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EVALUATION OF IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF CORDIA MONOICA (ROXB.) LEAVES

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ABSTRACT
The present exploration was carried out to determine the *in-vitro* anti-inflammatory activity of *Cordia monoica* (Roxb.) leaves. Ethanol and Ethyl acetate extract of *Cordia monoica* leaves were taken for the study. The *in-vitro* anti-inflammatory assay was evaluated using protein denaturation assay and Proteinase inhibitory method. In both the methods, the ethanolic extract exhibited better activity than ethyl acetate when compared with standard (Aspirin). The extracts of *Cordia monoica* leaves showed dose dependent activity. The activity increased with increasing concentration. The phytochemical compounds such as phenol, tannin, flavanoid may be responsible for the anti-inflammatory activity. Thus it can be concluded that *Cordia monoica* leaves possess remarkable anti-inflammatory activity.

KEYWORDS: *Cordia monoica*, IC_{50}, protein denaturation, proteinase inhibition, Aspirin.

INTRODUCTION
Inflammation is a response to infection, destruction or injury which is characterised by pain, heat, redness, swelling and disturbed physiological functions. When living tissues are subjected to trauma, Inflammation occurs which leads to injury of cells. An acute inflammation builds up into chronic inflammation on persisting.[1] The inflammation process consists of step by step mechanism such as degeneration, exudation and proliferation. Inflammation is elicited by the chemical mediators released from the injured tissue, and also from antigen- antibody interactions.[2]

Acute inflammation ensues in vascular tissues and is mainly initiated due to mechanical damage, chemical damage, insect bites, infections due to microorganism and radiations. Chronic inflammation is long-term and inflammatory changes takes place in the connective tissue of joints and heart. They include rheumatic arthritis, Ostero arthritis, rheumatic fever and systemic lupus erythematous.[3]

There is a surplus production of oxygen, hydroxyl, hydrogen peroxide ions and increased activation of phagocytes during inflammatory disorders.[4] These free radicals will initiate lipid peroxidation that result in tissue damage. The production of mediators and chemotactic factors further activates an immune response.[5] In Arthritic conditions, these reactive oxygen species activate matrix metallo proteinase that leads to tissue damage.[6]

The drugs used for inflammatory conditions are non-steroidal anti-inflammatory drugs which cause adverse effects, especially gastric irritation.[2] Natural products have contributed to the development of modern medicine. Several investigations have proven that many medicinal plants have anti-inflammatory activity in the past few decades. Scientific reports support that drugs from medicinal plants were able to decrease inflammation.[7]

Cordia monoica Roxb. belonging to Boraginaceae family is a multi-stemmed evergreen shrub or small tree. *Cordia monoica* Roxb. distributed worldwide is found mainly in India, Sri Lanka and Africa. In India, the distribution is widely in southern part of all districts of Tamil Nadu, Andhra Pradesh and Kerala.[8] *Cordia monoica* Roxb. have several uses in traditional medicine. The crushed leaves with a cup of water are orally given to treat a local illness termed as MICH. MICH is a febrile disease with symptoms such as sweating, headache and fever.[9] The

leaf preparations of several species of *Cordia* are used in traditional medicine as remedies for some tumoral formations.[10],[11] Based on the traditional use, the present investigation is carried out to determine the *in-vitro* anti-inflammatory effect of ethanolic extract of *C. monoica* leaves.

MATERIALS AND METHODS

Collection of Plant Material

Cordia monoica Roxb. (*C.monoica*) belonging to Boraginaceae family is a shrub, widely distributed in most districts of Tamil Nadu on rocky hill sides. The plant materials were collected during the month of June. The leaves of the plant were collected from Maruthamalai Hills of Coimbatore, Tamil Nadu, India. Flowering shoots of the plants were also collected for identification. The collected plant material was identified and their authenticity was confirmed by comparing the voucher specimen at the Botanical survey of India, Coimbatore, Tamil Nadu, India (BSI/SRC/5/23/2014-15/Tech/512). The collected specimens were deposited in the Department of Biotechnology, Sri Ramakrishna College of Arts and Science, Coimbatore, Tamil Nadu, India.

Extraction Process

The collected leaves of *C. monoica* were cleaned to remove adhering dust and then dried under shade. Then the dried leaves were powdered in mechanical grinder fine enough to pass through a No.40 sieve for powder analysis. Coarse leaf powder was used for further extraction process and pharmacological studies. 50gm air dried coarse leaf powder was mixed with 100 ml of ethanol and ethyl acetate. The extraction was carried out in a closed macerated flask for 24 hours, shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, the mixture was filtered rapidly taking precautions against loss of the solvent. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. The extract is stored and used for further analysis.[12,13]

Inhibition of Protein Denaturation[14]

0.5ml of the reaction mixture consisted of 0.45ml bovine serum albumin (5% aqueous solution) and 0.05ml of *C. monoica* leaf extract (100-500µg/ml). pH was adjusted to 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 3min. After cooling the samples, 2.5ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660nm. For control tests 0.05ml of distilled water was used instead of extracts while product control tests lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

Percentage inhibition = 100 – ((O.D of plant sample – O.D of product control)/O.D of Control) x 100

The IC_{50} value was defined as the concentration of the sample extract to inhibit 50% of protein denaturation under assay condition.

Proteinase Inhibitory Activity[14]

2.0 ml of the reaction mixture contained 0.06mg trypsin; 1.0ml of 25mM tris – HCl buffer (pH – 7.4) and 1.0ml aqueous solution of *C. monoica* leaf extract (100-500µg/ml). The mixtures were incubated at 37°C for 5min and then added 1.0ml of 0.8% (w/v) casein. The mixtures were incubated for another 20min. The reaction was inhibited by adding 2.0ml of 70% (v/v) perchloric acid. The cloudy suspension was centrifuged. Absorbance of the supernatant was read at 280nm against buffer used as blank. The percentage of inhibition was calculated as follows.

Percentage inhibition = 100 – ((O.D of plant sample – O.D of product control)/O.D of Control) x 100

The IC_{50} value was defined as the concentration of the sample extract to inhibit 50% of protein denaturation under assay condition.

Statistical Analysis

All the values are expressed as mean SEM.

RESULT

Protein Denaturation Assay

Protein denaturation involves non-covalent change in the structure of protein. It alters the secondary, tertiary and quaternary structure of proteins, thereby leads to destruction of protein function. As a part of the investigation on anti-inflammatory activity, protein denaturation inhibition was studied with ethanol and ethyl acetate extract of *C. monoica* leaves at different doses (100-500 µg/ml). The result was dose dependent and maximum inhibition was achieved at a dose 500 µg/ml (Table 1 & Fig 1). The IC_{50} value for ethanol extract was 141±5.10µg/ml and for ethyl acetate it was 172.44 ± 2.74µg/ml. Gallic acid acts as standard with an IC_{50} value of 56.70±0.88µg/ml (Table 3).

Table: 1: Protein Denaturation inhibiting activity of *Cordia monoica* Leaf Extract.

Concentration (µg/ml)	Percentage activity of *Cordia monoica* leaves		
	CML Ethanol extract	CML Ethyl acetate extract	*Standard Aspirin
100	5.51 ± 0.59	7.56 ± 0.59	12.35 ± 0.46
200	22.56 ± 0.97	17.69 ± 1.02	14.99 ± 0.15
300	30.51 ± 0.59	30.26 ± 0.80	28.19 ± 0.15
400	44.36 ± 0.59	36.67 ± 0.59	42.81 ± 0.21
500	46.79 ± 1.24	44.87 ± 0.59	54.06 ± 0.21

CML – Cordia monoica leaf extract. Data are expressed as mean ± SD (n=3).
Dose dependent increase of *standard from 25-250 µg/ml.

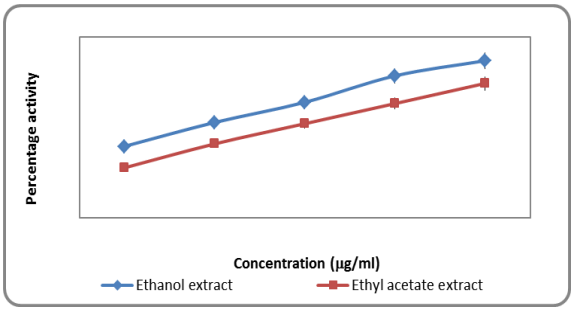

Fig. 1: Protein denaturation activity of *Cordia monoica* leaves.

Proteinase inhibitory action

Proteinase enzymes are responsible for hydrolysis of peptide bond changing the primary protein structure.[15]

The assay was carried out with ethanol and ethyl acetate extract of *C. monoica* leaves. The proteinase inhibitory activity was found to be increased with increase in concentration of extract. The ethanol extract showed an IC_{50} value of 97.74 ± 2.04 µg/ml and for ethyl acetate it was 106.95 ± 0.68 µg/ml (Table-3). The result was in comparison with standard Gallic acid. It is evident that the leaf extracts of *C. monoica* inhibits proteinase activity by preventing the hydrolysis of protein structure. Fig 2 and Table 2 depicts the proteinase inhibitory action of *C. monoica* leaf extract.

Table 2: Proteinase inhibitory activities.

Concentration (µg/ml)	Percentage activity of *Cordia monoica* leaves		
	CML Ethanol extract	CML Ethyl acetate extract	*Standard Aspirin
100	23.75 ± 2.50	16.67 ± 1.91	15.53 ± 1.74
200	31.67 ± 1.91	24.58 ± 1.91	31.06 ± 1.74
300	38.33 ± 1.91	31.25 ± 1.25	43.56 ± 2.37
400	47.08 ± 1.91	37.92 ± 1.44	60.61 ± 1.31
500	52.08 ± 0.72	44.58 ± 1.91	68.56 ± 1.74

CML – Cordia monoica leaf extract. Data are expressed as mean± SD (n=3). Dose dependent increase of *standard from 25-250 µg/ml.

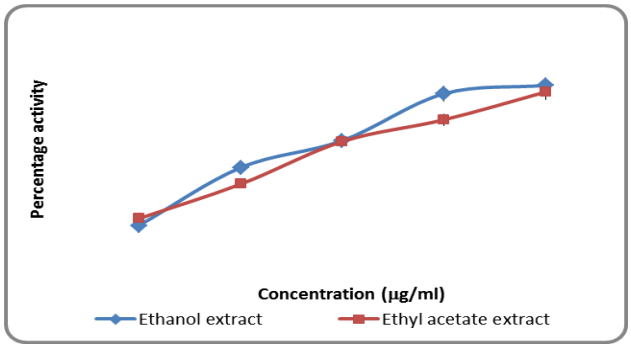

Fig. 2 Proteinase denaturation assay of *Cordia monoica* Leaves.

Table 3: IC_{50} value of *in-vitro* anti-inflammatory activity.

In-vitro Anti-Inflammatory	IC_{50} (µg/ml)		
	CML Ethanol extract	CML Ethyl acetate extract	Standard Aspirin
Protein Denaturation assay	141.76 ± 5.10	172.44 ± 2.74	56.70 ± 0.88
Proteinase Inhibitory activity	97.74 ± 2.04	106.95 ± 0.68	46.03± 0.22

CML – Cordia monoica leaf extract

DISCUSSION

The major cause of inflammation may be attributed to the denaturation of protein and is well-documented,[16] In certain rheumatic diseases auto antigens are produced due to protein denaturation. A number of anti-inflammatory drugs are known to inhibit the denaturation of proteins.

Protein denaturation assay

The denaturation of bovine serum albumin with the ethanol and ethyl acetate extract showed significant activity. The inflammatory drug (Salicylic acid, phenyl butazone, etc) has shown dose dependent ability to thermally induced protein denaturation.[17] The *C. monoica* extract may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal constituents

secrete bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damage.[18]

Proteinase inhibition assay

Proteinases are an enzyme that is present in all organisms and succours in many physiological processes from meek breakdown of food proteins to highly controlled cascade mechanism. Proteinase activity involves the hydrolysis of peptide bonds either specific peptide bond or the breakdown of complete peptide to amino acids. The activity ends up with either digesting the protein to its principal components or destroying the function of proteins.

Proteinase plays a noteworthy role in arthritic reactions. Neutrophil acts as a base for Proteinase enzyme. They transfer many serine proteinases in their lysosomal granules. It is involved in the progress of tissue damage during an inflammatory reactions and significant level of protection was provided by proteinase inhibition.[19] Therefore proteinase inhibitors are used in the treatment of inflammation process to inhibit tissue damage caused by proteinase enzymes.

The preliminary phytochemical studies revealed the presence of phenol, tannin, flavonoid, steroid and protein, which may be responsible for the anti-inflammatory activity.[20] The GC-MS analysis also resulted with various bioactive compounds such as hexadecanoic acid, carotene, tetracosohexane hexamethyl and neophytadiene that exhibit anti-inflammatory property.[21] The activity of ethanol extract of *C. monoica* leaves may be attributed due to the presence of these compounds. Thus, the studies proved the efficacy *C. monoica* leaves to treat inflammation.

CONCLUSION

Both ethanol and ethyl acetate showed potent anti-inflammatory activity. Based on the IC_{50} values ethanol extract of *C. monoica* leaves has less IC_{50} values which indicate progressive anti-inflammatory activity. An *in-vivo* study reveals the anti-inflammatory activity in depth. Therefore, further studies and compound isolation may be carried on with ethanol extract of *C. monoica* leaves which will pave a way for synthesis of anti-inflammatory drug.

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