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Original Research Article

Pharmacognostic Evaluation and Anti-inflammatory Potential of Hydroalcoholic Extracts and Fractions of *Oxalis corneculata*

Aditya Mallappa kamble[1]*, Ravi Shankar Rao[1], Rohit Kishor Patil[1], Nivedita Shivaji More[2]

[1]Sunrise Pharmacy College Alwar, Rajasthan, India, 301001
[2]Balasaheb Desai College of Pharmacy, Pal, Kolhapur, Maharashtra, India, 416209

***Corresponding Author:** Aditya Mallappa kamble
Sunrise Pharmacy College Alwar, Rajasthan, India, 301001

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Abstract: *Oxalis corniculata* has been traditionally recognized for its medicinal properties, yet its anti- inflammatory potential remains underexplored. This study investigates its pharmacognostic characteristics and anti-inflammatory activity, focusing on enzyme inhibition and modulation of key inflammatory pathways. The extract was obtained using ultrasound-assisted extraction (UAE), with optimization enhancing the yield of total phenolic and flavonoid content. The anti-inflammatory potential was evaluated through in vitro human red blood cell (HRBC) membrane stabilization and protein denaturation inhibition assays. Furthermore, in silico molecular docking and network pharmacology analyses were conducted to elucidate interactions between bioactive compounds and key inflammatory targets. The optimized UAE method significantly improved the extraction efficiency of bioactive compounds. The extract exhibited strong anti-inflammatory activity, with IC_{50} values of 65.27 µg/mL (HRBC assay) and 68.003 µg/mL (protein denaturation assay), comparable to standard anti-inflammatory agents. Network pharmacology analysis identified Beta- Sitosterol, apigenin, betulic acid, and swertisin as key active compounds interacting with crucial inflammatory mediators, including IL6, COX-2, NF-κB1, STAT3, MAPK8, TNF, NOS2, and TLR4. Pathway enrichment analysis revealed significant involvement of the IL- 17 signaling pathway, HIF-1 signaling, cytokine regulation, oxidative stress response, and immune signaling, highlighting the extract's multi-targeted anti-inflammatory action. *Oxalis corniculata* demonstrates significant anti-inflammatory activity through enzyme inhibition and modulation of inflammatory pathways. Its polypharmacological nature supports its potential as a natural therapeutic agent for inflammation-related disorders. Further in vivo and clinical studies are warranted to validate its efficacy and therapeutic applications.

Keywords: Ultrasound-Assisted Extraction, Oxalis Corniculata, Anti-Inflammatory, Network Pharmacology.

INTRODUCTION

Inflammation is an essential biological response that plays a crucial role in the body's defense mechanism against harmful stimuli, including pathogens, damaged cells, and irritants. This response is characterized by a complex interplay among various immune cells, blood vessels, and molecular mediators [1]. Under normal circumstances, inflammation serves to protect the body, promote healing, and restore homeostasis. However, when this response becomes dysregulated—either through excessive activation or prolonged duration—it can lead to chronic inflammatory diseases [2].

Chronic inflammatory conditions, such as rheumatoid arthritis, inflammatory bowel disease (IBD), asthma, and cardiovascular diseases, represent significant health challenges that affect millions of individuals worldwide [3]. The underlying mechanisms of these diseases are multifactorial, with genetic predisposition, environmental influences, and lifestyle choices playing pivotal roles in their etiology. For instance, autoimmune disorders like rheumatoid arthritis occur when the immune system mistakenly identifies healthy tissues as threats, leading to persistent inflammation, joint damage, and debilitating pain. Inflammatory bowel disease encompasses conditions such as Crohn's disease and ulcerative colitis,

where chronic inflammation of the gastrointestinal tract results in severe digestive issues and can significantly impair quality of life [4, 5].

Lifestyle factors have also been shown to exacerbate inflammation. Obesity, for example, is increasingly recognized as a pro-inflammatory state [6]. Excess adipose tissue produces various inflammatory mediators, which can lead to systemic inflammation and contribute to the development of metabolic syndrome—a cluster of conditions including insulin resistance, hypertension, and dyslipidemia. These factors not only heighten the risk of developing chronic inflammatory diseases but also worsen outcomes for those already affected [7-9].

Current estimates suggest that inflammatory diseases will increasingly affect a larger segment of the global population [10]. Projections indicate a rise in incidence and prevalence due to factors such as aging populations, urbanization, and changing lifestyle habits [11]. For instance, as populations age, the prevalence of age-related inflammatory diseases is expected to increase, thereby imposing a substantial burden on healthcare systems. Furthermore, urbanization often correlates with lifestyle changes that include decreased physical activity and poor dietary choices, both of which are associated with higher inflammation levels [12, 13]. As a result, inflammatory conditions are anticipated to account for a considerable portion of the global burden of disease, with rising numbers of individuals suffering from these conditions over the coming decades.

This anticipated increase underscores the urgent need for effective therapeutic strategies, particularly those that minimize the adverse effects commonly associated with conventional anti-inflammatory medications, such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. While these medications are effective in managing acute inflammatory symptoms, they can lead to significant side effects, including gastrointestinal bleeding, cardiovascular issues, and long-term dependence. Consequently, there is a pressing demand for natural products that exhibit anti-inflammatory properties, which could be utilized as complementary or alternative therapies [14-16].

In this context, *Oxalis corniculata* emerges as a promising candidate. Known for its extensive use in traditional medicine, this herb has been employed for various ailments, particularly those involving inflammation. *Oxalis corniculata* is rich in a diverse array of bioactive compounds, including flavonoids, alkaloids, terpenoids, and phenolic acids, which are believed to contribute to its medicinal properties [17]. For instance, flavonoids are well- documented for their anti-inflammatory and antioxidant effects, potentially mitigating oxidative stress and inflammatory responses [18]. Alkaloids, on the other hand, may exert analgesic effects and modulate immune responses, while terpenoids have been recognized for their potential to influence inflammatory pathways [19].

The use of hydroalcoholic extracts is particularly relevant, as this extraction method effectively combines water and alcohol to capture a broader spectrum of bioactive compounds from the plant. By enhancing the solubility of both polar and non-polar compounds, hydroalcoholic extracts can significantly improve the bioavailability and therapeutic potential of these phytochemicals [20]. Understanding the mechanisms through which hydroalcoholic extracts of *Oxalis corniculata* exert their effects is essential for elucidating their potential role in managing inflammatory diseases [21]. Mechanistic studies may focus on identifying specific molecular targets and signaling pathways involved in inflammation. For example, examining how the extracts influence the NF-κB pathway—a critical regulator of inflammatory responses—can provide insights into their anti- inflammatory mechanisms. Additionally, in vitro and in vivo models can be employed to further assess the efficacy of the extracts in reducing inflammation and improving clinical outcomes [22].

Through detailed pharmacognostic studies, including the identification and characterization of active constituents within *Oxalis corniculata*, this research aims to provide valuable insights into its therapeutic applications [23]. By focusing on both the pharmacological and mechanistic aspects of the extracts and their fractions, we seek to bridge the gap between traditional knowledge and modern scientific inquiry. This integration of traditional and scientific perspectives can ultimately contribute to the development of effective, safe, and accessible treatments for individuals suffering from inflammatory diseases. Furthermore, the findings from this research may support the standardization and quality control of herbal preparations, ensuring that they meet safety and efficacy standards for clinical use.

The significance of this research extends beyond the scope of *Oxalis corniculata*. As the scientific community continues to explore the intricate relationships between plants and health, the insights gained from this investigation could pave the way for discovering novel anti-inflammatory agents that may complement existing therapies. By rigorously investigating the pharmacognosy and anti-inflammatory effects of hydroalcoholic extracts of *Oxalis corniculata*, we aim to uncover the valuable therapeutic secrets held within this resilient herb. Ultimately, this study aspires to foster greater public awareness and appreciation for the medicinal potential of indigenous plants, encouraging their conservation and sustainable use [24].

In summary, the rising prevalence of inflammatory diseases necessitates the exploration of alternative therapeutic approaches, particularly those rooted in traditional medicine. *Oxalis corniculata* presents a promising opportunity for such exploration, given its rich history in herbal remedies and its diverse array of bioactive compounds. Through comprehensive pharmacognostic research and mechanistic studies, this investigation aims to validate the therapeutic potential of *Oxalis corniculata*, contributing to the development of safe, effective, and accessible treatments for inflammatory diseases, and bridging the gap between traditional knowledge and modern scientific practice.

MATERIALS AND METHODS

Pharmacognostical Studies

Materials:

Chemicals: Hydrochloric acid (HCl), Alcohol, Ether, KBr, Quercetin, Aluminum Chloride 0.1 M Phosphate buffer, Dinitrosalicylic acid (DNS), Sodium hydroxide, Potassium sodium, Acrobose¸Tartrate and Phloroglucinol HCl.

Instruments: UV-Visible spectroscopy, Fourier transform Infra-red spectroscopy, Fluorescence microscope and Muffle furnace.

Methods

1. Collection and Authentication of Plant Material: *Oxalis corniculata* were procured from the local market in Kolhapur and authenticated by the Agharkar Research Institute, Pune, Maharashtra. The collected plant materials underwent a thorough preparation process, including washing to remove impurities, draining to eliminate excess moisture, and slicing for uniform drying. The sliced samples were then dried under controlled conditions to preserve their bioactive compounds. Once dried, the material was finely blended to obtain a uniform powder and subsequently sieved to ensure consistency. This prepared plant material was then utilized for further pharmacognostic and anti-inflammatory studies, ensuring quality and reliability in experimental evaluations.

2. Processing of Plant Material: Whole plant material was washed thoroughly with tap water. Further cleaned material was shade dried after complete drying, the plant material was made in to coarse powder and stored until further use [35].

Pharmacognostic Evaluation

Plant material was evaluated for various pharmacognostic parameters as mentioned below as per standard procedures.

Morphological Characteristics

The freshly collected plant material was subjected to organoleptic evaluation such as color, odour, taste and some extra Characteristics features are evaluated.

Microscopical Characters

The Powdered plant material was studied for various microscopical characters with the help of Trinocular microscope as per standard procedures and reported [36].

Physicochemical Parameters

The powder was evaluated for various physicochemical parameters which includes

- Loss on Drying
- Ash values
- Extractive value

- **Loss on Drying**

The mass is lost due to heat expressed as a w/w percentage. The correctly weighed 2gm sample was poured onto a petri dish and placed in a hot air oven at 105°c for 2hours losses on drying of the specimens to the initial volumes were calculated [37].

$$\%\text{Loss of drying} = \frac{\text{Final volume}}{\text{Initial volume}} X100$$

Ash Value

Total Ash

Weighing 2 gm at temperature of 500 to 600°c were engraved in a crucible in a muffle furnace until carbon free ash was obtained. It was then cooled, weighed, and the percentage of total ash was calculated relative to the air-dried drug [38].

$$\%\text{Total ash} = \frac{\text{Weight of total ash}}{\text{Weight of crude drug}} \times 100$$

Acid Insoluble Ash

The ashes obtained above where boiled with 25 ml of hydrochloric acid for 5 minutes and filtered using an ashless filter paper. The insoluble material placed on the filter paper was washed with hot water and the filter paper was lit to a constant weight in a muffle furnace. The percentage of acid insoluble Ash was calculated relative to the air dried drug [39].

$$\%\text{Acid insoluble Ash} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of crude drug}} \times 100$$

Water Soluble Ash

The ashes obtained were boiled with 25 ml of water for 5 minutes and the insoluble material collected on the filter paper without Ash washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°c in a muffle furnace. The difference in the weight of ash and the weight of water insoluble material give rise to water soluble ash. The percentage of water soluble as was calculated relatively to air dried powder [40].

$$\%\text{Water soluble Ash} = \frac{\text{Weight of total ash} - \text{Weight of water soluble ash}}{\text{Weight of crude drug}} \times 100$$

Extractive Value

Alcohol Soluble Extractive

Weighed with precision, the 4g sample was macerated in a conical flask with 100 ml of alcohol for 24 hours, shaking continuously at 6 hour intervals, and then allowing it to stand for 18 hours and filter quickly. The 25 ml filter was evaporated until it dried in a porcelain dish and dried to a constant weight at 105 °c. The percentage of alcohol-insoluble extract was calculated relative to the air dried material [41].

$$\%\text{ Alcohol soluble extractive} = \frac{\text{Weight of extract}}{\text{Weight of plant material}} \times 100$$

Water Soluble Extractive

The Preferably weighed 4g sample was successively macerated with 100 ml of water in a conical flask with shaking it was then boiled slowly for 1 hour in a water bath, cooled and weighed. The 25 ml filtrate was evaporated until it dried in a porcelain dish and dried to constant weight at 105°c. The percentage of water soluble extract was calculated relative to the air dried material [42].

$$\%\text{ Water soluble extractive} = \frac{\text{Weight of extract}}{\text{Weight of plant material}} \times 100$$

Ether Soluble Extractive

Weighed with precision, the 4g sample was macerated in a conical flask with 100 ml of ether for 24 hours with shaking. It was then boiled slowly for 1 hour in a water bath, cooled and weighed. The 25 ml filtrate was evaporated until it dried in a porcelain dish and dried to constant weight at 105°c. The percentage of chloroform soluble extract was calculated relative to the air tried material [43].

$$\%\text{ Ether soluble extractive} = \frac{\text{Weight of extract}}{\text{Weight of plant material}} \times 100$$

Preliminary Phytochemical Screening

Initial phytochemical screening helps to identify the types of secondary metabolites that are present in plants. There is discussion of the numerous chemical tests that were performed.

- **Test for Carbohydrates:**
  a. **Molisch's test:** Few drops of alcoholic alpha naphthol solution, few drops of concentrated sulphuric acid were

added given sample was taken in a test tube. The test result gave a purple or violet-coloured.

b. **Benedict's test:** Added Benedict's reagent to the given sample in a test tube, heated the test tube in a boiling water bath. Red precipitation was formed.
c. **Fehling's Test:** Filtrates were heated with Fehling's A & B solutions; formation of red precipitate indicates the presence of reducing sugars.

- **Detection of alkaloids Alkaloids Test**
  - **Mayer's Test:** Mayer's reagent added to sample yellow ppt was formed.
  - **Dragendroff's Test:** Dragendroff's reagent added to sample orange ppt was formed.
  - **Wagner's Test:** Wagner's reagent added to sample reddish brown ppt was formed.
  - **Hager's Test:** Hager's reagent added to sample yellow ppt was formed.

- **Test for Steroids Sterols**

a] Libermann-Burchad Test: Added 2ml chloroform, 2 drops of concentrated sulphuric acid, 10 drops of acetic anhydride to the extract. The chloroform layer showed a Bluish red color.
b] Salwoski Test: Added 1-2 drops of concentrated sulphuric acid & chloroform to extract. The bluish-red color was formed.

Glycosides Test
c] Legal Test: Added sodium nitroprusside, pyridine to the extract, pinkish-red color was formed.
d] Baljet Test: Added picric acid to the given sample. The orange color showed a positive result.

- **Flavonoids Test**

a] Shinoda test: 1-2 drops of concentrated hydrochloric acid, magnesium turnings to extract. The red color showed a positive result.
b] Ferric chloride test: Added ferric chloride to the sample greenish-black color was formed.

- **Test for Tannin:**

Gelatin Test: Added 10% sodium chloride in 1% gelatin solution to the extract gave white precipitation.

- **Detection of Phenols**

Ferric chloride test: 1ml of sample 3-4 drops of ferric chloride added in clean test tube formation of blue colour indicates the presence of phenols.

Quantitative Estimation of Phytoconstituents

Tannins, flavonoids, phenols, alkaloids, and several other aromatic compounds or secondary metabolites present in plants that serve as a defence Mechanism against a variety of bacteria, insects, and herbivores. These secondary metabolites may contribute to the therapeutic capabilities of plants. Some common and other different ailments may be treated using medicinal herbs.

Estimation of Total Phenolic Content

Plants include phenolic compounds, well-known phytochemicals. They are composed of benzoic acid, cinnamic acid, coumarins, tannins and lignin's. Plant phenolic compounds are divided into simple phenols, and polyphenols based on the total amount of phenol units in the molecule. Total phenolics are detected chemically, and phenolic components are recognised and quantified using spectrophotometric and chromatographic methods. An aromatic ring with one or more hydroxyl groups functions as the fundamental structural component of phenolic compounds. The biological features of these chemicals include antioxidant, anti- aging, anti-carcinogen, protection against autoimmune, immunological, and cardiovascular illnesses, as well as protection against brain disorders including Parkinson's, Alzheimer's, and Huntington's diseases [44].

Instrument: Shimadzu UV Visible spectrophotometer, Model 1800

Reagents
Folin Ciocalteu Reagent (1N)

An equivalent volume of distilled water was used to dilute the readily accessible Folin Ciocalteu Reagent (2N). The finished solution has been placed in a brown bottle and maintained in a at 4°C refrigerator. Sodium carbonate solution (10%)

Procedure

Gallic acid was weighed and diluted in water to create a solution with a concentration of 1 mg/ml. This solution was further diluted to produce concentrations ranging from 2 to 10 µg/ml. Folin Ciocalteu reagent (0.5 ml) was added to

the solutions and allowed to stand for 15 minutes. Then, 1 ml of 10% sodium carbonate solution was added and the mixtures were made up to 10 ml with distilled water. They were allowed to stand for 30 minutes at room temperature, and the total phenols were determined by spectrophotometrically measuring the absorbance at 760 nm with the reagent as the blank. The extract of *Oxalis corniculata* was also weighed and diluted to get a solution of 1 mg/ml. Different concentrations of the solution were taken in separate test tubes and the same procedure was repeated. A calibration curve was generated by plotting the concentration of gallic acid versus absorbance, and a linear regression equation was determined using regression analysis. The total phenol content was calculated using the linear regression equation and expressed as mg of gallic acid equivalent per gm of extract (mg GAE/g). The results obtained were presented in Table 3.

Estimation of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) is typically determined using the Aluminum Chloride ($AlCl_3$) Colorimetric Assay, which measures the flavonoids' ability to form stable complexes with aluminum chloride, resulting in a color change measurable at 415 nm [45].

Materials Required:

Plant Extract/Sample

- Aluminum chloride ($AlCl_3$, 10%)
- Sodium nitrite ($NaNO_2$, 5%)
- Sodium hydroxide (NaOH, 1M)
- Methanol (80%)
- Quercetin or Rutin (Standard flavonoid compound)
- UV-Visible Spectrophotometer
- Distilled water

Procedure:

Preparation of Standard Solution:

1. A stock solution of quercetin/rutin (standard flavonoid) is prepared in methanol at a concentration of 1 mg/mL.
2. A calibration curve is generated by preparing standard dilutions (e.g., 10, 20, 40, 60, 80, and 100 µg/mL).

Sample Preparation:

The plant extract is dissolved in 80% methanol to obtain a suitable concentration for analysis.

Reaction Setup:

1. 0.5 mL of sample or standard solution is taken in a test tube.
2. 2 mL of distilled water is added.
3. 0.15 mL of 5% $NaNO_2$ is mixed and incubated for 5 minutes at room temperature.
4. 0.15 mL of 10% $AlCl_3$ is then added, followed by another incubation for 5 minutes.
5. 1 mL of 1M NaOH is added to the reaction mixture, and the total volume is adjusted to 5 mL with distilled water.
6. The solution is mixed thoroughly and allowed to stand for 15 minutes.

Absorbance Measurement: The absorbance of the reaction mixture is measured at 415 nm using a UV- Visible spectrophotometer.

Calculation of Total Flavonoid Content: A standard calibration curve is used to determine the flavonoid content in the plant extract.

TFC is expressed as mg quercetin equivalent (QE) per gram of dry weight (mg QE/g DW) using the formula:
$TFC=C\times V/M$

Where:

C = Concentration from the standard curve (mg/mL)
V = Volume of extract (mL)
M = Mass of plant extract used (g)

***IN VITRO* ANTIOXIDANT ACTIVITY**

Since antioxidants neutralize or eliminate "reactive oxygen species" (ROS) or free radicals before they cause cell harm, they have become an essential component of our life today. The oxidation caused by ROS leads to DNA mutations, membrane protein damage, and cell membrane disintegration, which accelerates the ageing process and the progression of a number of diseases, including arteriosclerosis, cancer, Anti-inflammatory, liver damage, inflammation, skin damage,

coronary heart disease, and arthritis. Antioxidants are chemical substances that slow down the lipid oxidation process in food systems.

Antioxidants are defined as compounds that fight oxidation or suppress processes induced by oxygen or peroxides; many of these substances are preservatives used in a variety of goods. Antioxidants are compounds that are added to products for the purpose of preventing degradation caused by the action of oxygen in the air [46]. They can be synthetic or natural. Examples include enzymes or other chemical compounds like vitamin E or -carotene.

A chemical entity known as a free radical possesses one or more unpaired electrons and is capable of independent life. Free radicals can react with molecules without regard to their structure since they are less stable than non-radicals [47]. Once generated, radicals can either react with other radicals or with molecules that are not radicals through a variety of interactions. Covalent bonds are created when two radicals meet and share one electron. Non- radical compounds are the most prevalent found in *in vivo*. A radical change from being a radical to being a non-radical by giving or taking an unpaired electron from other molecules. A new radical form at the same moment.

MATERIALS AND METHODS

Method 1. Free radical scavenging activity using diphenyl picrylhydrazyl (DPPH) free radical

According to Brand Williams, the capacity of the extracts decreases the colour of DPPH serves as a measure of their free radical scavenging capability [48].

Principle

The DPPH stable free radical technique is a simple, quick and accurate way to evaluate a compound's or plant extract's level of antioxidant activity. The stable 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical is used in a straightforward approach that has been devised to assess the antioxidant activity of plants [49]. The odd electron of the DPPH free radical causes an important absorption maximum with a purple colour at 517 nm odd electron of DPPH-H reacts with hydrogen atom of sample purple colour of DPPH turns into yellow. Regarding the quantity of electrons caught, the ensuing decolorization is stoichiometric [50].

DPPH + AH= DPPH -H + A

Procedure:

Oxalis corniculata sample stock solution with a 10 mg/ml concentration was prepared. 4ml of DPPH was added to 1ml of test samples at varying doses. Control was made without the sample. In the case of a blank, ethanol was used instead of DPPH. incubate the sample for 30 mints. readings were then determined at 517 nm. Using the formula, the percentage of scavenging was determined. Ascorbic acid used as reference.

% Inhibition = [(Control-Test)/Control] x 100.

Using linear regression analysis, the sample concentration needed to achieve a reduction of 50% in absorbance (IC_{50}) was determined. The results obtained are presented in table 5 and fig 4.

Method 2: Nitric Oxide (NO) Scavenging Assay

The nitric oxide (NO) scavenging activity of the test samples was evaluated using the sodium nitroprusside (SNP) assay method, following the standard protocol with slight modifications [51].

Chemicals and Reagents

Sodium Nitroprusside (SNP)
Griess reagent (Sulfanilamide, Phosphoric acid, Naphthyl ethylenediamine dihydrochloride)
Phosphate-buffered saline (PBS, pH 7.4)
Ascorbic acid or gallic acid (Standard)
Dimethyl sulfoxide (DMSO) (if required for sample solubilization)

Preparation of Solutions

Sodium Nitroprusside Solution: A 10 mM solution of SNP was prepared in phosphate-buffered saline (PBS, pH 7.4).
Griess Reagent: A freshly prepared mixture of equal volumes of 1% sulfanilamide in 5% phosphoric acid and 0.1% Naphthyl ethylenediamine dihydrochloride (NED) was used.
Standard Solution: Ascorbic acid or gallic acid was prepared in different concentrations (e.g., 10–100 µg/mL) as a reference antioxidant.
Test Sample Preparation: The plant extract or phytosome formulation was prepared in PBS or DMSO to obtain different concentrations (10–100 µg/mL).

Assay Procedure

Reaction Mixture Preparation:

A total of 1 mL of different concentrations of the test sample (or standard) was mixed with 1 mL of 10 mM SNP solution in PBS.

The mixture was incubated at 25°C for 150 minutes under light to allow the production of nitric oxide.

Nitric Oxide Detection:

After incubation, 0.5 mL of the reaction mixture was mixed with 0.5 mL of freshly prepared Griess reagent.

The mixture was incubated in the dark for 10 minutes at room temperature.

Absorbance Measurement:

The absorbance of the pink-coloured complex was measured at 546 nm using a UV-Visible spectrophotometer.

A control (without test sample) and blank (without SNP) were also prepared.

Calculation of % Inhibition

The percentage inhibition of nitric oxide production was calculated using the following equation:

% Inhibition= [(Control-Test)/Control] x 100.

Where:

Ac = Absorbance of the control (without extract)

As = Absorbance of the sample or standard

In vitro anti-inflammatory activity

Method 1: The Human Red Blood Cell (HRBC) Membrane Stabilization Method:

The HRBC membrane stabilization method evaluates the anti-inflammatory potential of a test compound by assessing its ability to protect red blood cells from hypotonicity-induced hemolysis. In inflammatory conditions, lysosomal enzymes released from destabilized cell membranes contribute to tissue damage. Since the erythrocyte membrane is structurally similar to lysosomal membranes, its stabilization reflects the ability of a compound to prevent lysosomal rupture and subsequent inflammatory damage. When exposed to a hypotonic solution, RBCs undergo osmotic stress, leading to hemolysis. However, anti-inflammatory agents strengthen the membrane by preventing leakage of hemoglobin, which is quantified spectrophotometrically. Higher membrane stabilization indicates a stronger protective effect, suggesting potential anti-inflammatory properties of the test compound.

The human red blood cell (HRBC) membrane stabilization method:

Procedure for Human Red Blood Cell (HRBC) Membrane Stabilization Method [52, 53]

Preparation of HRBC Suspension

Collect fresh human blood from a healthy volunteer in an anticoagulant-containing tube (e.g., heparinized tube).

Centrifuge the blood at 3000 rpm for 10 minutes at 4°C to separate the plasma.

Discard the supernatant and wash the packed red blood cells (RBCs) three times with normal saline (0.9% NaCl) by centrifugation at 3000 rpm for 10 minutes each time reconstitute the washed RBCs in isotonic saline to obtain a 10% v/v suspension.

Hypotonicity-Induced Hemolysis Assay

Prepare hypotonic solution by diluting phosphate buffer saline (PBS) pH 7.4 or using distilled water.

Take 0.5 mL of the prepared HRBC suspension in different test tubes.

Add 1 mL of the test sample at different concentrations (prepared in isotonic saline).

For the control, replace the test sample with 1 mL of isotonic saline.

For the standard, add 1 mL of diclofenac sodium (or aspirin) solution as a reference anti-inflammatory drug.

Add 5 mL of hypotonic solution to all tubes and incubate at 37°C for 30 minutes.

Centrifuge at 3000 rpm for 10 minutes to separate the unlysed cells.

Collect the supernatant and measure the absorbance at 540 nm using a UV-Vis spectrophotometer.

Calculation of Membrane Stabilization (%)

The percentage of membrane stabilization (protection) is calculated using the formula:

$$\%\text{Protection} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100$$

Method 2:

Protein Denaturation Method:

The protein denaturation method evaluates the anti-inflammatory potential of a compound by assessing its ability to inhibit heat-induced denaturation of proteins. During inflammation, tissue proteins undergo structural alterations, leading to inflammatory responses. Since denaturation of proteins like egg albumin mimics these changes, stabilizing them reflects the potential of a compound to prevent protein degradation. In this assay, egg albumin is exposed to heat (70°C) in the presence of test compounds, and the degree of denaturation is measured spectrophotometrically at 660 nm. Compounds that inhibit denaturation show reduced absorbance, indicating their ability to protect proteins from thermal damage, thereby suggesting their anti-inflammatory potential [54, 55].

Procedure for Protein Denaturation Method

Preparation of Reagents

Phosphate Buffer (pH 6.3): Prepare by dissolving appropriate amounts of NaH_2PO_4 and Na_2HPO_4 in distilled water.
Egg Albumin Solution (5% v/v): Collect fresh hen's egg, separate the albumin, and dilute to 5% v/v with phosphate buffer.
Test Samples: Prepare different concentrations of the test compound in phosphate buffer.
Standard Drug: Prepare diclofenac sodium or aspirin solution as a reference anti- inflammatory agent.

Experimental Setup

Take 0.2 mL of egg albumin solution in different test tubes.
Add 2.8 mL of phosphate buffer (pH 6.3) to each tube.
Add 2 mL of test sample at varying concentrations to respective tubes.
For control, replace the test sample with 2 mL of phosphate buffer.
For standard, add 2 mL of diclofenac sodium (or aspirin) solution instead of the test sample.
Incubate all tubes at 37°C for 15 minutes.
Heat the tubes in a water bath at 70°C for 5 minutes to induce protein denaturation.
Allow the tubes to cool at room temperature.
Measure the absorbance at 660 nm using a UV-Vis spectrophotometer.
Calculation of Inhibition (%)

The percentage inhibition of protein denaturation is calculated using the formula:

$$\%\text{Protection} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100$$

RESULTS AND DISCUSSION

1. Morphological Evaluation of *Oxalis corniculata* Extract

The morphological evaluation is an important tool for the identification and characterization of plant extracts. The morphological characteristics of the *Oxalis corniculata* extract are summarized in Table 01.

Table 01: Morphological Characters of *Oxalis corniculata* Extract

Parameters	Observation
Color	Dark brown/Greenish-brown (depending on extraction method)
Odor	Mild characteristic herbal odor
Taste	Slightly sour due to oxalic acid content
Size	Semi-solid (in case of concentrated extract) or fine powder (after drying)
Shape	Amorphous or crystalline (depending on drying technique)

These morphological attributes aid in the preliminary identification and standardization of *Oxalis corniculata* extract for pharmacognostic and phytochemical studies.

2. Physicochemical Evaluation of *Oxalis Corniculata* Extract

Physicochemical parameters are crucial for the standardization and quality assessment of plant-based materials, ensuring consistency, purity, and efficacy. These parameters help in identifying adulteration, degradation, and overall chemical composition. The physicochemical properties of *Oxalis corniculata* extract, including moisture content, ash values, and extractive yields, are detailed in Table 02.

Table 02: Physicochemical Parameters of *Oxalis corniculata* Extract

Parameter	Observation (% w/w)
Moisture Content	8.38 ± 0.36
Total Ash Value	7.16 ± 0.31

Acid Insoluble Ash	0.6 ± 0.0
Water Soluble Ash	1.13 ± 0.36
Alcohol-Soluble Extractive	7.74 ± 0.28
Water-Soluble Extractive	5.00 ± 0.1
Ether-Soluble Extractive	1.12 ± 0.0

Preliminary Phytochemical Screnning

Phytochemical evaluation is essential for identifying the bioactive compounds present in *Oxalis corniculata*. These metabolites play a crucial role in therapeutic applications, contributing to the plant's anti-inflammatory, antioxidant, antimicrobial, and metabolic- regulating properties. The qualitative phytochemical analysis of *Oxalis corniculata* leaf extract reveals the presence of carbohydrates, alkaloids, flavonoids, steroids, proteins, tannins, and saponins, as shown in Table 03.

Table 03: Phytochemical Investigations of *Oxalis corniculata* Extract

Phytochemicals	Presence (+)	Significance & Mechanism
Carbohydrates	+	Serve as an energy source, contribute to metabolic functions, and provide structural stability to plant-derived formulations.
Alkaloids	+	Possess anti-inflammatory, analgesic, and antimicrobial properties by interacting with cellular receptors and modulating biological pathways.
Flavonoids	+	Act as potent antioxidants that neutralize free radicals, reduce oxidative stress, and exhibit anti-inflammatory, anti-cancer, and cardioprotective effects.
Steroids	+	Contribute to hormonal balance, membrane stabilization, and possess anti-inflammatory and immune-modulating activities.
Proteins	+	Essential for enzymatic reactions, cellular functions, and contribute to structural and metabolic processes.
Tannins	+	Exhibit astringent, antimicrobial, and antioxidant properties by binding to proteins and metal ions, reducing inflammation and microbial growth.
Saponins	+	Known for their foaming properties, they enhance bioavailability, act as natural emulsifiers, and contribute to antioxidant and immune-boosting activities.

Total Flavonoids Content

The total flavonoid content (TFC) of *Oxalis corniculata* represents the cumulative amount of flavonoid compounds present in the extract, measured in terms of quercetin equivalents (QE). Flavonoids are bioactive polyphenolic compounds known for their antioxidant, anti- inflammatory, anti-inflammatory, and cardioprotective properties.

Table 04: Total Flavonoid content

Concentration (µg/mL)	Absorbance
0	0
2	0.085
4	0.154
6	0.249
8	0.330
10	0.398
Sample	**Absorbance**
Sample	0.62

Sample	Conc. µg/ml	V=Vol. of sample (ml)	W=Wt. of dry extract per ml(g)	Sample absorbance	Y=mx+c	C_1=QC Conc. C(mg/ml)	C_1=QC Conc. C(µg/ml)	TFC (mg/100 mg QE) $C=C_1V/m$
F4	1000	1	0.001	0.62	y = 0.0403x+ 0.0012	15.355	0.0153	18.34

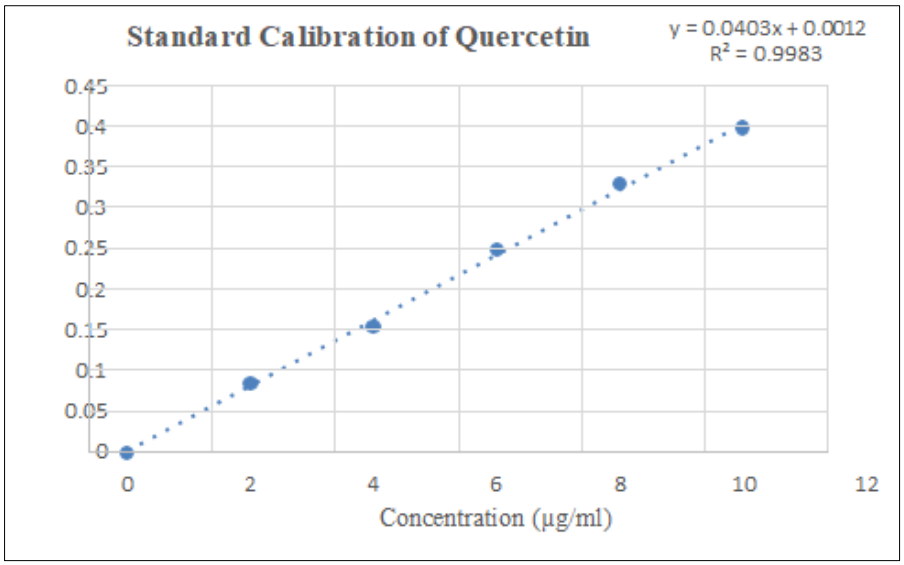

Figure 1: Total Flavonoid content by $AlCl_3$ Method

Determination of Total Phenolic Content

Determination of Total Phenolic Content (TPC) of *Oxalis Corniculata*

The total phenolic content (TPC) of *Oxalis corniculata* extract represents the cumulative concentration of phenolic compounds, which are essential for antioxidant, anti- inflammatory, and anti-inflammatory properties.

Analysis of Total Phenolic Content (TPC)

The total phenolic content is expressed in mg of Gallic Acid Equivalents (GAE) per gram of extract, as determined by the calibration curve of standard gallic acid (Figure 2).

S. No	Conc. of Gallic acid in µg/ml	Absorbance at 760 nm	Amount of total Phenolic content in terms mg gallic acid equivalent/ g of extract*
1	2	0.116	
2	4	0.186	
3	6	0.246	37.97±0.38
4	8	0.325	
5	10	0.386	
6	sample	0.092	

Fig. 2: Calibration curve of Gallic acid

*Values are mean ± SD (n = 3)

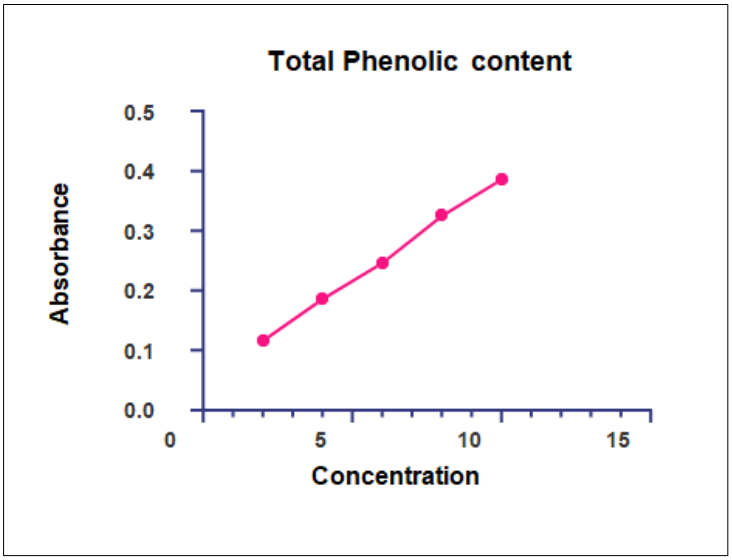

Figure 3: Calibration curve of gallic acid

The linear regression equation was found to be Y=0.034x+0.0481while the correlation coefficient was found to be 0.9985. The amount of phenol content present in the extract in terms mg GAE/g of extract was found to be 37.97±0.38 by using the above linear regression equation.

Antioxidant Activity by DPPH method:

Method 1: DPPH free Radical Scavenging Assay

The DPPH free radical scavenging assay of *Oxalis corniculata* extract demonstrated a concentration-dependent increase in antioxidant activity. At 10 µg/mL, the extract showed 36.07% inhibition, which increased to 43.65% at 20 µg/mL, 49.34% at 30 µg/mL, 65.99% at 40 µg/mL, and 68.83% at 50 µg/mL. Comparatively, ascorbic acid exhibited a slightly stronger effect, with 21.35% inhibition at 10 µg/mL, reaching 79.87% at 50 µg/mL. The IC_{50} value, which represents the concentration required to inhibit 50% of DPPH radicals, was 37.295 µg/mL for the extract and 28.945 µg/mL for ascorbic acid, indicating that while *Oxalis corniculata* possesses significant antioxidant potential, it is slightly less potent than ascorbic acid. This result confirms the presence of bioactive compounds such as flavonoids and phenolics contributing to its free radical scavenging activity.

Table 5: Percentage inhibition of *Oxalis corniculata* extract and std ascorbic acid against DPPH at 517nm.

Sl.no	Conc (µg/ml)	Ascorbic acid	Hydroalcoholic extract
1	10	21.34707	36.06919
2	20	36.29003	43.65109
3	30	52.33714	49.33714
4	40	65.9919	65.9919
5	50	79.8675	68.82591
	IC50	28.945	37.295

Method 2: Nitric Oxide (NO) Scavenging Assay

The Nitric Oxide (NO) Scavenging Assay of *Oxalis corniculata* extract demonstrated its potential as an antioxidant by inhibiting nitric oxide radicals in a concentration-dependent manner. At 10 µg/mL, the extract showed 19.21% inhibition, increasing to 32.24% at 20 µg/mL, 43.24% at 30 µg/mL, 53.70% at 40 µg/mL, and 65.50% at 50 µg/mL. In comparison, the standard antioxidant, ascorbic acid, exhibited stronger scavenging activity, with 38.73% inhibition at 10 µg/mL, increasing progressively to 72.96% at 50 µg/mL. The IC_{50} value, which indicates the concentration required to inhibit 50% of nitric oxide radicals, was 43.9539 µg/mL for the extract and 23.724 µg/mL for ascorbic acid, suggesting that a higher concentration of the extract is required to achieve the same level of NO scavenging as ascorbic acid.

Table 6: Percentage inhibition of Nitric Oxide (NO) Scavenging Assay

Concentration	Ascorbic acid	Sample
10	38.73391	19.20601
20	45.65451	32.24249
30	56.97425	43.24034
40	65.34335	53.70172
50	72.96137	65.50429
Ic50 value	23.724	43.9539

Anti-Inflammatory Activity

Human Red Blood Cell (HRBC) Membrane Stabilization Method

The Human Red Blood Cell (HRBC) Membrane Stabilization Method evaluates the anti- inflammatory potential of *Oxalis corniculata* by assessing its ability to protect erythrocyte membranes from hypotonic-induced lysis. The percentage inhibition of hemolysis by the extract increased in a dose-dependent manner. At 20 µg/mL, the extract exhibited 14.98% inhibition, which gradually rose to 29.47% at 40 µg/mL, 43.17% at 60 µg/mL, 58.55% at 80 µg/mL, and 75.79% at 100 µg/mL. In comparison, the standard (likely diclofenac or aspirin) demonstrated a higher stabilizing effect, starting at 30.18% inhibition at 20 µg/mL, reaching 90.12% at 100 µg/mL. The IC_{50} value, which represents the concentration needed to achieve 50% membrane stabilization, was found to be 65.28 µg/mL for the extract and 47.57 µg/mL for the standard, indicating that the extract requires a higher concentration to achieve similar stabilization.

Table 7: Percentage inhibition of Human Red Blood Cell (HRBC) Membrane Stabilization Method

Concentration	Standard	Sample
20	30.18077	14.98559
40	43.69924	29.47341
60	58.29185	43.17527
80	74.53498	58.55384

Concentration	Standard	Sample
100	90.12313	75.79251
IC50 VALUE	47.57166	65.2791

Method 2: Protein Denaturation Method

The Protein Denaturation Method is used to evaluate the anti-inflammatory potential of *Oxalis corniculata* by measuring its ability to prevent heat-induced protein denaturation, which is a key process in inflammation. The percentage inhibition of protein denaturation increased in a concentration-dependent manner for both the extract and the standard. The extract exhibited 21.91% inhibition at 20 µg/mL, increasing to 33.88% at 40 µg/mL, 46.71% at 60 µg/mL, 57.04% at 80 µg/mL, and 67.55% at 100 µg/mL. In comparison, the standard (likely diclofenac or aspirin) showed a higher inhibitory effect, starting at 34.43% inhibition at 20 µg/mL and reaching 79.57% at 100 µg/mL.

Table 8: Percentage inhibition of Protein Denaturation Method

Concentration	Standard	Sample
20	34.43892	21.91819
40	46.40685	33.88612
60	58.04312	46.71089
80	68.13156	57.04809
100	79.57345	67.55113
IC50 VALUE	46.92857	68.00385

Network pharmacology

Mining of Phytoconstituents and Targets

The components of *Oxalis corniculata* were obtained from Dr Duke, IMPPAT and Published articles, a total of 13 bioactive components were obtained. The chemical composition of *Oxalis corniculata* was confirmed using the PubChem. The detailed information given in table 7. The targets of identified compounds were screen out by using digep pred software.

Identification of Anti-Inflammatory Targets

A total of 9373 genes were obtained from the Gene Card databases using "Anti inflammatory," as keyword; These were compared with the target genes of the active ingredients to obtain potential Anti inflammatory target genes for each active ingredient with the help of venny 2.0. After comprehensive acquisition of the potential Anti inflammatory target genes of *Oxalis corniculata*, a total 67 potential targets related to the treatment of Anti inflammatory were selected as candidate targets.

Protein-Protein Interaction and Cluster Analysis

Protein-Protein Interaction Network of *Oxalis Corniculata* - Anti inflammatory Regulated Genes

The Protein-Protein Interaction (PPI) network represents functional connections among proteins involved in inflammatory pathways, where nodes signify proteins and edges denote interactions, including experimental, co-expression, or predicted associations.

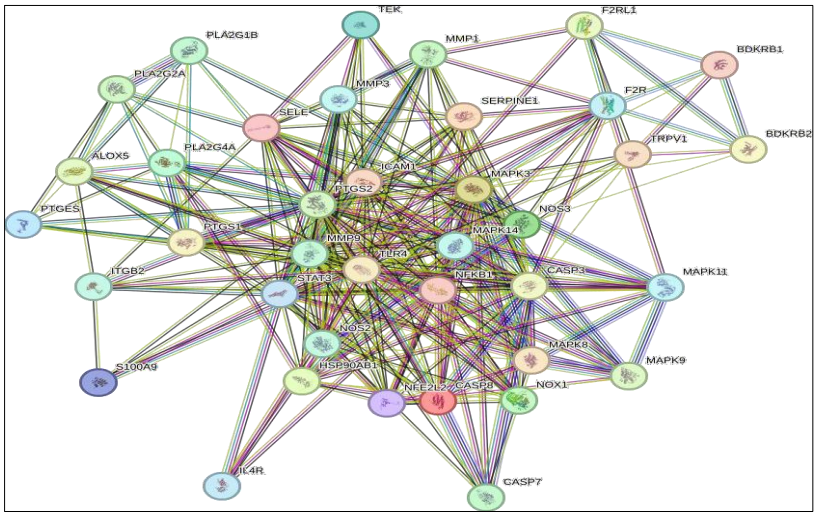

Figure 3: The Protein protein interaction of common targets between phytoconstituents and Anti inflammatory. (A) *Oxalis corniculata* - Anti inflammatory PPI network

GO and KEGG Pathway Enrichment Analyses of Cluster 1 for Anti Inflammatory Gene Ontology (GO) Analysis (Biological Process)

The Gene Ontology (GO) Biological Process enrichment analysis in the context of anti- inflammatory treatment identifies key biological pathways and mechanisms that regulate inflammation. The visual representation (bubble plot) highlights enriched GO terms, where larger bubbles indicate a higher gene count involved in that process, and color intensity represents the statistical significance (False Discovery Rate, FDR).

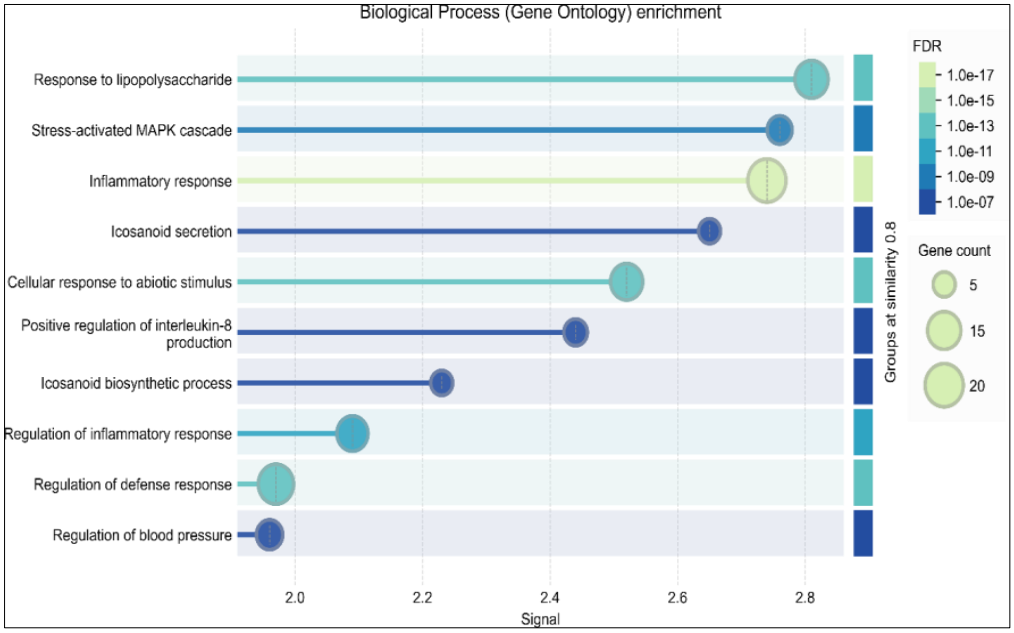

Figure 4: Gene Ontology (GO) analysis (Biological process)

Gene Ontology (GO) Analysis (Molecular Function)

Gene Ontology (GO) Molecular Function in Anti-Inflammatory Treatment

The GO Molecular Function enrichment analysis identifies key molecular activities of proteins involved in inflammation regulation. The bubble plot represents enriched molecular functions, where larger bubbles correspond to a higher number of associated genes, and color intensity denotes statistical significance (False Discovery Rate, FDR).

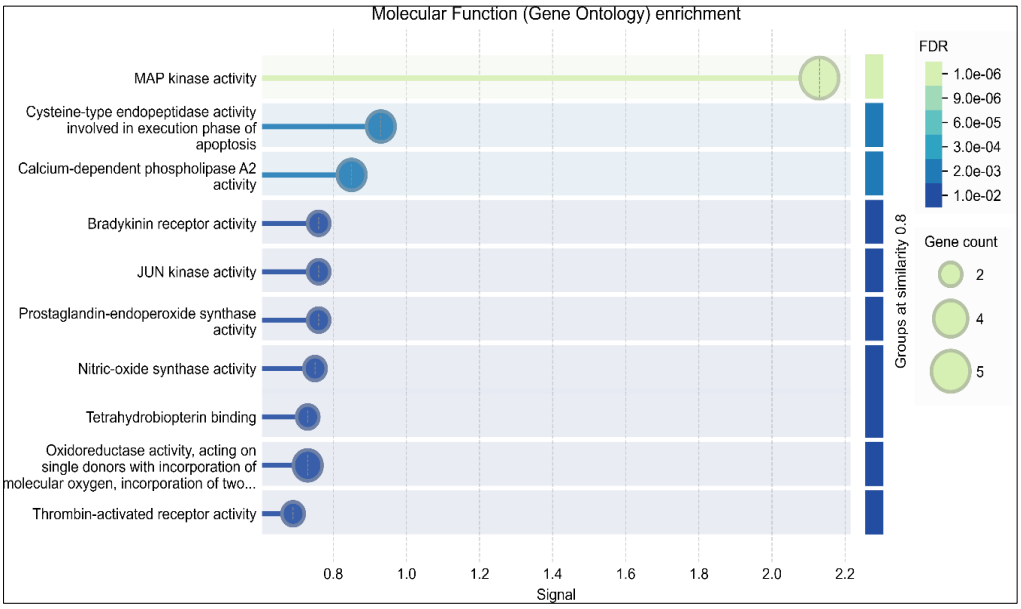

Figure 5: Gene Ontology (GO) analysis (molecular function)

Gene Ontology (GO) Analysis (Cellular Component)

The GO Cellular Component analysis identifies subcellular locations where key molecular functions occur during the anti-inflammatory response, with enriched components represented by bubble size (gene count) and color intensity

(FDR significance). Key components include the plasma membrane, where inflammatory receptors like TNF and IL- 6 mediate immune signaling; the cytoplasm, where pathways such as NF-κB, MAPK, and JAK-STAT function before nuclear translocation; and the nucleus, where transcription factors like NF-κB, Nrf2, and AP-1 regulate cytokine and enzyme expression.

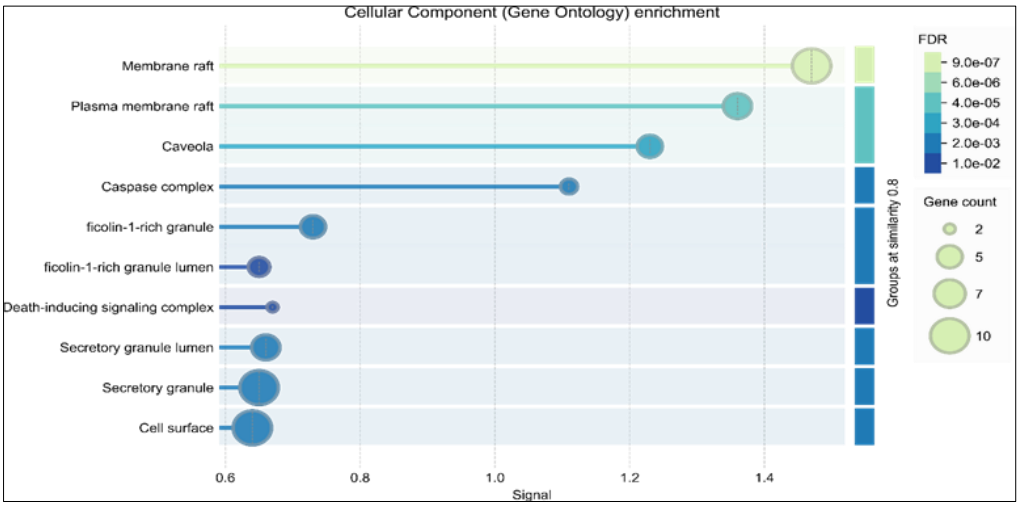

Figure 6: Gene Ontology (GO) analysis (cellular component)

KEGG Pathway Enrichment Analyses

KEGG Pathways in Anti-Inflammatory Treatment

The KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis identifies biological pathways significantly involved in the anti-inflammatory response. The bubble plot illustrates enriched pathways, with bubble size indicating the number of associated genes and color intensity reflecting statistical significance (FDR).

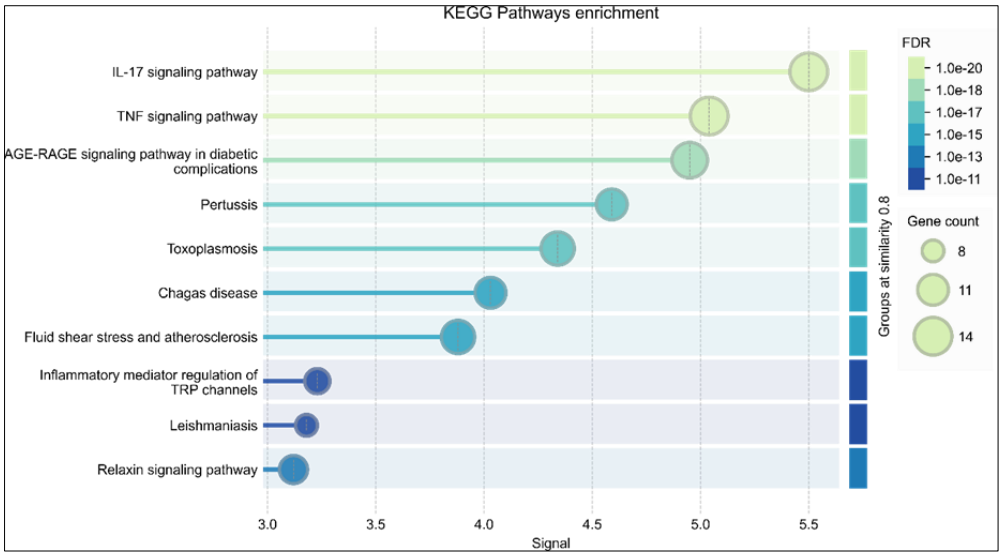

Figure 7: KEGG pathway enrichment analyses

Network Construction and Analysis

The network visualization provides an integrated view of the molecular interactions underlying anti-inflammatory treatment, emphasizing key targets, pathways, and bioactive compounds. NF-κB1, STAT3, MAPK8, TNF, NOS2, and TLR4 emerge as the most significantly modulated targets, playing crucial roles in regulating inflammatory responses by influencing cytokine production, oxidative stress, and immune signaling. These targets are involved in orchestrating inflammatory cascades that dictate the expression of pro- inflammatory mediators, including TNF-α, IL-6, and COX-2, which contribute to chronic inflammation and tissue damage if left unchecked.

The NF-κB signaling pathway is a central player in inflammation, controlling the expression of numerous pro-inflammatory genes and acting as a bridge between immune responses and oxidative stress. Activation of NF-κB leads to

increased production of cytokines, adhesion molecules, and enzymes such as inducible nitric oxide synthase (NOS2) and cyclooxygenase-2 (COX-2), both of which contribute to inflammatory processes.

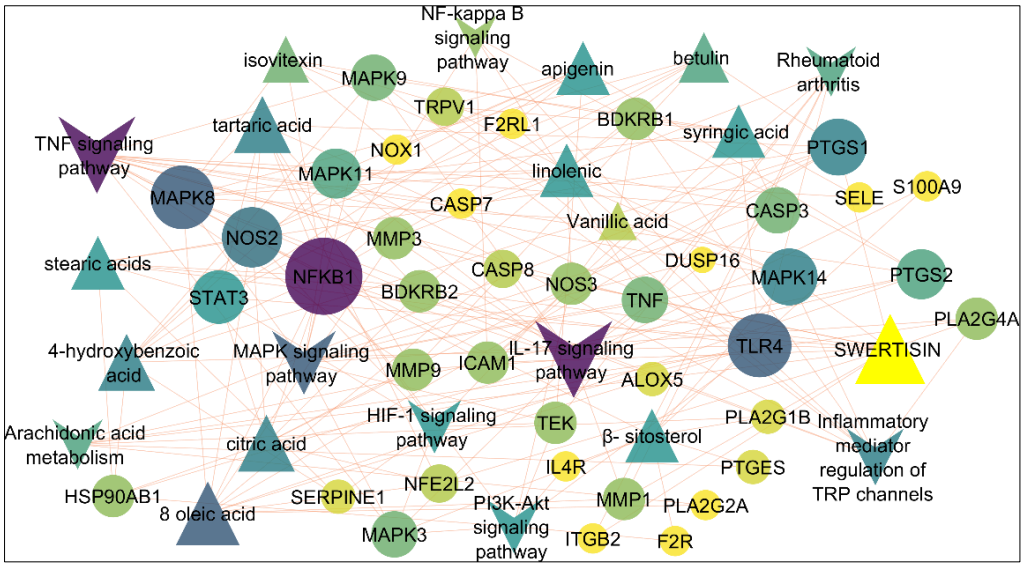

Figure 8: Network construction between Phytoconstituents, Targets and Pathways in Anti inflammatory.

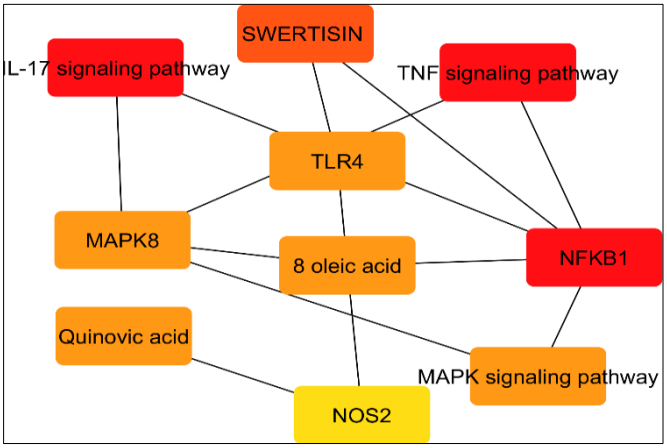

Figure 9: Cytohubba analysis of merged network by selecting top 10 Nodes of Anti inflammatory

CONCLUSION

The present study explored the pharmacognosy and anti-inflammatory potential of *Oxalis cornicualta* utilizing an optimized ultrasound-assisted extraction (UAE) method. The extraction parameters were carefully optimized to maximize the yield of bioactive constituents, particularly total phenolic and flavonoid content, which are known for their therapeutic properties. The in-silico analysis provided insights into the anti-inflammatory mechanisms, highlighting the interaction of *Oxalis cornicualta* phytoconstituents with key molecular targets involved in glucose metabolism. The findings support the potential of *Oxalis cornicualta i* as a promising natural candidate for inflammatory management. Further *in vivo* investigations are warranted to validate its efficacy and safety for potential pharmacological applications.

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