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Evaluation of Anti-microbial and anti- inflammatory activity of methanol leaf extract of *Ipomoea aquatica* Forsk

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ABSTRACT

Pain can be acute or chronic may be conceptualized as either nociceptive or neuropathic in origin. A natural supplement with a blend of ingredients can not only provide pain relief, but without stomach bleeding or liver damage that is associated with over the counter meds like non steroidal anti-inflammatory drugs NSAIDS (Celebrex, alleve, ibuprofen). This study is aimed to assess the anti-microbial and anti-inflammatory effects of *Ipomoea aquatica Forsk* (IAF) .The antimicrobial property of the IAF was studied against Gram-positive and Gram-negative microorganisms using the agar disc diffusion method, in which methanolic leaf extract of IAF has shown bigger zone of inhibition (15 – 25 mm) than aqueous leaf extract (IAF) (08–19 mm). The anti-inflammatory effect was evaluated in carrageenin-induced rat paw edema model. The methanolic and aqueous extracts of IAF was administered orally (p.o.) at 200mg/kg. Pretreatment with a single dose of IAF produced significant dose-dependent anti-inflammatory effects on carrageenin-induced rat hind paw edema. Crude methanolic and aqueous extract (200 mg/kg) and indomethacin (5 mg/kg) inhibited significantly ($p < 0.05$) the formation of the carrageenin-induced rat paw edema, measured in third hour of experiment (peak of edema formation). These results demonstrate that IAF possesses anti-microbial and anti-inflammatory effects and has no obvious acute toxicity, which advanced our understanding of the folk use of IAF in treating various inflammatory disorders.

Keywords: *Ipomoea aquatica* Forsk, Antimicrobial activity; Anti-inflammatory activity; non steroidal anti-inflammatory drugs; Methanolic leaf extract; aqueous leaf extract.

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INTRODUCTION

Inflammation is typically thought of as a swelling, painful or otherwise uncomfortable situation – perhaps in your joints, sinus or intestine. But for most people, inflammation occurs without any symptoms. Inflammation is defined classically as a protective reaction by the body, in response to some physical or chemical injury; acute inflammatory response begins immediately after cellular injury [1]. The number of chemical compounds, called phytochemicals, found within the plant kingdom is truly vast and their range of activity is equally as great. Some of the phytochemicals found in certain herbs and plants are reported to demonstrate pain and inflammation-reducing properties [2].

Ipomoea aquatica Forsk (IAF) belongs to the family Convolvulaceae grows wild and is cultivated throughout Southeast Asia and is a widely consumed vegetable in the region. Many of the waters where IAF grows serve as recipients for domestic and other types of waste water. Water spinach is also supposed to possess an insulin-like activity according to indigenous medicine in Sri Lanka [3]. Only a very few scientific studies have been conducted on its medicinal aspects. These include the inhibition of effects on liver diseases[4], constipation[5].IA is considered a tonic the species contains several vitamins, including A, B, C, E, and "U" (S-methyl-methionine), and is used to treat gastric and intestinal disorders[6–7].The species also contains aliphatic pyrrolidine amides, carotenoids, hentriacontane, β-sitosterol and its glycosides, prostaglandin, leukotrine, N-trans- and N-cis feruloyltyramines [8-11]. It is runner type plant with numerous small flowers[12-13] .The current study was undertaken to evaluate the anti-microbial and anti- inflammatory activity of methanolic extract IAF by, till now no pharmacological evaluation has been done on IAF especially in leaf for its anti- inflammatory activity. This prompted us to pursue the activity and was examined for their efficacy and for determination of their possible mechanism of action.

MATERIALS AND METHODS

Plant material

The fresh leaf's of IAF were collected from (Peranakkavur,Ramakrishna mudaliar street, Changlepet, Tamilnadu,India) western Ghats of South India during June 2009 . The plant was identified and authenticated by Dr.sasikala Ethirajulu,Captain Sreenivasan research foundation, Chennai, Tamilnadu ,India.The specimen voucher was deposited in the Department of Pharmacology and toxicology, C.L.Baid Metha College of Pharmacy, Chennai,Tamil nadu,India.

Preparation of the methanolic extract of IAF

The fresh leafs of IAF (2 kg) were collected and washed well to remove any adhering foreign particles and soil materials. The washed leafs were dried under shade. After drying, it was coarsely powdered. Air dried Powdered drug was divided in to two equal parts (1 kg each); one part was macerated with methanol (90 % v/v) in glass percolator and allowed to stand at room temperature for about 24 hours. Then the extract obtained was filtered, concentrated by rotary vacuum pump to get the solid mass. The percentage yield was 19.6 %, second part of powdered leaf was cold macerated with distilled water then the same procedure was repeated as mentioned above. The percentage yield was 10.5%.

Phytochemical screening

The freshly prepared methanol and aqueous leaf extract of *Ipomoea aquatica Forsk* (MIAF) and (AIAF) was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Mayer's, Hager's, and Dragendorffs reagent; Flavonoids with the use of sodium acetate, ferric chloride, amyl alcohol; Phenolic compounds and tannins with lead acetate and gelatin; carbohydrate with Molish's,Fehling's and Benedict's reagent; proteins and amino acids

with Millon's,Biuret, and xanthoprotein test.Saponins was tested using hemolysis method; Gum was tested using Molishs reagent and Ruthenium red;Coumarin by 10% sodium hydroxide and Quinones by Concentrated Sulphuric acid. These were identified by characteristic color changes using standard procedures [14].

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The screening results were as follows: Alkaloids + ve; Carbohydrates + ve; Proteins and amino acids +ve; Steroids - ve; Sterols + ve; Phenols + ve; Flavonoids + ve; Gums and mucilage + ve; Glycosides + ve; Saponins – ve; Terpenes + ve and Tannins + ve
Where + ve and – ve indicates the presence and absence of compounds.

Microorganisms and animals

Swiss albino rats of either sex, weighing (150–200 g body weight) were obtained from animal house of C.L.Baid Metha College of pharmacy, Chennai, Tamil nadu, India. Animals were kept in raised mesh bottom cages to prevent coprophagy .The animals were maintained in colony cages at 26±2 ◦C, relative humidity 45–55% maintained under 12:12 h light and dark cycle. The animals were fed with Standard animal feed (Hindustan Lever, Chennai, Tamil nadu, India.) and water ad libitum. All the animals were acclimatized to the laboratory conditions prior to experimentation. Sixteen hours before the experiments, they were fasted overnight, but allowed free access to water. The body weight and fasting blood glucose levels of all the rats were determined before the start of the experiment. All the experiments were conducted in accordance with the ethical guidelines of the International association for Study of Pain [15] .All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).The study was approved by the Institutional Animal Ethical Committee (Ref No: IAEC/XII/05/CLBMCP/2008-2009-dated: 06-06-2009).

Bacterial (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proeus vulgaris*) were procured from Jasmine Diagnostic Labs (Microbial Type Culture Collection) Chennai,Tamil Nadu, India.

Chemicals and drugs

Methanol was obtained from Merck Ltd. (Mumbai, India). Cloxicillin ,Amoxiclav, Cefuroxime, Cefixim were from Tablets IndiaP.vt,Limited(Chennai,Tamil Nadu,India) were used as reference antibiotics (RA) against bacteria.The nutrient agar was from Himedia (Mumbai, India). Indomethacin was from Merck.

Acute toxicity studies

Acute toxicity study was performed for the extracts to ascertain safe dose by acute oral toxic class method of Organization of Economic Co-operation and Development, as per 423 guidelines (OECD) 12. A single administration of starting dose of 2000 mg/kg body weight/po of the MEIAF and AEIAF was administered to 3 female mice and observed for 3 days. There was no considerable change in body weight before and after treatment and no sign of toxicity were observed. When the experiment was repeated again with same dose level, 2000 mg/kg body weight/po of plant extract for 7 more days and observed for fourteen days no change was observed from the experiments [16].

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Anti microbial activity

Sensitivity test: agar disc diffusion assay

The disc diffusion method was followed to evaluate anti-microbial activities using a range of microorganisms. Sterile Discs (Whatman, 6 mm) were impregnated with 10µl of reconstituted crude extracts (1mg/ml) and placed on the surface of Muller–Hilton agar dispersion plates inoculated with microbes. Each extract was tested in triplicate. Control discs contained pure DMSO (100%). Standard antibiotics, Cloxicillin, Amoxiclav, Cefuroxime, and Cefixime (30 µg disc^{-}1), were used to eliminate variation between plates. Agar plates containing bacteria were incubated at 37°C for 24 h. Inhibition zones were recorded as the diameter of growth-free zones(IZ), including the diameter of the discs, in mm, at the end of the incubation period [17].

Anti-inflammatory test

Carrageenin-induced paw edema in rats

The anti-inflammatory effects of the extracts were assessed using the carrageenin induced paw edema in rats. The animals used for each of the tests were divided into four groups with each group containing five rats. The control group I and the reference (standard) group II received normal saline (10 ml/kg) and indomethacin (5 mg/kg,) respectively. While the test groups (III and IV) were treated with 200 mg/kg of methanolic and aqueous leaf extracts, respectively. Saline, extract and indomethacin were all administered orally. Carrageenin-induced pedal inflammation was produced according to the method described by winters [18]. An injection of 0.1 ml of 1% carrageenin suspension was made into the right hind foot of each rat under the subplantar aponeurosis. The control, reference and test groups were treated orally with saline, indomethacin and the extract 1 h before carrageenin injection. Measurement of paw size was carried out by wrapping a piece of cotton thread round the paw and the length of the thread corresponding to the paw circumference was determined using a meter rule [19]. Measurement was done immediately before and 1–5 h following carrageenin injection. The inhibitory activity was calculated according to the following formula [20].

$$\text{Percentage inhibition} = \frac{(Ct - C0)\ \text{control} - (Ct - Co)\ \text{treated}}{(Ct - C0)\ \text{control}} \times 100$$

Statistical analysis

All values are expressed as mean ±SEM. Data were analyzed by non-parametric ANOVA followed by Dunnett's multiple comparison tests, and other data was evaluated using Graph Pad PRISM software. A p-value <0.05 was considered significantly different.

RESULTS

Effect on Anti microbial activity

The methanolic and aqueous extracts from the leafs of IAF has shown inhibition effects on the growth of all the organisms tested, but their efficiency in inhibition was varied from one organism to another. In almost all, the tested organisms' growth was inhibited by both MEIAF and AEIAF has shown higher range of inhibition diameter (IDZ) from 08 to 19 mm, where as MEIAF has shown inhibition range of 15–20mm. *Staphylococcus aureus* is more sensitive and *Escherichia coli* is least sensitive to MEIAF.Where as AEIAF has shown inhibition range of 08-19mm. *Bacillus subtilis* is more sensitive and *Escherichia coli* are least sensitive to AEIAF. Cloxicillin, Amoxiclav,

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Cefuroxime, Cefixime ranged from 20-28 mm at a concentration of 30µg/zone. All IZD corresponding to test organisms are tabulated in Table 1.

Effect on carrageenin-induced rat paw edema

In the anti-inflammatory tests, the result show that oral pretreatment with a single dose of IAF produced significant dose-dependent anti-inflammatory effects on carrageenin-induced rat hind paw edema. Crude methanolic and aqueous extract (200 and 400 mg/kg) and indomethacin (5 mg/kg) inhibited significantly ($p < 0.05$) .The formation of the carrageenin-induced rat paw edema, measured in third hour of experiment (peak of edema formation) as well as in the fifth hour of inflammatory phases.MEIAF at the dose of 200 mg/kg shows 55.07% inhibitory activity when compare AEIAF which shows 49.27% when compared to standard 63.76%,where as MEIAF at the dose of 400 mg/kg shows 81.17% inhibitory activity when compare AEIAF which shows 76.47% when compare to the standard 89.41%,these results impacted that MAIAF has potential anti-inflammatory activity when compare to AEIAF(Table 2).

Table 1: Antimicrobial activity MEIAF and AEIAF on different microbes and their corresponding IZD.

S. No.	Microbe	IZD (mm)		
		MEIAF	AEIAF	Standard
1	*Staphylococcus aureus*	25	18	28
2	*Bacillus subtilis*	20	19	26
3	*Pseudomonas aeruginosa*	18	11	24
4	*Proteus vulgaris*	16	14	24
5	*Escherichia coli*	15	08	20

Ipomoea aquatica Forsk leaf extracts were screened for antimicrobial activity. All the test organisms are more sensitive to MEIAF than the AEIAF. *Bacillus substilis* is equally sensitive to both the extracts. Cloxicillin, Amoxiclav, Cefuroxime, and Cefixime are the standards (values are mean ± S.D).

Table 2: Effect of the methanolic and aqueous leaf extract *Ipomoea aquatica Forsk* on carrageenin-induced paw edema in rats

Groups	Dose (Orally)	Initial paw size (cm)	Paw edema (mm)		Inhibition (%)	
		3 h	5 h	3 h	5 h	
(I) Control (saline)	-	2.0±0.1	6.9±0.5	8.5±0.2	-	-
(II) Indomethacin	5mg/kg	1.9±0.0	2.5±0.1**	0.9±0.5**	63.76	89.41
(III) MEIAF	200mg/kg	1.9±0.6	3.1±0.6**	1.6±0.6**	55.07	81.17
(IV) AEIAF	200mg/kg	1.8±0.0	3.5±0.7*	2.0±0.4*	49.27	76.47

Each value is the mean ± S.E.M of five rats. * $P < 0.05$ compared with control. ** $P < 0.001$ compared with Student's *t*-test.

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DISCUSSION AND CONCLUSION

Results obtained in the present study have shown that both the extracts, MEIAF and AEIAF, are active against the growth of the microbes such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Escherichia coli*. Both extracts possess promising anti-microbial activity. Hence, the external application of these extracts on the wound prevented the microbes to invade through the wound, resulting protection of wound against the infections of the various microorganisms, because the presence of replicating microorganisms within a wound that cause host injury includes *Staphylococcus aureus*, *Beta-hemolytic Streptococcus*, *E. coli*, *Klebsiella*, *Pseudomonas*, *Acinetobacter*, *Stenotrophomonas*.etc. Hence, the synergistic effect of both anti-microbial and anti-inflammatory activity accelerated the wound-healing process. The overall result of the antimicrobial indicates that both the extracts can be useful in the development of antimicrobial drugs. This could be confirmed by the results of the IZD determination (Table 1). The presence of antimicrobially active metabolites classes such as flavonoids, phenols, terpenoids, alkaloids, glycosides might explain the wide spectrum of activity of the tested extracts. However, the isolation of the active principles will confirm this hypothesis and provide more explanation on mechanism of action of these extracts.

The anti-inflammatory effect of a methanolic and aqueous extract of IAF in experimental animal models is proposed by local injection of carrageenin into rat hind paw induces acute inflammatory responses such as edema. The acute inflammatory responses induced by carrageenin injection involve three phases of chemical mediator release in an orderly sequence [21]. For the first 1.5 h an initial phase takes place with the release of histamine and serotonin and for the subsequent 1.5–2.5 h a second phase is mediated by bradykinin. The third and final phase occurs between 2.5 and 5 h and is presumably mediated by prostaglandins (PGs). In the present study the anti-inflammatory activity of IAF took place at 3 and 5 h after carrageenin injection, suggesting that its action mechanism may involve multiple anti-inflammatory factors and mediators. This is in consistent with the generally believed thinking that herbal preparations usually have multitargets [22].

In the above acute inflammatory models, IAF showed anti-inflammatory activity similar to the standard drug indomethacin, a known nonselective COX inhibitor. These data suggest that IAF has an anti-inflammatory property probably like indomethacin, acting through the inhibition of the inflammatory mediators of the acute phase of inflammation.

In conclusion, our results indicate that IAF has anti-microbial and anti-inflammatory effects, which provide pharmacological evidence for folk uses of *Ipomoea aquatica* Forsk. In the treatment of various inflammatory disorders such as arthritis, sprains and injuries. Further molecular and cellular experiments are warranted to explore its action mechanisms. Identification of its active components is also warranted.

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REFERENCES

[1] Dr. Phil Maffetone .The ABCs of Chronic Inflammation, www.philmaffetone.com.
[2] Blazso G, Gabor M. Acta Physiol Acad Sci Hung 1980; 56(2):235-40.
[3] Malalavidhane TS,Wickram singh DN,Jansz ER. J Ethno pharmacol 2000; 72: 293-98.
[4] Badruzzaman, SM, Husain W. Fitoterapia 1992; 63: 245-47.
[5] Samuelsson G, Farah MH, Claeson P, Hagos M, Thulin M, Hedberg Oet al. J Ethnopharmacol 1992; 37:47-70.
[6] Westphal, E. Ipomoea aquatica Forsskal in Plant Resources in South-East Asia. Number Vegetables. Edited by J.S. Siemonsma K. Piluek. Wageningen,Pudoc Scientific Publishers, 1993; 181-84.

[7] Bruemmer J H and Roe R. Protein extraction from water spinach (Ipomoea aquatica). Proceedings of the Florida State Horticultural Society, Florida 1979; 72: 140-43.
[8] Chen BH, Chen YY. Food Chemistry 1992; 45:129-34.
[9] Snyder GH, Morton JF, Gentung WG. Proc Florida State Hort Soc 1981; 94:230-35.
[10] Sundar Rao K, Dominic R, Singh K. J Agric Food Chem 1990; 38:2137-39.
[11]Tofern B,Mann P, Kaloga M, Jenett-Siems K,Wigge L, Eich E. Phytochemistry 1990; 52:1437-41.
[12]Wills RBH, Rangga A. Food Chemistry 1996; 56: 451-55.
[13]Merrill ED. Philippine J Sci 1939; 59:451-53
[14]Trease GE and Evans WC.In phenols and phenolic glycosides.Text book of Pharmacognosy, London, and ELBS 1989; 223-49.
[15]Zimmerman, M. Pain 1983, 109– 10.
[16]Donald Ecobichon J. The Basis of Toxicity Testing, NewYork, CRC press, 1997; 43–49.
[17]Salie F, Eagles PFK, Leng HMJ. J Ethanopharmacol 1996;l52: 27–33
[18]Winter CA., Risley E A and Nuss GW. Proc Soc Biol Med 1962: 11: 544-47.
[19]Hess SM, Milonig RC. Assay for anti - inflammatory drugs In: Lepow, I.H Ward, P.A. (eds.), Inflammation, Mechanisms and control. Academic press, New York 1972; 1 - 2.
[20]Olajide OA, Awe SO, Makinde JM., Ekhelar AI, Olusola A, Morebise O & Okpako DT. J Ethnopharmacol 2000; 71 (1-2): 179-186.
[21]Di Rosa M, Willoughby DA. J Pharm Pharmacol 1971; 23:297-8.
[22]Huang YL, Chen CC, Chen YJ, Huang RL, Shith BJ. J Nat Prod 2001; 64: 903–06.