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# PHYTOCHEMICAL ANALYSIS AND EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *MOLLUGO OPPOSITIFOLIA* EXTRACTS

Biochemistry

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## ABSTRACT

Inflammation is a part of the body's immune response that involves the destruction of tissues and organs. It can be considered as part of the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells or irritants. The search for natural compounds and phytochemicals that are able to interfere with these mechanisms by preventing a prolonged inflammation could be useful for human health. *Mollugo oppositifolia* belongs to the family Molluginaceae. The present study was aimed to find the phytochemicals present and the evaluation of anti-inflammatory property of *Mollugo oppositifolia* extract using HRBC suspension method. The phytochemical analysis of the plant showed that the major constituents are the phenols, glycosides, tannins, terpenoids and steroids. The plant extract showed significant anti-inflammatory activity.

## KEYWORDS

Inflammation, Phytochemicals, Natural compounds, *Mollugo oppositifolia.*

**Introduction:** Nature has given us a very large number of diverse types of plant sources that grow in different parts of the world. Medicines produced from plants are without side effects and are known to cure a large number of diseases very effectively. Inflammation is a natural, protective process that involves destruction of tissues, damage to organs etc. It involves various local inflammatory mediators including arachidonic acid, prostaglandins and leukotrienes (1). The mediators of the process of inflammation induce heat, redness, swelling and pain. Inflammation may be either acute inflammation or chronic inflammation. Acute inflammation is of short duration whereas chronic inflammation is of long duration (2). The visual changes seen during the process of inflammation can be described well. The sensation of heat is caused due to the increased movement of blood through dilated vessels which also results in increased redness. Edema occurs due to the increased passage of fluid from the dilated blood vessels to surrounding tissues. Pain is due to the direct effects of mediators, either from initial damage or that resulting from the inflammatory response itself, and also due to the stretching of sensory nerves caused by oedema. These destructive processes involve some major cells of the immune system including T cells, B cells, basophils, neutrophils and mast cells, etc. These events are controlled by extracellular mediators and regulators like cytokines, growth factors, eicosanoids like leukotrines and prostaglandins. There are various synthetic drugs available for the treatment of inflammation. Traditionally the standard treatment for rheumatoid arthritis involves the use of non-steroidal anti-inflammatory drug such as aspirin for pain relief and also the use of corticosteroids in attempt to reduce other symptoms of the disease (3).

Prostaglandins are a family of chemicals that are produced by the cells of the body and have several important functions. They promote inflammation that is necessary for healing, but also results in pain, and fever, support the blood clotting function of platelets and protect the lining of the stomach from the damaging effects of acid. Nonsteroidal anti-inflammatory drugs (NSAIDs) block the COX enzymes and reduce prostaglandins throughout the body. As a consequence ongoing inflammation, pain, and fever are reduced. Since the prostaglandins that protect the stomach and support platelets and blood clotting also are reduced, NSAIDs can cause ulcers in the stomach and promote bleeding. The examples of NSAIDs includes aspirin, ibuprofen, diclofenac, naproxen etc. the side effects caused by these drugs include kidney failure, nausea, diarrhea, constipation, drowsiness, liver failure, ulcers etc (4).

Hence the identification of plants with anti- inflammatory activity and without side effects plays an important role in the treatment of inflammation. The inflammation is mainly due to the lysis of lysosomal membrane. Since the human erythrocyte membrane is similar to the lysosomal membrane, the Human Red Cell suspension method was used to assess the *invitro* anti-inflammatory activity of plants (5).

Plants produce a wide range of secondary metabolites which are called as phytochemicals. They have the ability to cure a large number of diseases. They can be effectively used in the treatment of a number of diseases and conditions (6). The plant *Mollugo oppositifolia* belongs to the family *Molluginaceae* and is commonly known as carpetweed. It is a common weed growing in wet and warm areas all over the world. The plant is known to be edible.

## Materials and methods

### Collection and preparation of extract

The plant was collected from Ayanavaram, Chennai and authenticated by a scientist from Botanical Survey of India, Coimbatore, Tamil Nadu, India. The whole plant was washed to remove dust particles and kept under sun for an hour. 100g of plant sample was taken and crushed in a mixer- grinder. The blended plant material was transferred to a conical flask and was covered with a cotton plug. It was then extracted with 50% ethanol and kept overnight on a rotary shaker. The extract was then evaporated to remove the solvent in a rotary vaccum evaporator at a temperature of 60 ºC. The extract was filtered and then used for experiment.

### Qualitative and Quantitative test for Phytochemicals

The filtered extract was used for the following tests.

### Qualitative tests

I) **Test for Phenol**

To 1ml of the plant extract added 20µl of 1% ferric chloride. The appearance of bluish black precipitate indicates the presence of phenol (7).

ii) **Test for Flavonoids**

To 1ml of the extract added few drops of 1% sodium hydroxide solution. The appearance of yellow colour indicates the presence of flavonoids (7).

iii) **Test for Terpenoids**

To 0.5ml of the plant extract added 2ml of chloroform and 3ml of concentrated sulphuric acid along the sides of the test tubes. The appearance of reddish brown colour at the interface indicates the presence of terpenoids (7).

iv) **Test for Quinones**

To 1ml of the plant extract added few drops of concentrated hydrochloric acid. The presence of yellow precipitate indicates the presence of quinines (8).

v) **Test for Glycosides**

To 2ml of the plant extract added 1ml of glacial acetic acid. To that added 1% ferric chloride solution drop by drop and then added concentrated sulphuric acid along the sides of the test tube. The

appearance of greenish blue colour indicates the presence of glycosides (9).

vi) **Test for Tannins**
To 1ml of the extract added 10ml of distilled water. The solution was then filtered and then added few drops of 0.1% ferric chloride slowly to the filtrate. The appearance of brownish green colour indicates the presence of tannins

vii) **Test for Saponins**
To 1ml of the plant sample added 2ml of distilled water. The solution was shaken and then added three drops of coconut oil; the solution was shaken again and then observed for formation of emulsion. The formation of emulsion indicates the presence of saponins (8).

**Quantitative tests**
**Total phenolic content**
The total phenolic content was estimated by folin's ciocalteau assay. To 0.2ml of plant sample added 1ml of folin's ciocalteau reagent. The mixture was left undisturbed for 10 minutes and then added 0.8ml of sodium bicarbonate solution. The reaction turns the sample blue in colour. The intensity of the colour is proportional to the phenolic concentration. After incubation for 30 minutes, the colour intensity was measured in colorimeter at 743nm against reagent blank.

Gallic acid was used as a standard solution in varying concentrations. It was similarly treated like the plant sample and the intensity of the colour developed was read in a colorimeter (10).

**Total Flavonoid content**
The total flavonoid content was measured by aluminium trichloride method. In a series of test tubes taken 100µl of the plant extract and added few drops of 20% aluminium chloride followed by the addition of few drops of acetic acid. After 10 minutes, the intensity of the colour developed was read at 410nm against blank solution. A standard solution was prepared using quercetin and treated similarly like the plant sample (11).

***In-vitro* Anti-inflammatory activity**
Invitro anti – inflammatory activity was estimated by HRBC suspension method. Blood was collected from a healthy human volunteer and the collected blood was mixed with equal volume of Alsever's solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL). It was then centrifuged at 3000rpm for 10 minutes to remove the packed cells. The cells were then carefully removed and washed in fresh normal saline. The process of centrifugation and washing was repeated five times until clear supernatants were obtained. A 10% v/v human erythrocyte suspension was prepared using isosaline. The assay mixtures consisted of 2ml of hyposaline, 1ml of 0.15M sodium phosphate buffer at pH 7.4, 0.5ml of HRBC suspension and 100, 200µl of sample respectively. Diclofenac sodium was used as a standard drug. The reaction mixtures were incubated at 37ºC for 45 minutes followed by centrifugation at 3000rpm for 10 minutes. The absorbance of lysed haemoglobin was read at 560nm. The membrane stabilization was calculated using the following formulae (12).

Percent of protection = 100 – (OD of test ÷ OD of control × 100).

**Results and Discussion**
**Table-1 Phytochemical Analysis of *Mollugo oppositifolia***

| Phytochemicals | Qualitative Determination |
|---|---|
| Phenol | + |
| Flavonoids | + |
| Quinone | + |
| Terpenoids | + |
| Glycosides | + |
| Tannins | + |
| Saponins | + |

+ indicates 'presence'

The ethanolic extract of the plant was tested for phytochemicals and it was found that it contains phenols, flavonoids, quinone, terpenoids, glycosides, tannins and saponins. The results are similar to the study in which it was found that the plant extracts contain terpenoid derivatives and glycoside (13) (14).

**Quantitative tests of Phenol and Flavonoids**
Phenolic compounds are one of the most ubiquitous plant secondary metabolites that are known to possess various biological activities including antioxidant activity (15). Flavonoid compounds also have numerous biological activities including cardio protective role (16) and hence the total phenol and flavonoid content was estimated and presented in the table2.

**Quantitative tests for Phenol and Flavonoids**

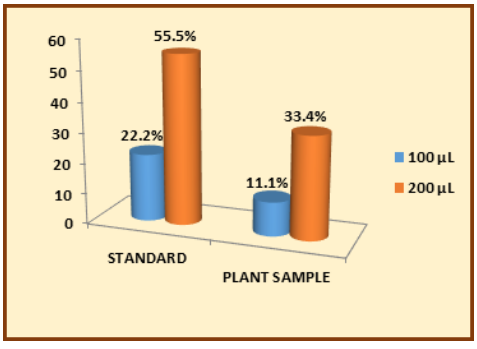
**Table-2 Estimation of Phenols and Flavonoids**

| Phytochemical | Amount/100g |
|---|---|
| Phenol (g equivalent to gallic acid standard) | 18.36g |
| Flavonoids (g equivalent to quercetin standard) | 0.205g |

***In-vitro* Anti-inflammatory activity**
HRBC suspension assay has been considered as a valid assay for the assessment of anti-inflammatory potential of the plant extracts (17). The anti-inflammatory potential of the plant extracts were tested and the standard solution consisted of Diclofenac sodium. The plants showed significant anti-inflammatory activity and the results are given in the Figure 1.

% Protection of haemolysis from HRBC membrane stabilization method.

**Figure-1 *In vitro* Anti-inflammatory activity**




% protection by HRBC Suspension assay

**Summary and Conclusion**
From the present study it can be concluded that *Mollugo oppositifolia* contains various phytochemicals namely phenols, flavonoids, quinones, terpenoids, glycosides, tannins and saponins. The plant extract showed significant anti inflammatory activity. The phytochem icals present in *Mollugo oppositifolia* might be responsible for the anti-inflammatory property.

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