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Evaluation of *In-vitro* Anti-Arthritic Potential of Aerial Parts of *Ipomoeapes-caprae* (L.) R.Brand Estabilishment of Its Mechanism of Action

N Deepak Venkataraman*[1], W Clement Atlee[1], T Purushoth Prabhu[2], G Surya[3], R Kannan[1], I Sheik Nasar[1]

[1]Dept. Of Pharmacology, C.L.Baid Metha College of Pharmacy, Chennai-97
[2]Dept. Of Pharmacognosy, C.L.Baid Metha College of Pharmacy, Chennai-97
[3]Dept. Of Pharmaceutical Analysis, C.L.Baid Metha College of Pharmacy, Chennai-97

ABSTRACT

pomoea pes-caprae (IP) is a fabulous plant which was traditionally used in various inflammatory conditions such asRheumatoid arthritis, Alkylosing spondylitis, Osteoarthritis, Gout etcand also in conditions such asPain,Ulcer, Cancer and Wounds. The antinociceptive, anti-inflammatory and anti-oxidant activities of IP have already been scientifically proven. The present*invitro* anti-arthritic study ofethanolic extract of leaves and stems ofIPwas undertaken to substantiate its folkloric uses in the treatment of arthritis. The ethanolic extract of leaves of IP (EELIP) was found to possess potent *invitro*anti-arthritic potential whereas stem extract (EESIP) was found to be moderately effective when compared with the standard diclofenac sodium. The percentage inhibitions of EELIP and EESIP at the dose of 2000 mcg/kg were found to be 82.94 and 55.47 respectively. Preliminary phytochemical studies revealed the presence of tannins. The immunosuppressive effect of tannins could be a reason for the anti-arthritic activity.

Keywords: Ipomoea pes-caprae, Rheumatoid arthritis, Anti-arthritic, Anti-inflammatory, Antinociceptive, Immunosuppressive effect.

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INTRODUCTION

Rheumatoid Arthritis is a chronic systemic disease primarily of the joints, usually polyarticular, marked by inflammatory changes in the synovial membranes and articular structures and by atrophy and rarefaction of the bones. It is characterized by inflammation, pain and over activation of the immune system. It affects approximately 1% and 0.9% of the world and indian populations respectively [1]. Market available many steriodal and non-steroidal anti-inflammatory analgesic medications are currently used to treat RA along with disease-modifying antirheumatic drugs (DMARDs) such as anti-tumour necrosis factor (TNF)-α therapy (etanercept, infliximab and adalimumab), anti-CD20 therapy (rituximab) and abatacept. But all these agents are less appropriate or associated with many side effects. Plant remedies are often sought after because they have multiple mechanisms of action, fewer side effects and are cost effective.*Ipomoea pes-caprae*(IP) is a valuable medicinal plant, distributed in the tropics and subtropics regions and used in folk and tribal medicines. It is a pan tropical, trailing vine that routinely colonizes on sand dunes. It grows just above the high tide line along coastal beaches, forming large mats that assist in stabilizing sands. This is an evergreen perennial with a large, thick root that can be 10ft long and 2 inch in diameter. The entire plant is glabrous and somewhat fleshy. The stem runs along the ground rooting at the nodes with only the flowers being erect. [2] Traditionally *Ipomoea pes-caprae*is used in different ways like; the juice from the succulent leaves has been used as a first aid to treat jellyfish stings. Some Indians use it in ritual baths to alleviate evil spells. Leaves are used in rheumatism, and as stomachic and tonic. The extract of the leaves have the astringent, diuretic and laxative properties. It has biological activity like antioxidant, analgesic and anti-inflammatory, antispasmodic, anticancer, antinociceptive, antihistaminic, insulogenic and hypoglycemic[3]. It is also used in inhibition of platelet aggregation, diarrhoea, vomiting, and piles[4].The *invivo*&*invitro* anti-inflammatory have been reported [4]. The anti-nociceptive activities of IP have already been proved. [6]The compounds responsible for the anti-inflammatory and anti-nociceptive actions have also been isolated. 2-hydroxy-4,4,7-trimethyl-1(4H)-naphthalenone, (-)-mellein, eugenol and 4-vinyl-guaiacol were the Compounds inhibiting prostaglandin synthesis isolated from IP [7].Compounds such as glochidone, betulinic acid, alpha- and beta-amyrin acetate, isoquercitrin isolated from IP were found to be responsible for its anti-nociceptive properties. [8]IP was found to also possess hypoglycemic, anti-haemolytic, antispasmodic, anti-histamine, anti-cancer activities. [9]This study focuses on the *invitro* anti-arthritic potentials of ethanolic extracts of leaf (EELIP) and stem (EESIP) of *Ipomoea pes-caprae.*

MATERIALS AND METHODS

Preparation of Extract

Whole plant of IP were collected from coastal areas of district, Tamil Nadu and authenticated by Dr.P.Jayaraman (Botanist), Director PARC, West Tambaram, Chennai. The leaves and stems were segregated, dried, powdered and were extracted separately with ethanol using soxhlet apparatus for 48 hrs. The solvent was distilled at lower temperature

under reduced pressure and concentrated on water bath to get the crude extract which is stored in desiccator for future use.

Preliminary Phytochemical Tests

The ethanolic extracts were subjected to phytochemical chemical tests to identify the phytoconstituents using standard qualitative reagents. (Table: 1) [10-11]

***In-vitro* Anti-Arthritic Activity by Inhibition of Protein Denaturation Method**

- The Test solution (0.5ml) consist of 0.45ml of Bovine serum albumin (5%W/V aqueous solution) and 0 .05ml of test solution (250mcg/ml).

- Test control solution (0.5ml) consist of 0.45ml of bovine serum albumin (5%W/V aqueous solution) and 0 .05ml of distilled water.

- Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution (250mcg/ml).

- Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0.05ml 0f Diclofenac sodium (250mcg/ml). All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°c for 20minutes and the temperature was increased to keep the samples at 57°c for 3minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416nm. [12-13]

The percentage inhibition of protein denaturation can be calculated as,

PERCENTAGE INHIBITION = [100-(OPTICAL DENITY OF TEST SOLUTION – OPTICAL DENSITY OF PRODUCT CONTROL) ÷ (OPTICAL DENSITY OF TEST CONTROL)] ×100.

The control represents 100% protein denaturation .The results were compared with standard Diclofenac sodium. The percentage inhibition of protein denaturation of different concentration was tabulated in (Table:2& Fig: 1)

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RESULTS AND DISCUSSION

Table 1: Preliminary Phytochemical Analysis

S.NO	TEST	EELIP	EESIP
I.	**ALKALOIDS**		
	Mayer's reagent	-	-
	Dragendorff's reagent	+	+
	Hager's reagent	+	+
	Wagner's reagent	-	-
II	**CARBOHYDRATES**		
	Molisch's test	+	+
	Fehling's test	+	+
	Benedict's test	+	+
III	**GLYCOSIDES**		
	Anthraquinone	+	+
	Cardiac	-	-
	Cyanogenetic	-	-
	Coumarin	-	-
IV	**PHYTOSTEROLS**		
	Salkowski test	+	+
	LibermanBurchard's test	+	+
V	**SAPONINS**	+	+
VI	**TANNINS**	+	+
VII	**PROTEINS AND FREE AMINO ACIDS**		
	Millon's test	+	+
	Biuret test	+	+
VIII	**GUMS AND MUCILAGE**	-	-
IX	**FLAVANOIDS**	+	+

The preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, sterols, terpenoids and glycosides both in EELIP and EESIP (Table 1).

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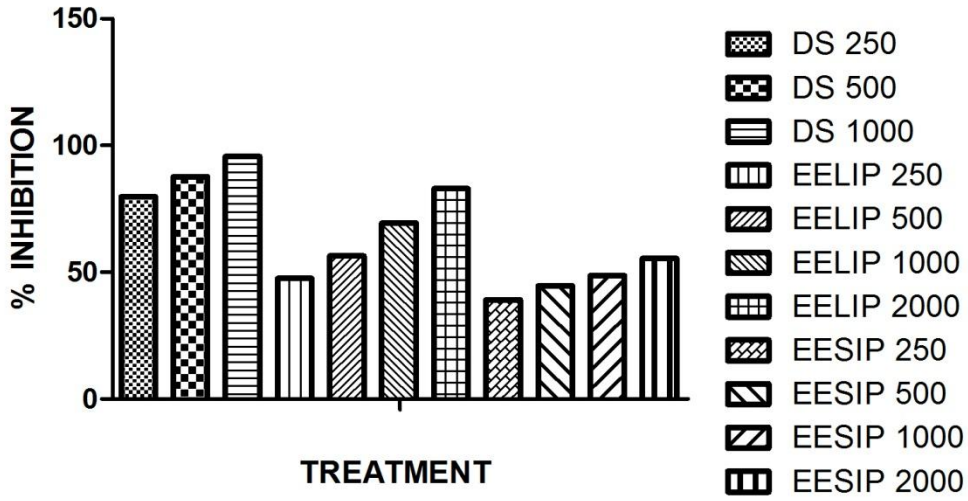
Table 2: Percentage Inhibition Of Protein Denaturation

TREATMENT	CONCENTRATION (mcg/ml)	% INHIBITION
Diclofenac sodium	250	79.82
	500	87.71
	1000	**95.63**
EELIP	250	47.57
	500	56.39
	1000	69.46
	2000	**82.94**
EESIP	250	39.17
	500	44.63
	1000	48.67
	2000	55.47

Fig 1: Percentage Inhibition Of Eelip & Eesip On Protein Denaturation



The ethanolic extract leaves of IP exhibits significant activity at 2000µg/ml (82.94 %)by inhibition of protein denaturation as opposed to the standard drug Diclofenac sodium. The production of auto antigen (like Rheumatoid Factor) in certain arthritic disease is because of protein denaturation[14-16]. Hence we can conclude that theethanolic extract of both leaves and stems of IP are capable of controlling the production of auto antigen and inhibits protein denaturation in rheumatoid arthritis. (Table: 2, Fig: 1)

CONCLUSION

Invitro studies on leaves and stems ofdemonstrate suppression of arthritis. The presence of active entities possessing immunosuppressive activities may be responsible for the *invitro* anti-arthritic effect. The preliminary screening study, revealed the presence oftannins. Tannins are also discussed for their implication of immune response such as immunosuppressive activity. [17] Hence the inhibition of protein denaturation mediated

through suppression of autoantigens may be due the presence of tannins.Thus it can be concluded that both leaf and stem extracts of *Ipomoea pes-caprae*have potent *invitro*anti-arthritic effects, further investigation is required to explore the *invivo* anti-arthritic potentials of the extracts.

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