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Investigation of *in-vitro* anti-inflammatory activity of *oxalis latifolia* kunth whole plant

Neelima S*[1], Bageswari B[2], Sainath Reddy C[2], Sai Suresh G[2], Haripriya M[2], Grace Angel N[2], Pradeep Kumar M[3].

[1]Department of Pharmacology, Vasavi Institute of Pharmaceutical Sciences, Vasavi Nagar, Peddapalli(Village), Sidhout (Mandal), YSR Kadapa (Dist)-516247, Andhra Pradesh, India.

[2]Vasavi Institute of Pharmaceutical Sciences, Vasavi Nagar, Peddapalli (Village), Sidhout (Mandal), YSR Kadapa (Dist)-516247, Andhra Pradesh, India.

[3]Department of Pharmaceutical Technology, Vasavi Institute of Pharmaceutical Sciences, Vasavi Nagar, Peddapalli (Village), Sidhout (Mandal), YSR Kadapa (Dist)-516247, Andhra Pradesh, India.

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Abstract

The primary purpose of the current study was to assess the *invitro* anti-inflammatory effects of methanolic extract of *Oxalis latifolia* Kunth (MEOL) using bovine serum and egg albumin protein denaturation method. In this method, anti-inflammatory was evaluated for the methanolic extract of *Oxalis latifolia* kunth (MEOL) whole plant and standard (Diclofenac sodium) at varying concentrations (100 - 500µg/ml). The bovine serum & egg albumin protein denaturation technique assessed MEOL for its anti-inflammatory activity. In both methods, it was found that an increase in the concentration of MEOL showed growth in the percentage of inhibition. The MEOL activity was compared to standards such as Diclofenac sodium. In this, it was evident that an increase in the concentration MEOL increased's anti-inflammatory activity of MEOL through inhibition of bovine serum & egg albumin protein denaturation. From the results, it was concluded that MEOL whole plant proved to have anti-inflammatory action by inhibiting bovine serum & egg albumin protein denaturation.

∗Corresponding Author
Name: Dr S. Neelima
Phone: +91 9701511457
Email: neelus1989@gmail.com

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INTRODUCTION

Inflammation, a body's protection system, is a wide variety of physiological and pathological processes characterized by four principal pointers, i.e., pain, redness, heat & swelling. Inflammation can occur due to pathogens such as bacteria, fungi, chemicals, or external injuries. Inflammation can be either acute or chronic [1]. The process is initiated by the augmented proclamation of chemical mediators, i.e., histamine, serotonin, leukotrienes, bradykinin, and prostaglandins from damaged tissue or cells. Apart from this, the

inflammatory mechanism involves a cascade of events in which arachidonic acid metabolism plays an important role. The Cyclooxygenase (COX) pathway of arachidonic acid releases prostaglandins & thromboxane A2, while the lipoxygenase (LOX) pathway releases leukotrienes. Non-steroidal anti-inflammatory drugs (NSAIDs) are mainly cast off for management or reduction of inflammation [2]. However, long term use of NSAIDs always results in serious adverse effects such as gastritis, hepatotoxicity, etc. So, herbs are given great importance compared to conventional drugs due to their fewer adverse effects. *Oxalis latifolia*kunth [OL], a perennial herb called Wooden sorrel, belongs to the family Oxalidaceae, which is native to Mexico, parts of central and South America, and the southern part of India [3]. The whole plant paste was traditionally used to treat pain by rubbing it on the wound.

This plant is also well known as a good appetizer for treating anaemia, dyspepsia, cancer, etc. The whole plant of *Oxalis latifolia*kunth was evaluated for cardiorelaxant, nematocidal, antifungal, anti-amoebic, anti-implantation, and antioxidant activities but not anti-inflammatory action. So, this research aims to assess the anti-inflammatory activity of the methanolic extract of *Oxalis latifolia*kunth whole plant using bovine serum & egg albumin protein denaturation technique [4].

MATERIALS AND METHODS

Materials:

Diclofenac sodium was obtained from Waksman Selman Pharmaceuticals Pvt. Ltd., Anantapur, India. Petroleum ether and 70% methanol were obtained from Changshu Hongsheng Fine Chemicals. All other reagents were procured from Himedia laboratories, which were of analytical grade.

Plant material:

The entire shrub of *Oxalis latifolia* was composed of local fields in Pullampeta, YSR Kadapa District, Andhra Pradesh. The identification & authentication of the collected leaves were conducted by Dr. K. Madhava Chetty from the Department of Botany at S.V. University, Tirupati. A voucher specimen (No. 0233) has been placed at the Department of Pharmacology, Vasavi Institute of Pharmaceutical Sciences, Vasavi Nagar, Andhra Pradesh, India.

Preparation of Methanolic Extract of *Oxalis latifoliakunth* (MEOL)

The entire plant of *Oxalis latifolia*Kunth was shade-dried under normal environmental conditions, then pulverized into a coarse powder &stored in opaque, tightly sealed jars for future use, as shown in **Figure 1**. Two hundred grams of the powdered plant material were placed in a flask, and 600 mL of 70% methanol was added to a one-lit round-bottom flask. Soxhlet assemblage was then set up for 10 to 15 cycles, as illustrated in **Figure 1**. The solvent was concentrated at a subordinate temperature under reduced pressure, as shown in **Figure 2**. Following this, the extract was cleaned, and the filtrate was concentrated with a water bath to attain the crude extract, which was stowed in a freezer for later use[5].

Figure 1 Dried plant and Powdered *Oxalis latifolia* kunth

Preliminary Phytochemical Screening:

The plant can be regarded as a biosynthetic laboratory, producing various amalgams.e., tannins, steroids, terpenoids, saponins, flavonoids, glycosides, alkaloids, carbohydrates, and phenolic compounds. These substances, known as secondary metabolites, are associated with therapeutic effects. To evaluate the presence or absence of primary and secondary metabolites, methanolic extract of *Oxalis latifolia* (MEOL) was exposed to a series of chemical tests [6].

***In-vitro* Anti-inflammatory action:** The *in vitro* anti-inflammatory action was assessed using bovine serum protein denaturation & egg albumin denaturation technique [7].

Bovine Serum Protein Denaturation Technique:

Preparation of Reagents

0.5% Bovine Serum Albumin (BSA):

Dissolved 500mg of BSA in 100 ml of water.

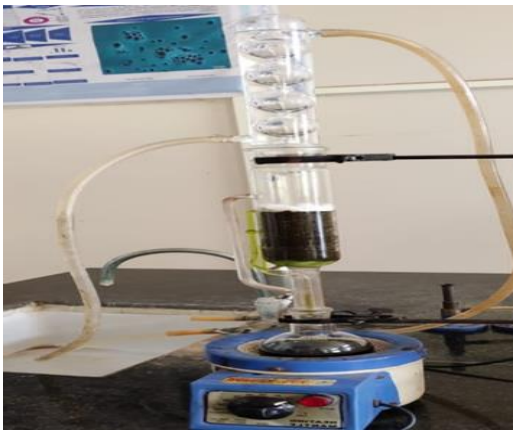

Figure 2 Extraction of OL whole plant

Phosphate Buffer Saline PH 6.3:

Dissolve 8 g of sodium chloride (NaCl), 0.2 g of KCl, 1.44 g of disodium hydrogen phosphate (Na_2HPO_4), and 0.24 g of potassium dihydrogen phosphate (KH_2PO_4) in 800 mL of water. Adjust the pH to 6.3 using 1 N HCl, then bring the total volume to 1000 mL with water [8].

Method:

0.5 ml test solution comprises 0.45 mL of bovine serum albumin (5% w/v aqueous solution) and 0.05 mL of MEOL at various concentrations (100, 200, 300, 400& 500 µg/mL). 0.5ml test control solution consists of 0.45 mL of bovine serum albumin and 0.05 mL of distilled water. 0.5ml standard solution includes 0.45 mL of bovine serum albumin &0.05 mL of diclofenac sodium (500 µg/mL) [9].

Procedure:

A volume of 0.05 mL of various concentrations (100 - 500 µg/mL) of MEOL and diclofenac sodium (500 µg/mL) was combined with 0.45 mL of 0.5% w/v BSA. The tasters were gestated at 37°C for 20 min, trailed by heating to 57°C for 3 min.

After freezing, 2.5 mL of phosphate buffer was added to each resolution. The absorbance was restrained using a UV-visible spectrophotometer at 255 nm. The control signifies 100% protein denaturation [10].

Egg Albumin Denaturation Technique:

The reaction mixture (5 mL) comprised 2 mL of the medication in multiple concentrations (100–500 µg/mL), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), and 0.2 mL of egg albumin (from a fresh hen's egg) [11]. The control was double-distilled water. After 15 minutes of incubation at 37 ± 2°C in a BOD incubator, the mixtures were stirred for five minutes at 70°C. After cooling, the vehicle was used as a blank to measure the absorbance at 660 nm. To assess absorbance, diclofenac sodium at concentrations of 100, 200, 300, 400, and 500 µg/mL was used the same way as the reference medication. The one that follows was applied to calculate the percentage inhibition of protein denaturation [12]:

$$\% \text{ inhibition } = 100 \times \frac{\text{V t}}{\text{V C} - 1}$$

Where V t = absorbance of the test sample

V c = absorbance of control

Three duplicates of each experiment were conducted, and the mean was calculated.

RESULTS:

Primary screening of MEOL for phytochemical constituents:

The obtained percentage yield in an extraction process of MEOL was 8.2% w/v.

The methanolic extract of *OL* (MEOL) was subjected to preliminary phytochemical examination for the existence of secondary

Table 1 Qualitative phytochemical screening of methanolic extract of OL whole plant

CONSTITUENT	MEOL
Tannins	+++
Steroids	-
Terpenoids	+++
Saponins	-
Anthraquinones	+
Flavonoids	+++
Glycosides	+
Alkaloids	+++
Carbohydrates	+++
Phenolics	+
Cardiac glycosides	+
Proteins	-

Table 2 Data showing Various concentrations and % inhibition of methanolic extract of *Oxalis latifolia* kunth

Sample	Concentrations in µg/ml	Absorbance	Percentage of Inhibition
STANDARD (Diclofenac)	100	0.0405±0.0021	42.14
	200	0.1415±0.0049	63.74
	300	0.161±0.0056	70.39
	400	0.403±0.0042	85.10
	500	0.414±0.0021	93.20
MEOL	100	0.029±0.0014	25.3
	200	0.0345±0.0021	35.4
	300	0.039±0.0014	45.4
	400	0.0505±0.0021	65.8
	500	0.06±0.0028	82.4

Table 3 Data showing Various concentrations and % inhibition of methanolic extracts of *Oxalis latifolia* kunth

SAMPLE	Concentration in µg/ml	Absorbance	Percentage of Inhibition
STANDARD (Diclofenac)	100	0.033±0.0028	65
	200	0.0365±0.0021	79
	300	0.078±0.0056	85
	400	0.095±0.0012	90
	500	0.121±0.0056	96
MEOL (70%)	100	0.04±0.0028	57
	200	0.048±0.0035	69
	300	0.096±0.0063	75
	400	0.184±0.0091	79
	500	0.299±0.0077	88

metabolites such as tannins, steroids, terpenoids, saponins, anthraquinone glycosides, alkaloids, carbohydrates, flavonoids by utilizing standard methods of analysis shown in **Error! Reference s ource not found.**.

***In-vitro* anti-inflammatory activity by Bovine Serum Denaturation Method**:

In *in-vitro* anti-inflammatory activity by Bovine Serum denaturation method at concentration of 100 - 500 µg/ml of methanolic extract of *Oxalis latifoliakunth* whole plant showed inhibition of bovine serum in a concentration-dependent manner whereas standard diclofenac at 100 - 500 µg/ml which showed 42.14, 63.74, 70.39, 85.10 and 93.2 % inhibition of denaturation of bovine serum and depicted in **Figure 3**.

***In-vitro* Anti-inflammatory Activity by Egg Albumin Denaturation Technique:**

In *in-vitro* anti-inflammatory action by Egg Albumin denaturation technique at a concentration of 100 - 500 µg/ml of methanolic extracts of *Oxalis latifolia* kunth whole plant showed inhibition of Egg Albumin in a dose-dependent manner whereas, standard diclofenac 100 - 500 µg/ml at which showed 82, 86, 90 and 96 % inhibition of Egg Albumin denaturation shown in **Table 3** and **Figure 4**.

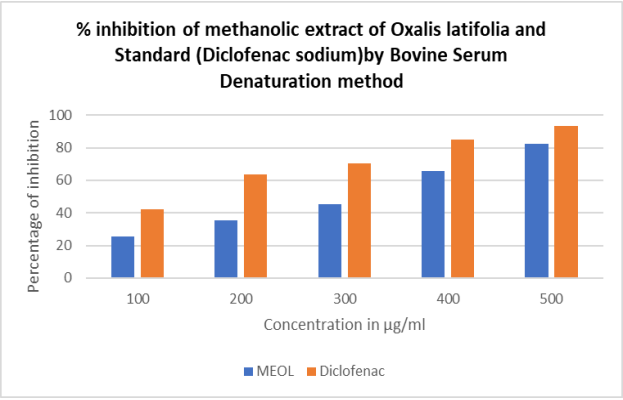

Figure 3 Percentage inhibition of MEOL and Standard by Bovine serum denaturation method

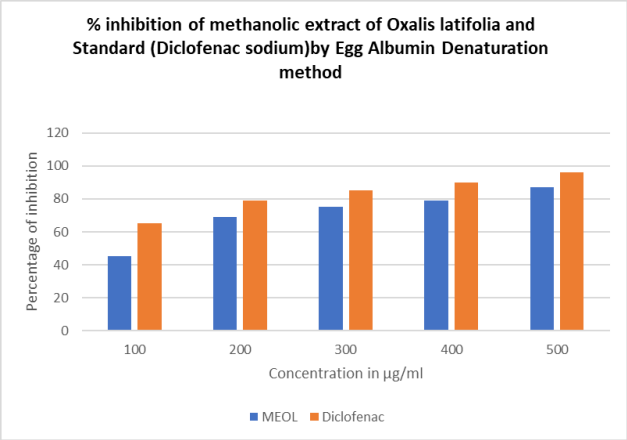

Figure 4 Percentage inhibition of MEOL and Standard by Egg Albumin denaturation method

DISCUSSION:

Indian texts, including Ayurveda and various ancient sources, have highlighted the use of herbal remedies for a wide range of health issues. *Oxalis latifolia* Kunth, a wooden sorrel in English and Pulichintha in Telugu, exhibits various pharmacological properties, including antidiabetic, antiulcer, antioxidant, nephroprotective, and hepatoprotective effects. Several bioactive compounds have been remote from diverse plant parts, showcasing notable pharmacological activities. Additional pharmacological studies employing modern techniques are needed to uncover the plant's full potential.

Our research aimed to evaluate the anti-inflammatory activity of methanolic extract of *Oxalis latifolia*whole plant by *in-vitro* models. The preliminary phytochemical studies of methanolic extracts highest presence of tannins, alkaloids, flavonoids, terpenoids, carbohydrates, cardiac glycosides & phytosterol, along with the absence of steroids &saponins. The inflammation is always a progression and correlated with the fragility of the lysosomal membranes, denaturation of proteins & release of inflammatory mediators.

MEOL was then evaluated for its ability to prevent protein denaturation in bovine serum & egg albumin solutions. Anti-inflammatory drugs have demonstrated a dose-dependent capacity to hinder thermally made protein denaturation. Therefore, protection against protein denaturation—previously considered a primary mechanism of action for NSAIDs before identification of their inhibitory effects on COX—may play a significant role in the antirheumatic properties of these drugs.

Rheumatoid arthritis (RA), a prevalent autoimmune disease, is characterized by chronic joint inflammation, leading to pannus formation that infiltrates lymphocytes and fibrinoid material within the synovial membrane.

Protein denaturation involves complex mechanisms, including alterations in electrostatic interactions, hydrophobic interactions, and hydrogen &disulfide bonds. This process can lead to the production of autoantigens in inflammatory conditions such as rheumatoid arthritis, cancer &diabetes. Consequently, inhibiting protein denaturation may help reduce inflammatory activity. In this study, NSAIDs were utilized as reference drugs, as they mitigate inflammation by inhibiting the activity of cyclooxygenase enzymes. However, these medications can also lead to adverse effects, including ulceration, bleeding, perforation, and obstruction. The fragility of lysosomal membranes, protein denaturation, and the subsequent release of inflammatory mediators can exacerbate inflammation in various diseases. Protein denaturation is a recognized contributor to inflammation, as most biological proteins lose their functional capabilities when denatured. In vivo, protein denaturation can lead to autoantigens formation, particularly in arthritic disorders. Serine proteinases from inflammatory cells, particularly neutrophils, are involved in several inflammatory disorders, including rheumatoid arthritis and pulmonary emphysema. Neutrophils are a significant source of serine proteinases and are predominantly found in lysosomes. A deficiency of protease inhibitors in circulation is a considerable risk factor for developing inflammatory disorders. Previous studies have shown that leukocyte proteinases contribute to tissue damage during inflammatory reactions and that proteinase inhibitors offer substantial protective effects.

In protein denaturation, the secondary & tertiary structures of proteins are disrupted by external factors such as heat, organic solvents, or strong acids and bases. In the present study, the plant extract demonstrated a high percentage of protein denaturation inhibition, comparable to diclofenac

sodium. The methanolic extract exhibited the most significant suppressive effect. Increases in absorbance of test samples compared to control suggest that the whole plant of *Oxalis latifolia*Kunth can effectively reduce thermal denaturation of proteins, mainly albumin.Agents that can avoid protein denaturation are promising candidates for anti-inflammatory activity. In this study, the methanolic extract of *Oxalis latifolia* (MEOL) demonstrated the capacity to inhibit protein denaturation. MEOL effectively inhibited heat-induced denaturation of both egg albumin &bovine serum albumin at varying concentrations between 100 and 500 µg/mL in a dose-dependent manner, with diclofenac sodium serving as the standard drug for comparison. The denaturation inhibition may be attributed to terpenoids, tannins, flavonoids& phenolic compounds found in MEOL.

CONCLUSION:

Protein denaturation inhibition was investigated to elucidate the anti-inflammatory mechanism of *Oxalis latifolia* Kunth. Our *in vitro* studies on the methanolic extracts of this plant revealed significant anti-inflammatory action, likely due to the presence of active compounds such as flavonoids, terpenoids, tannins&related polyphenols. Consequently, *Oxalis latifolia*Kunthhas the potential to serve as a potent anti-inflammatory agent. Further purification of its bioactive compounds is essential, as purified forms may exhibit enhanced activity. This study suggests that the phytoconstituents of the whole plant can be explored as lead compounds for developing effective anti-inflammatory drugs for treating various conditions, including rheumatoid arthritis.

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Author Contribution

All authors made substantial contributions to the conception, design, acquisition, analysis, or interpretation of data for the work. They were involved in drafting the manuscript or revising it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work, ensuring its accuracy and integrity.

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