



Research Article

IN VITRO ANTI-INFLAMMATORY, ANTI-ARTHRITIC ACTIVITY OF *NERIUM INDICUM* AND *LEUCAS ASPERA* A TRADITIONAL FOLKLORE MEDICINE

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ABSTRACT

The present study is aimed to evaluate the efficacy of *Nerium indicum* and *Leucas aspera* leaf extract and fractions for anti-arthritis activity using *in-vitro* inhibition of protein denaturation model and anti-inflammatory activity using albumin denaturation assay comparing with Diclofenac sodium used as a standard drug. Antiarthritic activity was carried out using 10-80 µg/ml concentration, whereas antiinflammatory activity at the concentration of 25-100 µg/ml. The results of the present study represents that the methanolic extract and fractions of both the plants demonstrates dose dependent inhibition. The maximum antiarthritic activity of *Nerium indicum* and *Leucas aspera* was found at the concentration of 80 µg/ml with 81.6% and 86.2% inhibition. Similarly, maximum antiinflammatory activity of *Nerium indicum* and *Leucas aspera* was observed at the concentration of 100 µg/ml with 84.4 and 87.2% inhibition. It is evident that the cause of inflammation and arthritis is mainly due to denaturation of tissue proteins and the present study expresses that denaturation of proteins is inhibited, inhibiting the progression of arthritis and reducing the cause of inflammation.

Key words: *in-vitro*, anti-inflammatory, antiarthritic, denaturation.

INTRODUCTION

Multiple drug resistance and increased side effects due to allopathic drugs has warned scientific community with a global alarm to identify molecules from natural sources to combat diseases with minimum or no side effects. Hence, folklore medicine has gained back its status after many decades due to increase usage of folklore and substantial decline in usage of allopathy drugs. Many plant products are used widely in folklore medicine because of their therapeutic potential. Thousands of phytochemicals have been isolated and characterized from plants, including fruits, greens and vegetables. The isolated phytochemicals are grouped into distinctive classes by the number and kind of constitutive atoms and the structure of the basic skeleton. Phytochemicals derived from the plants remain the basis for a large percentage of the commercial medications used today for the treatment of a wide range of diseases. In recent years, attention on plant investigation has improved all over the world and a lot of confirmations have shown the feasible of medicinal plants used in various traditional systems¹.

Thousands of plants have been mentioned in Ayurvedic medicinal systems one among them is *Nerium indicum* Mill². *Nerium indicum* is used as traditional medicine in different parts of the world, especially in India and China³. *Nerium indicum* is used for diverse treatments such as cardiac illnesses, asthma, corns, cancer, epilepsy, reproductive problems, gastrointestinal infections, antidiabetic, headache, and dermatological problems^{4, 5, 6, 7}.

Leucas aspera (Willd.) Linn. commonly known as "Thumbai"⁸ is distributed throughout India from the Himalayas down to Ceylon. The plant is used traditionally for various diseases and is effectively used for antidiabetic, antimicrobial, antinociceptive, antioxidant and cytotoxic activities^{9, 10, 11}. Though there are number of studies representing the traditional usage of *Nerium indicum* and *Leucas aspera*, the present work focuses on the antiinflammatory and anti-arthritis potential of these plants.

MATERIAL AND METHODS

Collection and preparation of plant material

Nerium indicum and *Leucas aspera* leaves were collected from Vellore District, Tamil Nadu, India. The plant materials were cleaned with distilled water, shade dried at room temperature and authenticated from the Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore Dt, Tamil Nadu and voucher specimens (CAHC-15Bc/2012 & CAHC 16Be/2012) were stored. The dried leaves were coarsely powdered by using electric blender and stored separately in an air-tight container for further use.

Extraction and fractionation

The powdered leaf sample of *Nerium indicum* and *Leucas aspera* were first macerated in methanol separately for 24 h for proper extraction, the residue was removed by filtration and the filtrate was concentrated under reduced pressure in a rotary evaporator at 60±10°C to yield the required quantity of crude extract. The methanolic extract was again macerated separately in hexane then with ethyl acetate and finally with Isopropyl alcohol. The filtrates

were concentrated using rotary evaporator at 60±10°C and the resultant extract was stored and used for further studies¹².

Chemicals and solvents

All the chemicals and solvents were of analytical grade procured from Fischer Inorganic and Aromatic Limited, Chennai, India.

In vitro anti-inflammatory activity-Albumin denaturation inhibition¹³

Albumin denaturation inhibition assay was followed in which the sample consisting of (25-200µg/mL) of *Nerium indicum/Leucas aspera*/fractions/Diclofenac-sodium mixed with 1% of bovine albumin in aqueous solution maintaining the pH 6.3 using 1N HCl. The samples were incubated at 37°C for 20 min and then by 51°C for 20 min cooled and the turbidity was measured spectrophotometrically at 660nm.

Percent inhibition of protein denaturation was calculated by using the formula

% inhibition = $[(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) / \text{Abs}_{\text{Control}}] \times 100$, where Abs control is the absorbance without sample, Abs sample is the absorbance of sample extract/standard.

In vitro anti-arthritis activity-Inhibition of protein denaturation method¹⁴

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *Nerium indicum/Leucas aspera*/fractions/Diclofenac-sodium (10 - 80 µg/ml) maintaining pH 6.3 using 1 N HCl. The samples were incubated at 37°C for 20 min, then at 57°C for 3 min, cooled and 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity formed during the process was measured at 416 nm spectrophotometrically. The percentage inhibition of protein denaturation was calculated using the below equation.

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

Table 1: *In vitro* anti-arthritis activity of *Nerium indicum* methanol extract and fractions on inhibition of protein denaturation

Sample	Concentration (µg/ml) (% of inhibition)			
	10	20	40	80
<i>Nerium indicum</i> Methanol extract	35.5	47.2	67.4	81.6
<i>Nerium indicum</i> Hexane fraction	18	24.6	35.2	42.4
<i>Nerium indicum</i> Ethyl acetate fraction	32.6	57.4	68.4	78.2
<i>Nerium indicum</i> Isopropyl alcohol fraction	34.8	42.4	61.6	80.2
Diclofenac sodium	38	46.2	55.8	84.6

Table 2: *In vitro* anti-inflammatory activity of *Nerium indicum* methanol leaf extract and fractions on albumin denaturation

Sample	Concentration (µg/ml) (% of inhibition)			
	25	50	100	200
<i>Nerium indicum</i> Methanol extract	24	36.4	58.8	84.4
<i>Nerium indicum</i> Hexane fraction	6.2	9.4	20.6	34.2
<i>Nerium indicum</i> Ethyl acetate fraction	18.6	42.2	58.2	76.6
<i>Nerium indicum</i> Isopropyl alcohol fraction	18.4	42.4	58.8	78.6
Diclofenac sodium	36.8	48.6	74.2	88.4

Table 3: *In vitro* anti-arthritis activity of *Leucas aspera* methanol leaf extract and fractions on inhibition of protein denaturation

Sample	Concentration (µg/ml) (% of inhibition)			
	10	20	40	80
<i>Nerium indicum</i> Methanol extract	32	49.6	67.4	86.2
<i>Nerium indicum</i> Hexane fraction	12.2	26.4	38.4	50
<i>Nerium indicum</i> Ethyl acetate fraction	26	48.8	64.6	78.2
<i>Nerium indicum</i> Isopropyl alcohol fraction	20.4	38.6	60.4	72.2
Diclofenac sodium	40.6	56.8	70.4	92.4

Table 4: *In vitro* anti-inflammatory activity of *Leucas aspera* methanol leaf extract and fractions on albumin denaturation

Sample	Concentration ($\mu\text{g/ml}$) (% of inhibition)			
	25	50	100	200
<i>Nerium indicum</i> Methanol extract	30.8	42.6	64.2	87.2
<i>Nerium indicum</i> Hexane fraction	16.4	28.6	40.8	52.2
<i>Nerium indicum</i> Ethyl acetate fraction	26.8	38.4	66.4	84.2
<i>Nerium indicum</i> Isopropyl alcohol fraction	22.8	40.6	64.2	86.4
Diclofenac sodium	36.8	48.6	74.2	88.4

RESULTS AND DISCUSSION

In vitro anti-arthritis and inflammatory activity of *Nerium indicum*

Table 1 depicts the effect of *Nerium indicum* on antiarthritic activity using protein denaturation assay. It is well known that denaturation of proteins is an important cause of arthritis and inflammation. To investigate the mechanism of antiarthritic activity, methanol extract of *Nerium indicum* and fractions collected Methanolic- Hexane fraction, Methanolic – Ethyl acetate fraction, and Methanolic- Isopropyl alcohol fraction were tested for protein denaturation studies. The results revealed dose dependent inhibition of extracts and fractions. At 80 $\mu\text{g/ml}$ concentration, methanolic extract demonstrated 81.6% inhibition, whereas hexane fraction 42.4%, ethyl acetate fraction 78.2% and isopropyl fraction 80.2% respectively. Minimum inhibition was observed by hexane fraction at all the tested concentration. The observed results were on par with the standard drug treated with diclofenac sodium.

Anti-inflammatory effect of methanolic extract of *Nerium indicum* and fractions were evaluated using *in-vitro* albumin denaturation assay and the results are represented in Table 2. Maximum inhibition of albumin denaturation was observed at the concentration of 200 $\mu\text{g/ml}$ with 84.4 % inhibition from methanolic extract and the minimum inhibition was observed by hexane fraction with 88.4% inhibition. Screening of various medicinal plants and compounds from plants has led to the discovery of new medicines which are effective and protect against various diseases¹⁵. Research has proved that inflammation is the primary physiological defense mechanism protecting the system against different stimuli¹⁶ Tissue proteins denaturation is found to be a potential cause of inflammation and arthritic diseases¹⁷. Hence the present study focuses and proves experimentally the traditional usage of both the folklore medicinal plants *Nerium indicum* and *Leucas aspera* against arthritis and inflammation and reveals the potential experimentally.

In vitro anti-arthritis and inflammatory activity of *Leucas aspera*

Antiarthritic activity of *Leucas aspera* on protein denaturation assay is represented in Table 3. The mechanism of antiarthritic activity was investigated using methanol extract of *Leucas aspera* and fractions collected Methanolic- Hexane fraction, Methanolic – Ethyl acetate fraction, and Methanolic- Isopropyl alcohol fraction were tested for protein denaturation studies. *In vitro* assay results revealed 56.2% inhibition with methanolic extract and the fractions demonstrated 50%, 78.2% and 72.2% inhibition at 80 $\mu\text{g/ml}$ concentration and the inhibition was found to be dose dependent.

Table 4 represents the results of anti-inflammatory effect of methanolic extract of *Leucas aspera* and fractions on *in-vitro* albumin denaturation assay. The results revealed dose dependent inhibition and maximum inhibition was observed at the concentration of 200 $\mu\text{g/ml}$ with 87.2% inhibition from methanolic extract, followed by ethyl acetate and isopropyl alcohol fraction with 84.2% and 86.4% inhibition. Whereas minimum inhibition was observed in hexane fractions with 52.2% inhibition. The common drugs used for inflammation and arthritis are analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), steroids and disease-modifying anti-rheumatic drugs (DMARDs)¹⁸. Though the progress in the treatment of arthritis by NSAIDs and other drugs, search for safer drugs continues due to limitations and side effects of synthetic drugs. These limitations and side effects have urged scientists all over the world to search for new drugs without these limitations. *In vitro* protein denaturation and albumin denaturation study using *Nerium indicum* and *Leucas aspera* has revealed tremendous potential to be a right candidate inhibiting protein denaturation preventing the primary cause for inflammation and arthritis.

CONCLUSION

Due to the global alarm against the side effects caused by allopathy drugs there is a trend of returning back to nature, focusing on traditional folklore medicine to cure various diseases among the scientific community. The current research explores the usage of *Nerium indicum* and *Leucas aspera* against arthritis and inflammation experimentally representing significant activity.

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