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PHYTOCHEMICAL SCREENING AND EVALUATION OF IN VITRO ANTI-INFLAMMATORY, AND ANTI-ARTHRITIC ACTIVITIES OF *MUNTINGIA CALABURA L.*

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ABSTRACT

Muntingia calabura L. is traditionally employed in the treatment of inflammatory disorders and joint-related ailments. The present study aimed to conduct phytochemical screening and evaluate the in vitro anti-inflammatory and anti-arthritic activities of *M. calabura* L. bark extract. The powdered plant material was extracted using a hydroalcoholic solvent and subjected to preliminary phytochemical screening, which revealed the presence of flavonoids, tannins, steroids, saponins, phenolic compounds, and proteins. The in vitro anti-inflammatory activity was evaluated using the human red blood cell (HRBC) membrane stabilization method, while anti-arthritic activity was assessed using bovine serum albumin denaturation and egg albumin denaturation methods at different concentrations of the extract. The extract demonstrated concentration-dependent membrane stabilization in the HRBC method, indicating significant anti-inflammatory potential. In the anti-arthritic models, the extract effectively inhibited

protein denaturation in both bovine serum albumin and egg albumin assays. The observed activities were comparable to those of the standard drug. The presence of phenolic compounds and flavonoids may contribute to the inhibition of protein denaturation and stabilization of biological membranes. These findings suggest that *M. calabura* L. possesses promising in vitro anti-inflammatory and anti-arthritic activities. Further investigations, including isolation of active constituents and in vivo studies, are warranted.

KEYWORDS: *Muntingia calabura*, phytochemical screening, vitro anti-inflammatory, anti-arthritic activity.

INTRODUCTION

Plants are the backbone of life on Earth and an essential resource for humans.[1] They play a crucial role in maintaining ecological balance and provide food, shelter, oxygen, and medicines necessary for survival. Since ancient times, plants have been extensively utilized in traditional systems of medicine, and even today a considerable proportion of the global population depends on plant-based remedies for primary healthcare needs. The growing interest in natural products has further emphasized the importance of medicinal plants in modern therapeutic research. Plants are a significant source of fine chemicals, which are widely used in the global pharmaceutical industry.[2] Natural products obtained from plants possess enormous structural diversity and biological activity, making them valuable leads for drug discovery. Many currently used drugs are either directly derived from plants or developed based on plant-derived compounds. Therefore, medicinal plants continue to serve as a promising source for the identification of novel bioactive molecules with therapeutic potential.

Medicinal plants exhibit curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary metabolites of plants.[3] These include alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids, and phenolic compounds. Such phytoconstituents are responsible for diverse pharmacological activities such as anti-inflammatory, antioxidant, antimicrobial, anti-ulcer, hepatoprotective, and anti-arthritic effects. Secondary metabolites are synthesized by plants as adaptive responses to environmental stress and play important roles in plant defense mechanisms. Unlike primary metabolites, secondary metabolites are not directly involved in growth and development but contribute to plant survival and ecological interactions. These compounds have attracted significant scientific attention due to their therapeutic potential and safety profile compared to

synthetic drugs.[4] Continuous phytochemical screening and pharmacological evaluation of medicinal plants therefore remain essential for discovering new drug candidates.

Man is the only clever living being that knows how to adapt to the changing environment of time and space.[5] By observing nature and utilizing plant resources, humans have developed traditional medicinal systems and advanced pharmaceutical sciences. The integration of ethnobotanical knowledge with modern analytical techniques has led to the identification of several potent therapeutic agents from plant sources. Hence, systematic investigation of medicinal plants is necessary to validate their traditional uses and to explore their potential for the development of safer and more effective drugs.

Inflammation means a protective response of the body to injury or infection, causing redness, swelling, heat, pain, and loss of function. Arthritis means a condition where joints become inflamed, leading to pain, stiffness, swelling, and reduced movement. Plant extracts contain numerous bioactive compounds including flavonoids, alkaloids, terpenoids, phenolic compounds, tannins, and glycosides that exhibit significant anti-inflammatory and antiarthritic activities. These phytochemicals may act by inhibiting inflammatory mediators, suppressing cytokine production, preventing oxidative stress, and stabilizing lysosomal membranes. Therefore, scientific evaluation of medicinal plants for their anti-inflammatory and antiarthritic activities is essential for the development of new therapeutic agents with improved safety profiles.

Muntingia calabura L. (family Muntingiaceae), commonly known as Jamaican cherry, is widely cultivated in warm regions of Asia and native to Central and South America.[6] The plant grows well in poor soils and tolerates drought conditions. Its fruits are used in food preparations, while leaves are consumed as tea. It has been reported to possess gastroprotective, antibacterial, antioxidant, antidiabetic, hepatoprotective, antinociceptive, and antiproliferative activities. Hence, the present study aimed to evaluate the anti-inflammatory and anti-arthritic activities of the hydroalcoholic extract of *M. calabura* L bark.

MATERIALS AND METHODS

Plant collection and authenticity

The whole plant of *M. calabura* L. was collected from Choutuppal regions of Yadadri Bhuvanagiri Dist. T.S, and was authenticated by Dr. K. Srinivasa Reddy, M. Sc., Ph. D, Asst.

Professor, Dept. of Botany, and Govt. Degree College for Woman, Nalgonda, and Telangana State.

Preparation of plant extract

The dried bark of *M. calabura* was collected, cleaned, and shade-dried. The dried material was then powdered using a mechanical grinder to obtain a coarse powder of suitable particle size for extraction. About 200 g of the powdered drug was accurately weighed and packed in a thimble made of filter paper. The thimble was placed in the main chamber of a Soxhlet extraction apparatus.

A hydroalcoholic solvent was placed in a round-bottom flask attached to the Soxhlet apparatus. The apparatus was assembled and heated on a heating mantle. The solvent was heated to its boiling point, and the vapours passed through the distillation arm into the condenser where they were cooled and condensed. The condensed solvent dripped into the thimble containing the powdered plant material. When the solvent level in the extraction chamber reached the siphon level, it automatically siphoned back into the round-bottom flask carrying the extracted constituents.

This cycle of extraction was repeated several times until the solvent in the siphon tube became colourless, indicating complete extraction of the phytoconstituents. After completion of extraction, the solvent was evaporated from the extract using a water bath to obtain the concentrated extract. The extract was then air-dried and stored in an airtight container for further studies.

Phytochemical screening

The extract was subjected to preliminary phytochemical screening in order to detect the presence of various phytoconstituents by using standard phytochemical procedures.[7,8]

HRBCs Membrane Stabilization Method

The human red blood cell membrane stabilization method was used for study of anti-inflammatory activity. The blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl in water) and centrifuged at 3,000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as

10% v/v suspension with isosaline. Various concentrations of extracts were prepared viz.,50, 100, 250 and 500 mcg/ ml using distilled water and to each concentration 1 ml of phosphate buffer (0.15M, pH 7.4), 2 ml hypo saline (0.36%) and 0.5 ml of HRBC suspension were added. Diclofenac was used as reference drug. Instead of hyposaline 2ml of distilled water was used in the control. All the assay mixtures were incubated at 37^0C for 30 min and centrifuged at 3,000 rpm for 20 min. The haemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm.[9,10] The percentage haemolysis was calculated by assuming the haemolysis produced in presence of distilled water as 100%.

Inhibition of Protein Denaturation (bovine serum method)

Test solution 0.5 ml of test solution consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of extracts in various concentrations (100, 200, 300, 400, and 500 µg/ml).

Test control solution 0.5 ml of test control solution consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of distilled water.

Standard solution 0.5 ml of standard solution consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of Diclofenac sodium solution (100 µg/ml).

The pH of the above solutions was adjusted to 6.5 using a small amount of 1N HCl. The samples were incubated at 37°C for 20 min and heated at 57°C for 3 min which were cooled, and 2.5 ml of phosphate buffer (pH 6.3) was added to it. Control represents 100% proteins. Their absorbance was measured at 660 nm using a pure blank after cooling. Diclofenac sodium (standard drug) was utilized as a reference medication and was handled as such for absorbance determination[11]. The following % inhibition of protein denaturation was calculated:

$$\textbf{Percentage inhibition} = \frac{\text{OD control - OD sample x 100}}{\text{OD control}}$$

Inhibition of albumin denaturation (egg albumin)

5 ml of test solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffer saline and 2 ml of in various concentrations of extracts (100, 200, 300, 400, and 500 µg/ml).

5 ml of test control solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffered saline and 2 ml of distilled water.

A standard solution of 5 mL contains 0.2 mL of egg albumin and 2.8 mL of phosphate buffer saline and Diclofenac sodium 100 µg/ml. The pH of the above solutions was adjusted to 6.4 using a small amount of 1N HCl. The samples were incubated at 37°C for 20 min and heated at 70°C for 5 min denaturations, and the results were compared with standard Diclofenac sodium.

Their absorbance was measured at 660 nm using a pure blank after cooling. Diclofenac sodium (standard drug) was utilized as a reference medication and was handled as such for absorbance determination.[11] The following % inhibition of protein denaturation was calculated:

Percentage inhibition = $\frac{\text{OD control - OD sample x 100}}{\text{OD control}}$

RESULTS

The preliminary phytochemical studies were carried out according to the procedures described. The extract was examined for the presence of various phytoconstituents such as proteins, steroids, saponins, tannins, phenolic compounds, flavonoids, etc. The results obtained are presented in Table 1.

Table 1: Phytochemical screening of *M. calabura L* bark.

S. No.	Chemical constituents	Hydro alcoholic extract
1.	Carbohydrates	Absent
2.	Proteins	Abundantly present
3.	Amino acids	Absent
4.	Tannins	Abundantly present
5.	Glycosides	Absent
6.	Alkaloids	Absent
7.	Flavonoids	Abundantly present
8.	Steroids	Abundantly present
9.	Saponins	Very little amount present

Table 2: Effect of hydro ethanolic extract on HRBC membrane haemolysis and membrane protection.

Conc. (µg/ml)	% Hemolysis of Diclofenac sodium	% Protection of Diclofenac sodium	% Hemolysis of hydro alcoholic extract	% Protection of hydro alcoholic extract
50	58.03	41.97	61.73	38.27
100	49.21	50.79	53.69	46.31
250	36.53	63.47	41.82	58.18
500	21.42	78.58	25.19	74.81

Table 3: Anti-arthritic activity of hydro alcoholic extract of *M. calabura* L. by bovine and egg serum albumin methods.

Effect of hydro alcoholic extracts.	(%) Inhibition by bovine serum method	(%) Inhibition by egg albumin method
Control	-----	----
Diclofenac sodium 100 µg/ml	87.08	82.30
100 µg/ml	29.11	19.73
200 µg/ml	38.32	27.62
300 µg/ml	47.09	34.41
400 µg/ml	65.47	51.18
500 µg/ml	79.91	72.42

DISCUSSION

Preliminary phytochemical screening of hydro alcoholic extract of *M. calabura* (HAEMC) revealed the presence of tannins, flavonoids, steroids, phenols and saponins, (Table 1). The HRBC membrane stabilization method is widely used to evaluate the in-vitro anti-inflammatory activity of plant extract. Stabilization of the human red blood cell membrane indicates the ability of the test sample to prevent haemolysis and thereby inhibit the release of inflammatory mediators. In the present study, the hydro-alcoholic extract of *M. calabura* L. was evaluated for its membrane stabilizing activity at different concentrations ranging from 50–500 µg/ml and compared with the standard drug Diclofenac sodium. The results obtained are presented in Table 2.

The hydro-alcoholic extract exhibited a concentration-dependent increase in membrane protection. At 50 µg/ml, the extract showed 38.27% protection, which gradually increased to 46.31% at 100 µg/ml and 58.18% at 250 µg/ml. The highest protection of 74.81% was observed at 500 µg/ml. Correspondingly, the percentage haemolysis decreased from 61.73% at 50 µg/ml to 25.19% at 500 µg/ml. The standard drug Diclofenac sodium also showed a similar concentration-dependent effect, producing 41.97% protection at 50 µg/ml and reaching a maximum protection of 78.58% at 500 µg/ml.

The results indicate that the hydro-alcoholic extract of the bark possesses significant membrane stabilizing activity, which may be attributed to the presence of phytoconstituents such as flavonoids, tannins, and phenolic compounds. These compounds are known to stabilize biological membranes and inhibit inflammatory processes by preventing the release of lysosomal enzymes. Thus, the findings suggest that the hydro-alcoholic extract of the bark

exhibits appreciable anti-inflammatory activity, although its activity is slightly lower than that of the standard drug Diclofenac sodium.

The anti-arthritic activity of the hydro-alcoholic extract of *M. calabura* L. was evaluated by bovine serum albumin and egg albumin denaturation methods. Protein denaturation is one of the important causes of inflammation and arthritis; therefore, the inhibition of protein denaturation indicates potential anti-arthritic activity. The results obtained are presented in Table 3.

The hydro-alcoholic extract showed a concentration-dependent increase in inhibition of protein denaturation. In the bovine serum albumin method, the extract exhibited 29.11% inhibition at 100 µg/ml, which gradually increased to 38.32% at 200 µg/ml, 47.09% at 300 µg/ml, 65.47% at 400 µg/ml, and reached a maximum of 79.91% inhibition at 500 µg/ml. Similarly, in the egg albumin method, the extract produced 19.73% inhibition at 100 µg/ml and increased progressively to 27.62%, 34.41%, 51.18%, and 72.42% inhibition at 200, 300, 400, and 500 µg/ml respectively.

The standard drug Diclofenac sodium (100 µg/ml) showed higher inhibition values of 87.08% in the bovine serum albumin method and 82.30% in the egg albumin method. Although the activity of the plant extract was comparatively lower than the standard drug, it demonstrated significant inhibition at higher concentrations. The observed anti-arthritic activity of the hydro-alcoholic extract may be attributed to the presence of phytoconstituents such as flavonoids, phenolic compounds, and tannins, which are known to stabilize proteins and inhibit inflammatory processes. Thus, the results suggest that the hydro-alcoholic extract of *M. calabura* possesses considerable anti-arthritic activity, which increases with increasing concentration of the extract.

CONCLUSION

The present study demonstrated that the hydro-alcoholic extract of *M. calabura* possesses significant anti-inflammatory and anti-arthritic activities in in-vitro models. The extract showed notable membrane stabilizing activity in the HRBC membrane stabilization method, indicating its potential to prevent the release of inflammatory mediators. In addition, the extract effectively inhibited protein denaturation in both bovine serum albumin and egg albumin methods, suggesting its potential anti-arthritic property. The activity of the extract increased with increasing concentration, showing a dose-dependent effect. Although the

activity was comparatively lower than the standard drug Diclofenac sodium, the extract exhibited appreciable pharmacological effects. These activities may be attributed to the presence of phytoconstituents such as flavonoids, phenolic compounds, and tannins. Therefore, *M. calabura* may serve as a potential natural source for the development of anti-inflammatory and anti-arthritic agents. Further studies are required to isolate the active constituents and to confirm the activity through in-vivo studies.

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