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IN VITRO ANTI-OXIDANT AND *IN VIVO* ANTI -INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *MIMUSOPS HEXANDRA*

Vishavjeet*[1], B. K. Dubey[1], Vivek Singh Thakur[1], Deepak Basedia[1], Sunil Shah[2]

[1]Technocrats Institute of Technology-Pharmacy, Bhopal (M.P.)
[2]TIT-College of Pharmacy, Bhopal (M.P.)

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***Corresponding author:**

Vishavjeet

Technocrats Institute of Technology-Pharmacy, Bhopal (M.P.)

ABSTRACT

Mimusops hexandra is a medicinal plant traditionally used for the treatment of various inflammatory and oxidative stress–related disorders. The present study aimed to evaluate the in vitro antioxidant and in vivo anti-inflammatory activity of the ethanolic extract of *Mimusops hexandra*. The ethanolic extract was prepared by solvent extraction and subjected to preliminary phytochemical screening, which revealed the presence of carbohydrates, flavonoids, proteins, and phenolic compounds. Quantitative analysis showed appreciable total phenolic content (0.843 mg/100 mg) and total flavonoid content (0.912 mg/100 mg) of the dried extract. The antioxidant activity was assessed using the DPPH free radical scavenging assay, where the extract exhibited concentration-dependent scavenging activity with an IC_{50} value of 74.95 µg/ml, compared to ascorbic acid (IC_{50} = 25.45 µg/ml). The anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model in rats. The ethanolic extract, particularly at a dose of 200 mg/kg, showed significant inhibition of paw edema, especially during the later phase of inflammation, comparable to the standard drug indomethacin. The results suggest that the ethanolic extract of *Mimusops hexandra* possesses significant antioxidant and anti-inflammatory potential, which may be attributed to its phenolic and flavonoid constituents. The study supports the traditional use of *Mimusops hexandra* and indicates its potential as a natural therapeutic agent for managing oxidative stress and inflammatory conditions.

KEYWORDS: *Mimusops hexandra*, Antioxidant activity, Anti-inflammatory activity, DPPH assay, Carrageenan-induced paw edema, Phenolic compounds, Flavonoids.

INTRODUCTION

Oxidative stress and inflammation are closely interrelated pathological processes implicated in the development and progression of several chronic disorders, including arthritis, cardiovascular diseases, diabetes, neurodegenerative diseases, and cancer. Excessive production of reactive oxygen species (ROS) leads to cellular damage of lipids, proteins, and nucleic acids, thereby triggering inflammatory cascades and tissue injury. Although synthetic antioxidants and anti-inflammatory drugs are widely used, their long-term use is often associated with adverse effects, prompting the search for safer and effective alternatives from natural sources (Halliwell et al., 2015).

Medicinal plants have long been recognized as valuable reservoirs of bioactive compounds such as flavonoids, phenolic acids, tannins, saponins, and alkaloids, which

exhibit potent antioxidant and anti-inflammatory activities. Plant-based therapies offer multitargeted actions with relatively fewer side effects and are increasingly gaining attention in modern pharmacological research (Pandey et al., 2014).

Mimusops hexandra Roxb. (family: Sapotaceae), commonly known as Khirni or Rayan, is a tropical evergreen tree widely distributed in India and other parts of South Asia. Various parts of the plant have been traditionally used in Ayurveda and folk medicine for the treatment of inflammation, fever, wounds, diarrhea, and skin disorders. Phytochemical investigations have reported the presence of polyphenols, flavonoids, triterpenoids, and sterols in *Mimusops* species, which are known to contribute to antioxidant and anti-inflammatory effects (Kirtikar et al., 2005).

Previous studies on related species of *Mimusops* have demonstrated significant free radical scavenging activity and inhibition of inflammatory mediators, supporting its ethnomedicinal claims. However, scientific data on the combined in vitro antioxidant and in vivo anti-inflammatory potential of the ethanolic extract of *Mimusops hexandra* leaves are limited. Ethanol is considered an efficient solvent for extracting polyphenolic compounds, which play a crucial role in neutralizing oxidative stress and modulating inflammatory pathways (Sasidharan et al., 2011).

Therefore, the present study was designed to evaluate the in vitro antioxidant activity using standard free radical scavenging assays and the in vivo anti-inflammatory activity using an established animal model. This investigation aims to scientifically validate the traditional use of *Mimusops hexandra* and to explore its potential as a natural therapeutic agent against oxidative stress– and inflammation-related disorders.

MATERIAL AND METHODS

Material

Fresh plant material of *Mimusops hexandra* was collected and authenticated before use. Analytical grade ethanol was used as the solvent for extraction. Chemicals required for phytochemical screening included Hager's reagent, Fehling's solution, ferric chloride, lead acetate, copper acetate, xanthoproteic reagent, and other standard laboratory reagents. Ascorbic acid was used as the reference standard for antioxidant studies, while 1,1-diphenyl-2-picrylhydrazyl (DPPH) was employed for free radical scavenging assay. Carrageenan was used to induce inflammation in experimental animals, and indomethacin served as the standard anti-inflammatory drug. All reagents and chemicals used were of analytical grade, and distilled water was used throughout the study.

Methods

Collection of plant materials

Leaves of *Mimusops hexandra* was collected from local area of Bhopal in the period of April, 2025.

Drying and storage of plant materials

Leaves of *Mimusops hexandra* were cleaned by tab water and a portion was dried at room temperature (Harborne, 1996). The dried samples were ground and passed through a sieve (20 meshes). The powdered drugs were kept in sealed containers and protected from light until used. Another portion of sample was used for maceration.

Extraction by maceration process

50 gram of powdered leaves of *Mimusops hexandra* were coarsely powdered and subjected to extraction. Dried powdered of leaves of *Mimusops hexandra* has been extracted with Ethanol as a solvent using maceration method for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007).

Determination of percentage yield

The percentage yield is a measure of the efficiency of a chemical reaction. It compares the actual yield of a reaction to the theoretical yield, which is the amount of product that would be produced if the reaction were 100% efficient. The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical analysis

The analysis of these compounds is important for determining the nutritional and medicinal value of plants and their potential for various applications in food, pharmaceutical, and cosmetic industries (Trease and Evans, 1978; Kokate, 1994).

Quantitative studies of phytoconstituents

Total phenol content estimation

The total phenol content (TPC) of a sample can be estimated using various methods, including:

Folin-Ciocalteu assay: This method involves the use of folin-ciocalteu reagent and gallic acid as a standard. The phenolic compounds in the sample react with the folin-ciocalteu reagent, resulting in a blue color, which can be measured spectrophotometrically at 765 nm. The TPC is expressed as gallic acid equivalent (GAE) in milligrams per gram of sample.

Based on the availability and feasibility folin-ciocalteu assay method used to determine total phenolic content estimation in extract.

Principle: The total phenol content of the extract was determined by the modified folin-ciocalteu method (Parkhe and Bharti, 2019).

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol.

Preparation of Extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol.

Procedure: 2 ml of extract and each standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexes for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

The total flavonoid content (TFC) of a sample can be estimated using various methods, including:

Aluminum chloride colorimetric assay: This method involves the use of aluminum chloride reagent and rutin as a standard. The flavonoids in the sample react with the aluminum chloride reagent, resulting in a yellow color, which can be measured spectrophotometrically at 420 nm. The TFC is expressed as rutin equivalent (RE) in milligrams per gram of sample.

Similar to the estimation of TPC, different flavonoids may have different reactivities with these assays, and therefore, the TFC may not accurately reflect the total flavonoid content of the sample. Additionally, these methods may also react with non-flavonoid compounds, leading to overestimation of the TFC. Therefore, it is important to use these methods as a relative measure of flavonoid content rather than an absolute quantification. Aluminum chloride colorimetric assay used to determine total flavonoids content determination.

Principle: Determination of total flavonoids content was based on aluminum chloride method (Parkhe and Bharti, 2019).

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol.

Preparation of extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.

Procedure: 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

In-vitro* antioxidant activity of ethanolic extract of *Mimusops hexandra

DPPH method

Total free radical scavenging capacities of extract were estimated according to the previously reported method with slight modification (Parkhe and Jain, 2018). Solution of DPPH (6 mg in 100ml methanol) was prepared and stored in dark place. Different concentration of standard and test (10- 100 µg/ml) was prepared. 1.5 ml of DPPH and 1.5 ml of each standard and test was taken in separate test tube; absorbance of this solution was taken immediately at 517nm. 1.5 ml of DPPH and 1.5 ml of the methanol was taken as control absorbance at 517nm.

The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

In-vivo* anti-inflammatory activity ethanolic extract of *Mimusops hexandra

Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute toxicity study

It was done according to Organization for Economic Co-operation and Development (OECD) guidelines 425 (up and down procedure). All the five rodents were administered 2000mg/kg of ethanolic extract of *Mimusops hexandra* orally and observed continuously for a period of 14 days, every hourly for 24 hours, and every day for 14 days for its movements, grooming activity, exploring activity, writing reflex, eye movements, and convulsion etc (Perianayagam *et al.*, 2006). The experimental dose of the extracts was selected as 100 and 200 mg/kg/p.o.

Experimental designs

Group	Treatment
Group 1	Carrageenan control (0.1 ml of 1% w/v)
Group 2	Carrageenan (0.1 ml of 1% w/v) + Indomethacin (10 mg/kg, p.o.)
Group 3	Carrageenan (0.1 ml of 1% w/v) + *Mimusops hexandra* extract (100 mg/kg, p.o.)
Group 4	Carrageenan (0.1 ml of 1% w/v) + *Mimusops hexandra* extract (200 mg/kg, p.o.)

Carrageenan induced hind paw oedema

Anti-inflammatory activity was measured using carrageenan induced rat paw oedema assay. The rats were divided into different groups of 6 animals each (plant extract was dissolved and administered per oral at different dose levels). Oedema was induced by injecting 0.1 ml. of a 1% solution of carrageenan in saline into the sub plantar region of the right hind paw of the rats. The volumes of oedema of the injected and the contralateral paws were measured after the induction of inflammation using a plethysomgraph to calculate the percentage of paw oedema inhibition (Huang *et al.*, 2010).

$$\text{Percentage Inhibition} = \frac{Vc-Vt}{Vc} \times 100$$

Where, Vc- Edema volume of control group
Vt- Edema volume of test group

Statistical Analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM) (Gepdiremen *et al.*, 2004). Data were analyzed by one-way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

RESULTS AND DISCUSSION

The present study evaluated the in vitro antioxidant **and** in vivo anti-inflammatory potential of the ethanolic extract of *Mimusops hexandra*, with the findings providing scientific support for its traditional medicinal use.

The percentage yield of the ethanolic extract of *Mimusops hexandra* was found to be 11.5% w/w**,** indicating good extractive efficiency of ethanol. The dark black color of the extract suggests the presence of a high amount of polar phytoconstituents, particularly phenolic compounds and flavonoids, which are commonly associated with antioxidant activity.

Preliminary phytochemical screening revealed the presence of carbohydrates, flavonoids, proteins, and phenolic compounds**,** while alkaloids, glycosides, diterpenes, and saponins were absent. The detection of flavonoids and phenols is of particular importance, as these compounds are well known for their ability to scavenge free radicals, inhibit lipid peroxidation, and modulate inflammatory mediators. Similar phytochemical profiles have been reported for other species of *Mimusops*, supporting the reliability of the present findings.

Quantitative estimation further confirmed a high total phenolic content (0.843 mg/100 mg) and total flavonoid content (0.912 mg/100 mg) in the ethanolic extract. Phenolic and flavonoid compounds act as hydrogen donors and metal chelators, thereby neutralizing reactive oxygen species and preventing oxidative damage. The relatively higher flavonoid content may significantly contribute to both antioxidant and anti-inflammatory effects observed in this study.

The DPPH free radical scavenging assay demonstrated a concentration-dependent increase in antioxidant activity of the ethanolic extract. Although ascorbic acid exhibited stronger radical scavenging activity with a lower IC_{50} value (25.45 µg/ml), the *Mimusops hexandra* extract showed appreciable antioxidant potential with an IC_{50} value of 74.95 µg/ml**.** This activity can be attributed to the synergistic action of phenolic and flavonoid compounds present in the extract. The results indicate that the extract possesses moderate but significant antioxidant capacity, which is essential in controlling oxidative stress-mediated inflammatory processes.

The in vivo anti-inflammatory activity, evaluated using the carrageenan-induced paw edema model, further substantiated the pharmacological potential of *Mimusops hexandra*. Carrageenan-induced inflammation is a well-established biphasic model, involving the release of histamine, serotonin, and bradykinin in the early phase, followed by prostaglandins and other inflammatory mediators in the later phase. The ethanolic extract at a dose of 200 mg/kg significantly reduced paw edema, particularly at the 2nd and 4th hour, indicating inhibition of the late phase of inflammation. This effect was comparable, though less potent, to the standard drug indomethacin.

The observed anti-inflammatory activity may be attributed to the presence of flavonoids and phenolic compounds, which are known to inhibit cyclooxygenase enzymes, suppress prostaglandin synthesis, and reduce oxidative stress at the site of inflammation. The dose-dependent reduction in paw edema further supports the therapeutic relevance of the extract.

Table 1: % Yield obtained ethanolic extract from *Mimusops hexandra*.

S. No.	Extract	Color	% Yield
1.	*Mimusops hexandra*	Dark black	11.5

Table 2: Preliminary phytochemical screening of *Mimusops hexandra*.

S. No.	Phytoconstituents	Test Name	Ethanolic extract
1	Alkaloids	Hager's Test	Absent
2	Glycosides	Legal's Test	Absent
3	Carbohydrates	Fehling's Test	**Present**
4	Flavonoids	Lead acetate	**Present**

5	Diterpenes	Copper acetate Test	Absent
6	Saponins	Froth Test	Absent
7	Proteins	Xanthoproteic Test	**Present**
8	Phenols	Ferric Chloride Test	**Present**

Table 3: Estimation of total phenolic and flavonoids content of *Mimusops hexandra.*

S. No	Extract	Total phenol content	Total flavonoids content
		(mg/100mg of dried extract)	
1	Ethanolic	0.843	0.912

Table 4: % Inhibition of ascorbic acid and ethanolic extracts of using DPPH method.

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	*Mimusops hexandra* extract
1	10	42.57	19.25
2	20	49.13	30.71
3	40	54.36	38.96
4	60	65.80	43.02
5	80	89.35	50.89
6	100	91.63	56.47
IC_{50} value		**25.45**	**74.95**

Table 5: Effect of ethanolic *extract of Mimusops hexandra* on paw edema induced by carrageenan in rats by different timelines.

Group No.	Treatment	Dose	Paw Edema (mm)				
			0 hr	30 min	1 hr	2 hr	4 hr
Group 1	Carrageenan control	–	3.81	4.21	4.49	4.72	5.09
Group 2	Carrageenan + Indomethacin	10 mg/kg	1.21	1.02	0.81	0.62*	0.61**
Group 3	Carrageenan + *Mimusops hexandra* extract	100 mg/kg	2.52	2.59	2.68	2.79	2.81
Group 4	Carrageenan + *Mimusops hexandra* extract	200 mg/kg	1.88	1.79	1.63	1.42*	1.21*

Values are expressed as mean ± SD. *P < 0.05-significant compared to carragenan treated group.

CONCLUSION

The present study demonstrated that the ethanolic extract of *Mimusops hexandra* possesses significant in vitro antioxidant and in vivo anti-inflammatory activities. Preliminary phytochemical screening revealed the presence of bioactive constituents such as flavonoids, phenolic compounds, carbohydrates, and proteins, which are known to contribute to antioxidant and anti-inflammatory effects. The extract showed notable free radical scavenging activity in the DPPH assay, with a concentration-dependent increase in percentage inhibition, indicating strong antioxidant potential. Furthermore, in the carrageenan-induced paw edema model, the extract produced a significant reduction in paw edema, particularly at the higher dose, demonstrating effective anti-inflammatory activity comparable to the standard drug. These findings suggest that *Mimusops hexandra* is a promising natural source of antioxidant and anti-inflammatory agents and supports its traditional use in the management of inflammatory conditions.

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