found in *Mucor* sp.

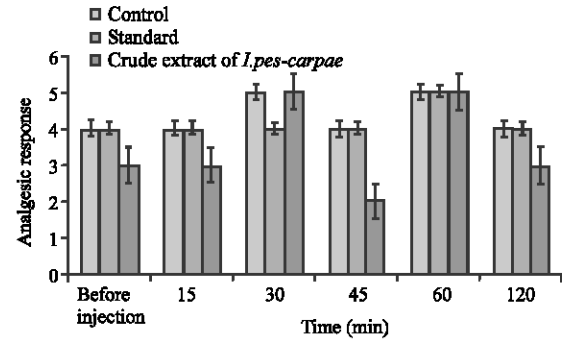


Fig. 1: Analgesic response in terms of tail flick method of *Ipomoea pes-caprae* extract at specific time intervals

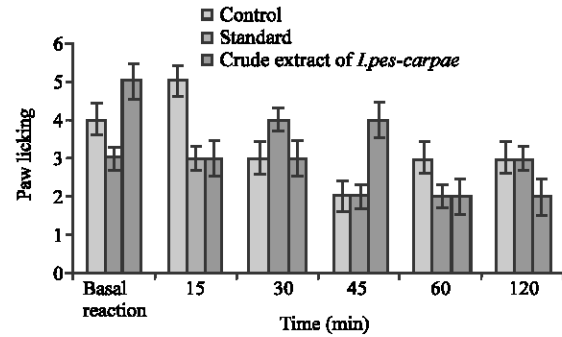


Fig. 2: Analgesic response (hot plate method) in terms of paw licking of the *Ipomoea pes-caprae* extract at specific time intervals

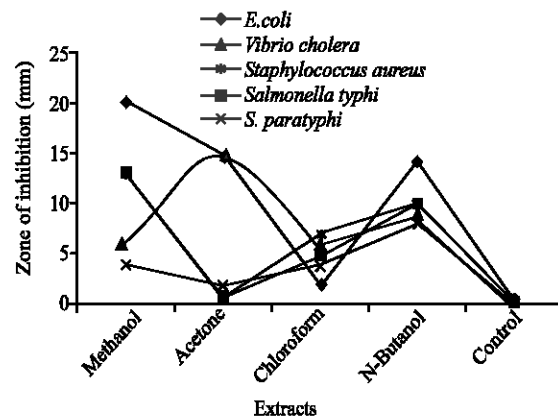


Fig. 3: Antibacterial activity of *Ipomoea pes-caprae* against human and shrimp pathogens

(1 mm). With respect to chloroform the maximum zone of inhibition was found in *Staphylococcus aureus* (7 mm) and minimum 2 mm showed *E. coli*. As regards the n-butanol extracts the maximum zone was shown in *Mucor* sp. (22 mm) and minimum zone was shown in *Rhizopis* sp. (8 mm).

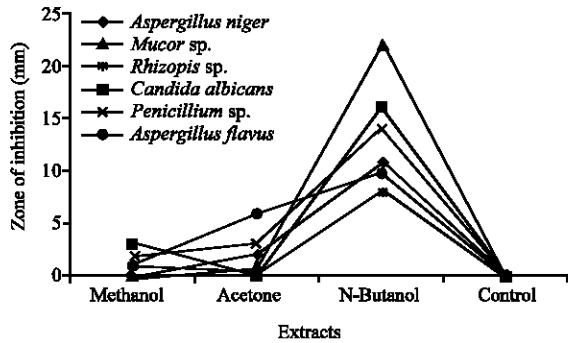


Fig. 4: Antifungal activity of *Ipomoea pes-caprae* against human pathogens

**Analgesic activity:** Analgesic activity of *Ipomoea pes-caprae* leaf extracts on tail flick and hot plate method were analysed by using mice. After administration of methanolic crude extracts i.p. at a dose level of 5 and 2 mg kg<sup>-1</sup> of body weight (a dose equal to the i.p. LD<sub>50</sub>) did not cause any mortality in mice.

The reaction time was noted at 15, 30, 45, 60 and 120 min after the methanolic crude extracts administration. Analgesic activity was expressed as a ratio between the difference in reaction time of envenomated rodents and control since analgesic potential is proportional to the difference in tail flick latency between the extracts and control (Fig. 1).

**Hot plate method:** In hot plate method, the analgesic ratio was recorded based on paw licking and jumping response of mice after administration. The maximum for paw licking Analgesic Response (AR) was recorded 4 in crude extract of *Ipomoea pes-caprae* flower extract after 45 min of administration and minimum of 1 AR showed in methanolic crude extracts at 60 min administration showed (Fig. 2). In jumping response the maximum of 6 AR jumping response showed 30 and 45 min after administration whereas minimum of 3 AR was recorded in 60 min was shown (Fig. 5).

Accumulating pharmacological data indicate that plants are a substantial source of active compounds capable of exerting potential therapeutic activity in the organism. In recent years, several reports on the antibacterial activity of marine plants have been published (Souza *et al.*, 2000).

Only a few reports are available for antibacterial activity of coastal plants (Padmakumar and Ayyakannu, 1985). In the present study, a broad screening of the coastal plant *Ipomoea pes-caprae* for antibacterial activity, antifungal activity and biomedical properties have been attempted. Crude extracts of *Ipomoea pes-caprae* was tested against five species of bacteria viz., *Staphylococcus aureus*, *S. paratyphi*, *E. coli*, *Salmonella typhi* and *Vibrio cholera*. The *Ipomoea pes-caprae*

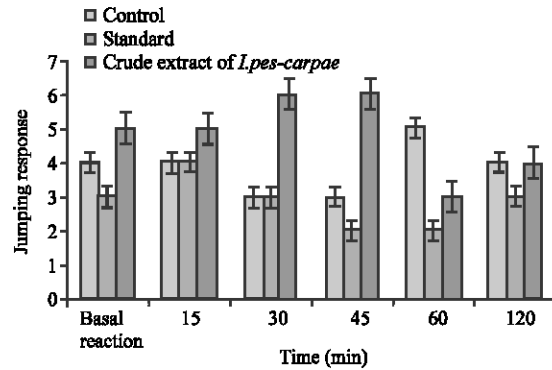


Fig. 5: Analgesic response (hot plate method) in terms of jump response of the *Ipomoea pes-caprae* extract at specific time intervals

against the pain associated to the local reaction after contact with venomous jellyfishes. The methanolic extract revealed potent and significant antinociceptive properties against acetic acid-induced abdominal constrictions and against the both phases (neurogenic and inflammatory) of formalin induced pain being equipotent to the some analgesic drugs (Souza *et al.*, 2000). Meckes *et al.* (1995) tested 135 plant species of which 6 showed highest activities against gram positive *Staphylococcus aureus*. Hussain and Tobji (1997) reported that none of the 10 plant extracts tested by them was active against gram negative bacteria. Recently Srinivasan and Lakshmanaperumalsamy (1999) assayed the crude extracts of 50 medicinal plants for antibacterial activity and they observed 54% of the extracts to be active. In the present study has revealed that the plant showing pronounced antibacterial activity against all human pathogens.

Several researchers have made a similar antifungal screening. A series of 100 Rwandese (Belgium) medicinal plants used by traditional healers to treat infections were screened for antifungal activity and 7% of the extracts ere found effective against *Candida albicans* (Vlietinck *et al.*, 1995). A test was made with a desert plant, *Phlomis floccuosa* and the chloroform extract was found to be the most potent one against fungi of the other extracts in water, ethyl acetate, ethyl alcohol (Mohammed, 1998). In the present investigation, antifungal activity of coastal plants showed the maximum activity in all extracts.

## CONCLUSION

*Ipomoea pes-caprae* exhibit anti-inflammatory activities and is a traditional medicinal plant used in Thailand for the treatment of various types of inflammation including jellyfish sting and dermatitis (Bandaranayake, 1998). The anti-inflammatory activity of an extract form the leaves of *Ipomoea pes-caprae* used as a traditional remedy for different types of inflammation

was investigated using experimental models of acute inflammation and also studied that *Ipomoea pes-caprae* has a significant anti-inflammatory activity, probably due to a reduction of prostaglandin and leukotriene formation. Pongprayoon *et al.* (1991) investigated that the *Ipomoea pes-caprae* as an anti-inflammatory agent in traditional medicine. Paula *et al.* (2003) extensively studied that the *I. imperati* extracts have local and systemic anti-inflammatory actions in mice and rats and showed the pharmacological mechanism involved in the anti-inflammatory effect may be related to the inhibition of PLA<sub>2</sub> and COX-2.

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