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In-vitro* Anti-inflammatory and Anti-arthritis Activity of Leaves of *Clerodendron Inerme

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ABSTRACT

Clerodendron inerme belongs to the family Verbenaceae found in southern region of India, which is used in the treatment of Veneral diseases, Rheumatism, Elephantiasis and Intermittent fever. The qualitative phyto-chemical screening showed the presence of alkaloid, steroids, phenols, flavanoids, tannins, carbohydrates, fixed oils and volatile oils. The Petroleum ether, Chloroform, Ethyl acetate, Ethanol and water fractions of the leaves of Clerodendron inerme were subjected to In vitro Anti-inflammatory activity by HRBC membrane stabilization method in various concentration i.e. 10,50,100,200,400,800,1000µg/ml. All the extracts showed positive response as compared to standard Diclofenac sodium. The Ethyl acetate and Ethanol extracts showed maximum activity. The order of effect of different extracts were represented as follows Ethyl acetate> Ethanol >Water> Chloroform> Petroleum ether. The Petroleum ether, Chloroform, Ethyl acetate, Ethanol and water fractions of the leaves of Clerodendron inerme were subjected to invitro anti- arthritis activity by protein denaturation method. All the extracts showed positive response .The effect was represented as follows Ethyl acetate> Chloroform>Ethanol> Water> Petroleum ether.

Keywords: Clerodendron inerme, anti-arthritis, anti-inflammatory, ethyl acetate

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INTRODUCTION

Cleodendron inerme (Family: Verbenaceae) commonly called as Garden Quinine. (Local Name: Pinasangam-koppi). It is one of the medicinal plant scientifically investigated by the medicinal plant researchers. It is a medicinally important plant used for the treatment of intermittent and remittent fever, venereal diseases and also used in rheumatism. The work on the chemical composition of the leaves revealed the presence of flavonoids and tannins. Many flavonoids have remarkable anti-arthritis activity. The present study was carried out to evaluating the anti-inflammatory and anti-arthritis activity of the leaves of Cleodendron inerme [1, 2].

Plant

The leaves of Cleodendron inerme were collected from the Sengottai, Tirunelveli, Tamil Nadu, India in the month of November 2009. The plant material was identified and authenticated by Mr. V.Chelladurai, Retired Research officer-botany, C.C.R.A.S. Govt. of India, Tirunelveli. The Collected plant material was free from diseases and also free from contamination of other plants.

Uses in traditional medicine

It is given in the form of tincture or decoction in intermittent and remittent fevers; it is used as a substitute for quinine and chiretta. Juice of leaves and root is employed as alterative in serofulous and venereal diseases. A poultice of the leaves is applied to resolve buboes. A batch of the leaves is recommended in mania and itches. Root boiled in oil is applied like liniment in rheumatism [3, 4].

The leaves warmed in sesame oil and pounded with garlic and pepper are applied in elephantiasis. The leaf juice is taken orally to relieve muscular pains and stiffness of legs. The leaves along with garlic, pepper, asafoetida are made into a fine paste and given orally to cattle in rheumatic pain and arthritis. The leaves and roots are also used for treating rheumatism and skin diseases.

A bibliographical survey showed that there is no report on anti-inflammatory and anti arthritic activity of leaves of Cleodendron inerme.

The present manuscript describes the anti-inflammatory and anti arthritic activity of leaves of Cleodendron inerme.

Reported activity

Ovicidal and nematocidal activities [5], Hepatoprotective activity [6], anticarcinogenic effects [7] and Anti fungal activity [8].

Prior isolated Constituents

phenylethanoid glycoside [9], Neo-clerodane diterpenoids [10], clerodane diterpene [11] and bioflavonoids [12].

MATERIALS AND METHODS

Extraction

The Cleodendron inerme leaves were air dried and powdered. It was extracted with different solvent system (Petroleum ether, Chloroform, Ethyl acetate, Ethanol and Water) by using successive solvent maceration. The Solvent was concentrated under reduced pressure to get the crude extract which is stored in desiccators for future use.

Procedure

To determine the anti-inflammatory activity by HRBC membrane stabilization method, the following four solutions were used [13].

1. **Test solution** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (pH7.4), 1ml of test extract (250mcg/ml) of final volume) in normal saline and 0.5ml of 10% w/v human red blood cells in isotonic saline.
2. **Product control** (4.5ml) consists of 2ml of hypotonic saline (0.25% w/v), 1ml of phosphate buffer (pH7.4) and 1ml of test extract (250mcg/ml of final volume) in normal saline and 0.5ml of isotonic saline.
3. **Test control** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (7.4pH) and 1ml of isotonic saline and 0.5ml of 10%w/v human red blood cells in isotonic saline.
4. **Standard solution** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (7.4pH) and 1ml of Diclofenac sodium (250mcg/ml of final volume) in normal saline and 0.5ml 10%w/v human red blood cells in isotonic saline.

The above four solutions were incubated at 56⁰c for 30 minutes. The tubes were then cooled running tap water for 30 minutes. After that they were centrifuged the supernatant liquid was separated and the absorbance of supernatant solution was measured at 560nm by UV-spectrophotometer. The percentage of membrane stability was calculated as follows:

$$\text{Percentage stabilization} = \frac{100 - (\text{OD of test solution} - \text{OD of product control}) \times 100}{\text{OD of test control}}$$

In vitro anti arthritic activity by inhibition of protein denaturation method [14]

1. **Test solution** (0.5ml) consists of 0.45ml of bovine serum albumin (5%w/v aqueous solution) and 0.05ml of test solution (250µg/ml).
2. **Test control** solution (0.5ml) consists of 0.45ml of bovine serum albumin (5%w/v aqueous solution) and 0.05ml of distilled water.
3. **Product control** (0.5ml) consists of 0.45ml of distilled water and 0.05ml of test solution (250mcg/ml).
4. **Standard solution** (0.5ml) consists of 0.45ml of bovine serum albumin (5%w/v aqueous solution) and 0.05ml Of Diclofenac sodium (250µg/ml).

All of the above solutions were adjusted to pH, 6.3 using a small amount of 1N HCl. The samples were incubated at 37°C for 20minutes and heated at 57°C for 3 minutes. After cooling, add 2.5ml of phosphate buffer to the above solutions. The absorbance of the above solutions was measured using UV-Visible spectrophotometer at 416nm. The percentage inhibition of protein denaturation was calculated using the formula.

$$\text{Percentage inhibition} = 100 - \left\{ \frac{\text{optical density of test solution} - \text{optical density of product control}}{\text{optical density of test control}} \right\} \times 100$$

The control represents 100%protein denaturation .The results were compared with Diclofenac sodium (200µg/ml) treated samples.

RESULTS

Invitro Anti- inflammatory Activity

The Percentage stabilization of different extracts of leaves of Clerodendron inerme by HRBC Membrane Stabilization is depicted in Table 1.

Table 1. The invitro anti-inflammatory activity of leaves of Clerodendron inerme by HRBC Membrane Stabilization Method

Concentration (µg/ml)	% inhibition					
	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Water	Diclofenac sodium
10	44.63	43.18	43.46	44.20	43.85	94.20
50	54.13	49.82	50.53	52.12	51.77	
100	61.38	55.02	60.35	60.74	63.50	
200	67.67	67.67	67.39	67.07	69.43	
400	73.29	73.29	73.32	74.84	74.98	
800	67.81	79.51	81.13	78.37	79.40	
1000	70.88	72.12	87.42	82.93	82.30	
IC ₅₀	272.9	693.3	571.9	602.9	607.6	

In-vitro Anti-arthritic Activity

The invitro anti-arthritic activity of Clerodendron inerme by protein denaturation method is shown in Table 2.

Table 2. The invitro anti-arthritic activity of Clerodendron inerme by protein denaturation method

Concentration (µg/ml)	% Protection					Diclofenac Sodium
	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Water	
10	43.06	43.17	49.25	45.72	43.53	92.20
50	50.25	52.23	55.82	51.76	52.37	
100	55.28	58.81	63.12	59.81	59.81	
200	60.53	63.55	70.45	65.96	66.28	
400	68.55	71.57	75.92	73.65	73.65	
800	70.38	78.04	79.01	82.24	78.65	
1000	78.94	88.46	89.25	87.10	82.31	
IC ₅₀ (µg/ml)	633.4	565.2	560.2	574.1	607.4	

DISCUSSION

Various fractions of the leaves of Clerodendron inerme were subjected to Invitro anti-inflammatory activity in various concentrations i.e.10, 50, 100, 200, 400, 800 & 1000 µg/ml. The percentage inhibition was found to be 73.29% (Petroleum ether), 79.51 % (Chloroform), 87.42% (Ethyl acetate), 82.93% (ethanol) 82.30%(water) and 92..4% (Diclofenac sodium). All the extracts showed positive response. The Ethyl acetate, Ethanol and Water extracts showed dose dependent response. Petroleum Ether and Chloroform extracts showed bi phasic response. This effect may be due to the presence of steroids, alkaloids and flavanoids present in various fractions. The effect was represented as follows

Ethyl acetate> Ethanol> Water> Chloroform>Petroleum ether

The IC₅₀ values were found to be 272.9 µg/ml for petroleum ether, 693.3µg/ml for Chloroform, 571.9µg/ml for Ethyl acetate, 602.9 µg/ml for ethanol and 607.6µg/ml for water extracts

Denaturation of protein is one of the cause of rheumatoid arthritis was well 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 Clerodendron inerme leaves is 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 78.94% (Petroleum ether),88.46 % (Chloroform), 89.25% (Ethyl acetate), and 87.10% (ethanol) 82.31%(water). And 92.20% (Diclofenac sodium).All the extracts showed 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



Ethyl acetate> Chloroform>Ethanol> Water> Petroleum ether

The IC₅₀ values were found to be 633.4 µg/ml for petroleum ether, 565.2µg/ml for Chloroform, 560.2µg/ml for Ethyl acetate, 574.1 µg/ml for ethanol and 607.4 µg/ml for water extracts

CONCLUSION

The In vitro studies on leaves of Clerodendron inerme showed the presence of significant anti-inflammatory and anti-arthritic activity. The ethyl acetate extract shows more anti-inflammatory and anti-arthritic activities. The Activity may be due to the presence of terpenoids, steroids, alkaloids, flavonoids and tannins. Our future aim is to isolate the chemical constituents responsible for the above activities and also to carry out the invivo investigation.

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