Original Article

IN VITRO ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY OF LEAVES OF PHYSALIS ANGULATA L.

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Abstract

Physalis angulata L. belongs to the family Solanaceae found in southern region of India, which is used in the treatment of variety of illnesses, such as malaria, asthma, hepatitis, dermatitis and rheumatism. The qualitative phyto-chemical screening showed the presence of steroids, flavonoids, and alkaloids. The Aqueous, Ethanol and Methanol fractions of the leaves of *Physalis angulata* L. were subjected to In vitro Anti-inflammatory activity by HRBC membrane stabilization method and in vitro anti-arthritic activity by protein denaturation method in various concentrations i.e. 10, 62.5, 125, 250, 500, 1000, 2000µg/ml. All the extracts showed positive response as compared to standard Diclofenac sodium. The Ethanol extract showed maximum activity. The order of effect of different extracts were represented as follows Ethanol > Water > Methanol.

Key words: *Physalis angulata* L, Protein denaturation, Anti-inflammatory activity, HRBC membrane stabilization.

Introduction

Physalis angulata L (Family: Solanaceae) is a wide spread indigenous herb. It is distributed throughout the tropical and subtropical area of the world. It grows up to 1m with small stem, cream-colored flowers and light yellowish & orange colored edible fruits wrapped by a layer of leaves.[1] It is a medicinally important plant used in traditional medicine as analgesic, anti-rheumatic to treat sore throat and abdominal pain. It is considered as antipyretic, anti-nociceptive, anti-diuretic and anti inflammatory for hepatitis and cervicitis.[2,3] The work on the chemical composition of the leaves revealed the presence of steroids, flavonoids and alkaloids. The present study was carried out to evaluate the anti-inflammatory and anti-arthritc activity of the various extracts of *Physalis angulata* L leaves. Rheumatoid arthritis is a chronic condition with multiple conditions that causes pain swelling, stiffness, and loss of function in joints[4].

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Anti- denaturation study is performed by using bovine serum albumin [BSA]. When BSA is heated it undergoes denaturation and express antigens associated with type-III hypersensitivity reaction and that is related to disease such as serum sickness, golmeralonephritis, rheumatoid arthritis and system lupus eruthematosus.[5]

Materials and methods

The plants of *Physalis angulata* L. of the family Solanaceae were obtained from chengalpattu area surroundings, Tamil nadu, India. The Authenticity of the plant species was confirmed by Professor .P.Jayaraman, Ph.D. Director of National Institute of Herbal Science, Chennai. The plant leaves were dried and ground to sawdust form, which was then kept in air-tight brown bottle until use.

Ethanol, Methanol, Bovine serum albumin (5%w/v aqueous solution), Diclofenac sodium, 1N Hydro chloric acid and Phosphate buffer.

Preparation of plant extracts

The dried leaves powder of *Physalis angulata* L was extracted with water, ethanol and methane. The extracts were filtered while the residue was further

extracted under the same conditions twice. Both extracts were evaporated under reduced pressure. The percentage yield of water, ethanol and methane were 22.46%, 15.26% and 13.68% respectively.

Preliminary phyto chemical screening

A portion of residue from each extract was subjected for phyto chemical analysis in order to see the presence of alkaloids, steroids and flavonoids.[6]

In vitro the anti-inflammatory activity by HRBC membrane stabilization method

The reaction mixture (4.5ml) consisted of 2ml of hypotonic saline (0.25% NaCl), 1 ml 0.15 M phosphate buffer (pH 7.4) and 1 ml of test solution (100 to 2000 mcg/ml of final volume) in normal saline. 0.5 ml of 10% rat RBC in normal saline was added. For control tests, 1ml of isotonic saline was used instead of test solutions while product control tests lacked red blood cells. The mixtures were incubated at 56°C for 30 minutes. The tubes were cooled under running tap water for 20 minutes. The mixtures were centrifuged and absorbance of the supernatants read at 560 nm [7]. Percent membrane stabilizing activity was calculated as follows,

$$\text{Percent stabilization} = 100 - \frac{\text{(O.D. of test - O.D. of product control)}}{\text{O.D. of control}} \times 100$$

The control represents 100% lysis. The result was compared with diclofenac sodium (250 mcg/ml) treated samples.

In vitro anti arthritic activity by inhibition of protein denaturation method

The reaction mixture (0.5ml) consisted of 0.45 ml of bovine serum albumin (5% aqueous solution) and 0.05ml of *Physalis angulata* leaf extracts (100 and 250mcg/ml of final volume). p^H was adjusted at 6.3 using a small amount of 1N HCL. The sample were incubated at 37°C for 20min and then heated at 57°C for 3 min. after cooling the sample, add 2.5ml of phosphate buffer solution in each test tube. Turbidity was measured spectrophotometrically at 600 nm for control tests 0.05ml distilled water was used instead of extracts while product control tests lacked bovine serum albumin.[8] The percentage inhibition of protein denaturation was calculated as follows,

$$\text{Percentage inhibition} = 100 - \frac{\text{(O.D. of test-O.D of product control)}}{\text{O.D. of control}} \times 100$$

The control represents 100% protein denaturation. The results were compared with diclofenac sodium (250mcg/ml).

Results and discussion

Protective effect on heat and hypotonic saline induced erythrocyte lysis is known to be a very good index of anti-inflammatory activity of any agent[4]. From the result of the present study, various fractions of the leaves of *Physalis angulata* L. were subjected to In vitro anti-inflammatory activity in various concentrations i.e. 10, 62.5, 125, 250, 500, 1000, 2000µg/ml and the percentage stabilization of different extracts of leaves of *Physalis angulata* L. by HRBC membrane stabilization is depicted in Table 1.

Table 1: In vitro Anti-Inflammatory Activity

Concentration (µg/ml)	Percentage inhibition			
	Methanol Extract	Ethanol Extract	Water Extract	Diclofenac sodium
62.5	24.9%	30.8%	27.4%	89.8%
125	32.6%	41.6%	35.9%	
250	43.9%	54.1%	44.9`%	
500	51.8%	70.4%	55.9%	
1000	62.8%	78.2%	67.8%	
2000	69.9%	85.9%	74.2%	

The percentage inhibition was found to be 69.9 % (methanol), 85.9% (ethanol), 74.2% (water) and 89.8% (Diclofenac sodium). All the extracts showed positive response and dose dependent response. This effect may be due to the presence of steroids, alkaloids and flavonoids present in various fractions. The effect was represented as follows Ethanol> Water> Methanol.

Denaturation of protein is one of the cause of rheumatoid arthritis was documented. Production of auto antigen in certain arthritic disease may due to denaturation of protein. The mechanism of denaturation probably involves alteration I electrostatic hydrogen, hydrophobic and disulphide bonding. From the result of the present study, it can be stated that all the extracts of *Physalis angulata* L. leaves are capable of controlling the production of auto antigen and thereby

it inhibit the denaturation of proteins and its effect was compared with the standard drug diclofenac sodium.

The percentage protection was found to be 68.9% (methanol), 82.9% (ethanol), 73.7% (water) and 92.20% (Diclofenac sodium). All the extracts exhibited dose dependant response. This effect may be due to the presence of steroids, alkaloids and flavonoids present in various fractions. The effect was represented as follows Ethanol> Water> Methanol. The invitro anti-arthritic activity of *Physalis angulata* L by protein denaturation method is shown in Table 2.

Table 2: In vitro Anti-Arthritic Activity

Concentration (µg/ml)	Percentage inhibition			
	Methanol Extract	Ethanol Extract	Water Extract	Diclofenac Sodium
62.5	22.9%	31.8%	28.4%	89.2%
125	33.6%	40.6%	34.8%	
250	42.8%	55.7%	46.4%	
500	53.8%	70.4%	55.9%	
1000	60.9%	76.6%	68.9%	
2000	68.9%	82.9%	73.7%	

Conclusion

The In vitro studies on leaves of Physalis *angulata* L. Showed the presence of significant anti-inflammatory and anti- arthritic activity. The Ethanol extract shows more anti inflammatory and anti-arthritic activities. The Activity may be due to the presence of steroids, flavonoids and alkaloid. Our future aim is to isolate the chemical constituents responsible for the anti inflammatory and anti-arthritic activities.

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