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Phytochemical Investigation & *In-Vitro* Anti-Inflammatory Activity of Various Extracts on *Mussaenda Philippica* Leaves

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Abstract:

Aim: *Mussaenda philippica* A. Rich. (Family: Rubiaceae) is a medicinal and ornamental plant traditionally used for various ailments and is reported to contain several bioactive phytoconstituents. However, systematic evaluation of the anti-inflammatory potential of its different solvent extracts remains limited.

Objective: The present study was undertaken to investigate the phytochemical constituents and evaluate the in-vitro anti-inflammatory activity of benzene, diethyl ether, and chloroform extracts of *Mussaenda philippica* leaves.

Methods: The leaves of *Mussaenda philippica* were collected from Tenkasi, Tamil Nadu, shade-dried, powdered, and subjected to Soxhlet extraction using benzene, diethyl ether, and chloroform. The percentage extractive values and preliminary phytochemical constituents were determined using standard qualitative chemical tests. The in-vitro anti-inflammatory activity of the extracts was evaluated by the albumin denaturation method at different concentrations, with diclofenac sodium used as the standard drug. The percentage inhibition of protein denaturation and IC_{50} values were determined.

Results: The percentage yields obtained by Soxhlet extraction were 3.48% for the benzene extract, 2.97% for the diethyl ether extract, and 4.25% for the chloroform extract. Phytochemical screening revealed the presence of phytosterols and flavonoids in all three extracts, while phenolic compounds and tannins were additionally detected in the chloroform extract. The benzene, diethyl ether, and chloroform extracts exhibited concentration-dependent inhibition of albumin denaturation. The IC_{50} values were 14.40 mg for the benzene extract, 13.30 mg for the diethyl ether extract, and 9.89 mg for the chloroform extract, whereas diclofenac sodium showed an IC_{50} value of 11.40 mg under the reported experimental conditions.

Conclusion: Among the three extracts investigated, the chloroform extract demonstrated the highest in-vitro anti-inflammatory activity, as evidenced by its lowest IC_{50} value. The observed activity may be associated with the phytoconstituents detected in the extract, particularly flavonoids, phenolic compounds, tannins, and phytosterols. Further studies are required to isolate the active constituents and validate the anti-inflammatory potential using additional in-vitro and in-vivo models.

Key words: Anti-inflammatory activity, Benzene, Chloroform, Diethyl ether, Indomethacin and *Mussaenda philippica*

INTRODUCTION

The worldwide use of medicinal plants has gained additional impact due to its natural origin and high therapeutic implications. *Mussaenda philippica* 'Aurorae' belonging to family *Rubiaceae* is a shrub, having small tube-like flowers, oblong-lanceolate, dark green, glossy leaves. It is well distributed throughout India, Southeast Asia and Philippines.

This plant is used for dysentery, jaundice, emollient, snake bites, stomach-ache and influenza as folk remedies. *Mussaenda* genuses have various species having distinct novel phytoconstituents which are preferred for the treatment as antioxidant, antimicrobial, anti-inflammatory agents.

Plant materials are easily available in rural areas. Due to readily available of resources i.e. plants / herbs the medicines in rural belt are cheaper than the alternative medicines like modern medicines. It is used in high doses to treat appendicitis and hepatitis. The most recognized compounds in *Mussaenda philippica* are the iridoids and triterpene saponins. The plant parts are being used in folkloric medication by the people for the treatment of different types of GIT disorders.

Inflammation is an adaptive response that is triggered by noxious stimuli and conditions, such as infection and tissue injury. Considerable progress has been made in understanding the cellular and molecular events that are involved in the acute inflammatory response to infection and, to a lesser extent, to tissue injury.

Inflammation is a natural process in which the body's white blood cells release chemicals into the bloodstream and around the affected tissues to protect against potential threats. These threats can include foreign bodies, irritants, and pathogens like bacteria, viruses, and other infectious organisms.

Tissue damage due to trauma, microbial invasion, or noxious compounds can induce acute inflammation. It starts rapidly, becomes severe in a short time and symptoms may last for a few days

Mussaenda philippica A. Rich plant belongs to genus *Mussaenda* and family Rubiaceae Commonly it is known as "Queen of Philippines, Bangkok rose, Tropical Dogwood *Mussaenda philippica* is otherwise called as vellaiyilai in Tamil. This plant is mostly seen in the Philippines, India etc. [12]

Mussaenda philippica is a perennial ornamental shrub that is well known for its attractive and colourful floral display. One of its distinctive features is the presence of enlarged, leaf-like sepals surrounding the flowers, which enhance its ornamental appearance. The plant is commonly cultivated in tropical and subtropical regions as a garden and landscape plant. It grows well under warm climatic conditions with adequate sunlight and moisture. The leaves are simple, green, and arranged oppositely along the stem.

MATERIALS AND METHODS

Table 1: Plant Materials

S. No	Plant Name	Family	Plant of Part Used	Collection Period	Place of Collection
1	*Mussaenda philippica* (pink)	Rubiaceae	Leaves	January 2026	Tenkasi

1. Solvents used for Extraction:

1. Benzene
2. Diethyl ether
3. Chloroform

2. Collection and Authentication of *Mussaenda philippica*

Mussaenda philippica leaves were gathered in Tenkasi, Tamil Nadu, Tirunelveli District. The Nandha College of Pharmacy, Erode District, Tamil Nadu, India, was used to create taxonomic differentiating evidence. A hermetically sealed container was used to store the plant powder. After being shade-dried, the leaves were ground into a coarse powder. Before being used, the powdered components were kept in an airtight polythene bag.

3. Extractive Value Analysis of Plant Powder from *Mussaenda philippica*

To investigate the distribution of different components of *Mussaenda philippica,* extractive values were recorded in petroleum ether, ethyl aceto acetate, chloroform, diethyl ether, acetone, ethanol and

benzene. A glass beaker containing precisely weighed 0.5g of coarsely powdered air-dried material was macerated with 25ml of various solvents for six hours. After repeatedly shackling, they were left to stand for eighteen hours. The mixture was quickly filtered. After that, it was moved to a flat-bottomed dish covered with tar and let to evaporate until completely dry in a water bath. For six hours, the residue was dried at 105°C. immediately weighed after being cooled for half an hour in a desiccator.

Fig.1: Extractive Value Analysis of Plant Powder

4. Calculation of Percentage Yield

The percentage yield was calculated for the various extracts with reference to the crude plant material taken using the formula given below.

$$\text{Percentage yield with reference to Crude Plant material} = \frac{\text{Weight in grams of extracts obtained}}{\text{Weight in grams of plant material taken}} \times 100$$

5. Preparation of Extract from *Mussaenda philippica*

Soxhlet extraction was performed on the leaf's sections of *Mussaenda philippica* using benzene, diethyl ether and chloroform as the solvent. A muslin cloth pouch containing around 500 g of air-dried powdered plant material was put into the thimble of a Soxhlet extraction machine. Benzene, diethyl ether and chloroform were used as the extracting solvent in a round bottom flask to which the equipment was attached. To keep the flask from bumping while heating, porcelain chips were inserted.

The extraction process was conducted between 40 and 60 degrees Celsius until the solvent in the siphon tube turned colorless, signifying a complete extraction. Once the extraction process was finished, the benzene, diethyl ether and chloroform extract were extracted by distilling the solvent, which was then recovered. A rotary vacuum evaporator was used to concentrate the extract to dryness under low pressure. The original weight of the air-dried plant material placed in the thimble was used to calculate the percentage yield after the dried extract was weighed. Before being used again, the dried extract was kept in an airtight container in the refrigerator. To ascertain whether different phytochemical elements were present or absent, preliminary phytochemical screening was done.

Fig.2: Soxhlet Extraction of Benzene Extract from *Mussaenda*

Fig.3: Soxhlet Extraction of Diethyl ether Extract from

Fig.4: Soxhlet Extraction of Chloroform Extract from

philippica Leaves *Mussaenda philippica* Leaves *Mussaenda philippica* Leaves

6. Phytochemical Analysis of Benzene, Diethyl ether and Chloroform extract of *Mussaenda philippica* [8]

The benzene, diethyl ether and chloroform extracts were subjected to preliminary phytochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to establishing and providing the identity of a substance. The pharmacological actions of crude drugs were determined by the nature of their constituents. The phytoconstituents are responsible for the desired therapeutic properties. To obtain these pharmacological effects, the plant materials itself or extract in a suitable solvent or isolated active constituent may be used.

The benzene, diethyl ether and chloroform extracts of were subjected to the following chemical tests, separately for the identification of various active constituents (Evans, 1997).

I. Tests for Alkaloids [8]

a) Dragendorff's Test

A fraction of the extracts was treated with Dragendorff's reagent and observed for the formation of yellow coloured precipitate. Indicates presence of alkaloids.

b) Wagner's Test

A fraction of the extracts was treated with Wagner's reagent and observed for the formation of a reddish-brown precipitate. Indicates presence of alkaloids.

c) Mayer's Test

A fraction of the extracts was treated with Mayer's reagent and observed for the formation of white precipitate or creamy coloured precipitate. Indicates presence of alkaloids. **d) Hager's Test**

A fraction of the extracts was treated with Hager's reagent and observed for the formation of yellow precipitate. Indicates presence of alkaloids.

II. Test for Carbohydrates [8]

a) Molisch's Test

To 2 mL of the extracts, 1 mL of α-naphthol solution were added, and sulphuric acid concentrated through the sides of test tube. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.

b) Fehling's Test

To 1 mL of the extracts, equal quantities of Fehling’s solution A and B were added, upon heating on water bath, formation of a brick red precipitate indicated the presence of carbohydrates.

c) Benedict's test

To 5 mL of Benedict's reagent, 1 mL of extracts solution were added and boiled for 2 minutes and cooled. Formation of a red precipitate showed the presence of carbohydrates.

III. Tests of Glycosides [8]

a) Legal Test

The extracts were dissolved in pyridine and sodium nitroprusside solution was added to make it alkaline. The formation of pink, red to red colour showed, the presence of glycosides. **b) Baljet Test**

To 1 mL of the test extracts were added with 1 mL sodium picrate solution and the yellow to orange colour revealed, the presence of glycosides.

c) Borntrager's Test

A few mL of dilute HCl was added to 1 mL of the extract's solution. It was then boiled, filtered and the filtrate was extracted with chloroform. The chloroform layer was then treated with 1 mL of ammonia. The formation of red colour showed the presence of anthraquinone glycosides.

d) Keller Killani Test

The extracts were dissolved in acetic acid containing traces of ferric chloride, and it was then transferred to a test tube containing sulphuric acid. At the junction, formation of a reddish-brown colour, which gradually became blue, confirmed the presence of glycosides.

IV. Tests for Phytosterol [8]

a) Liebermann Burchard's Test

Mixed 3 mL of test solution with 3 mL of acetic acid anhydride. It was heated and then cooled. Few drops of concentrated sulphuric acid were added. A blue colour showed, presence of phytosterol.

b) Salkowski's Test

Dissolve the extracts in chloroform and equal volume of concentrate sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer represented the steroid components in the test extract.

V. Test for Flavonoids [8]

a) Shinoda test

The dried powdered or extracts were treated with 5 mL of 95% ethanol. Few drops of concentrated hydrochloric acid and 0.5 g of magnesium turnings. Pink colour was observed, presence of flavonoids.

VI. Test for Tannins and Phenolic compounds [8]

a) Ferric chloride test

1 mL of the extracts were added with ferric chloride and observed the formation of a dark blue or greenish black colour showed, presence of phenolic compounds.

b) Gelatin Test

A fraction of the extracts was treated with 1% gelatin containing 10% NaCl and observed the precipitation indicates, presence of tannins.

VII. Tests for Proteins and Amino Acids [8]

a) Biuret Test

1 mL of the extracts were treated with 1 mL of 40% sodium hydroxide solution followed by 2 drops of 1% copper sulphate solution. Formation of a violet colour showed the presence of proteins.

b) Xanthoproteic Test

1 mL of the extracts were treated with 1 mL of concentrated nitric acid. A white precipitate is formed; it was boiled and cooled. Then 20% of sodium hydroxide or ammonia was subsequently added orange colour to indicate the presence of aromatic amino acids.

VIII. Test for Saponins [8]

About 1 mL of ethanolic extracts were diluted to 20 mL with distilled water and shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicated the presence of saponins.

IX. Test for Fixed Oils [8]

a) Spot Test

A small quantity of extracts was pressed between two filter papers. Oil stains on the filter paper indicated the presence of fixed oils.

b) Saponification Test

1 mL of the extracts were added with few drops of 0.5 N alcoholic potassium hydroxide along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hrs. The formation of soap or partial neutralization indicated the presence of fixed oils.

7. *IN-VITRO* ANTI-INFLAMMATORY STUDIES

I. Principle of *In-vitro* Egg Albumin Denaturation Method [25]

The most objective of the egg white's denaturation measure is to discover out in case certain operators or compounds can stop or moderate down the method of egg whites getting to be denatured beneath conditions. Denaturation alludes to the altar in a protein's structure that leads to a misfortune of its natural work. In this try, egg whites serve as a demonstrate protein, and denaturation happens when it is uncovered to extraordinary warm, changing pH levels, or other denaturing substances. Amid denaturation, the initial shape of egg whites is changed, influencing its physical properties and coming about in a misfortune of its utilitarian movement. The test surveys how well a medicate or compound can avoid or diminish the denaturation of egg whites, which makes a difference assesses its anti-inflammation impacts. The fundamental rule of the egg white's denaturation measure is that substances with antiinflammation properties might balance out protein structures and anticipate denaturation, a prepare regularly related with irritation and tissue harm. Hence, operators or compounds that altogether diminish egg whites' denaturation in this test might possibly display anti-inflammation impacts. Protein denaturation is accepted to be one of the components contributing to irritation. Non-steroidal anti-inflammation drugs (NSAIDs) not as it were avoiding protein denaturation but to restrain the COX enzyme at the same time. Different concentrations of the test can be blended with egg whites' arrangement beneath controlled exploratory conditions, permitting the responses to occur sometime recently measuring the absorbance to calculate the rate of restraint.[25]

II. Inhibition of Albumin Denaturation [25]

The response blend was made by combining 0.5 ml of Boswellic acid corrosive and a 0.45 ml fluid arrangement of 5% bovine egg whites. The pH of the blend, which was 6.3, was balanced with a bit of 0.1N HCl whereas keeping it at 37 ˚C for 20 minutes. After that, it warmed to 57 ˚C for 30 minutes. Once cooled, the arrangement was exchanged to 96-well plates, and the absorbance was measured at 660 nm. Standard was utilized as Diclofenac sodium (1000µg/ml) and the control contains 0.05ml refined water. The rate of restraint of egg whites' denaturation was calculated by the taking after equation,

Percentage of Inhibition (%)

$$= \frac{\text{Absorbance of Control - Absorbance of Test Sample}}{\text{Absorbance of Control}} \times 100$$

RESULTS AND DISCUSSION

1. Preparation of Extracts and the Calculation of Percentage Yield of *Mussaenda philippica*.

The *Mussaenda philippica* plant's shade-dried leaves were gathered from Tenkasi in Tamil Nadu, India's Tirunelveli District. In a Soxhlet device (Harborne, 1984), the powdered plant materials were successively extracted using benzene, diethyl ether and chloroform using the heated continuous percolation method for a full day. After concentrating the extract using a rotary evaporator, it was freeze-dried in a lyophilizer until dry power was achieved. Until it was needed again, the extract was kept in screw cap vials. Table No. 2 displays the % yield of *Mussaenda philippica* leaf plants.

Table 2: Percentage Yield of *Mussaenda philippica*

Plant name	Family	Parts Used	Method of extraction	Solvent system	Percentage yield (% W/W)
Mussaenda Philippica (Pink)	Rubiaceae	Leaf Powder	Continuous hot percolation in Soxhlet Apparatus	Benzene	3.48 %
				Diethyl ether	2.97 %
				Chloroform	4.25 %

2. Extractive Values of *Mussaenda philippica*

The extractive values are useful for estimating the chemical components of the crude medication and for determining which specific components are soluble in a certain solvent. In order to investigate the distribution of different components of *Mussaenda philippica,* extractive values were recorded in diethyl ether, petroleum ether, chloroform, benzene, acetone, ethyl acetate, ethanol. 3.47% w/w is the highest range of extractive values found in Petroleum ether extract.

Table 3: Extractive Values of Mussaenda *philippica*

S. No.	Experiment	Percentage values %			Average percentage yield (%) SEM
		1%W/W	2%W/W	3%W/W	
1	Diethyl ether extract	2.8	3.0	3.2	3.00 ± 0.12
2	Petroleum ether extract	3.3	3.5	3.6	3.47 ± 0.09
3	Chloroform extract	2.6	2.8	3.1	2.83 ± 0.15
4	Benzene extract	3.0	3.2	3.4	3.20 ± 0.09
5	Acetone extract	2.9	3.0	3.2	3.03 ± 0.09
6	Ethyl aceto acetate extract	3.1	3.3	3.7	3.37 ± 0.18
7	Ethanol extract	2.7	2.9	3.3	2.97 ± 0.18

3. Phytochemical Screening of Benzene, Diethyl Ether and Chloroform Extract of *Mussaenda philippica*

1. Phytochemical Screening of Benzene Extract of *Mussaenda philippica*

Phytosterols, Flavanoids and Fixed oils were found in the initial phytochemical examination of the benzene extract of leaf of the plant of *Mussaenda philippica*. Table 4: Screening for phytochemicals.

Table 4: Phytochemical Screening of Benzene Extract of *Mussaenda philippica*

S. No	Phytochemical test	Benzene Extract
1	Alkaloids	-
2	Carbohydrates	-
3	Glycosides	-
4	Phytosterols	+
5	Flavanoids	+
6	Phenolic compounds	-
7	Tannins	-
8	Protein	-
9	Amino acids	-
10	Saponins	-
11	Fixed oils	+

2. Phytochemical Screening of Diethyl ether Extract of *Mussaenda philippica*

Phytosterols, Flavanoids, Phenolic compounds and Fixed oils were found in the initial phytochemical examination of the Diethyl ether extract of leaf of the plant of *Mussaenda philippica*. Table 5: Screening for phytochemicals.

Table 5: Phytochemical Screening of Diethyl Ether Extract of *Mussaenda philippica*

S. No	Phytochemical test	Diethyl Ether Extract
1	Alkaloids	-
2	Carbohydrates	-
3	Glycosides	-
4	Phytosterols	+
5	Flavanoids	+

6	Phenolic compounds	+
7	Tannins	-
8	Protein	-
9	Amino acids	-
10	Saponins	-
11	Fixed oils	+

3. Phytochemical Screening of Chloroform Extract of *Mussaenda philippica*

Phytosterols, Flavanoids, Phenolic compounds, Tannins and Fixed oils were found in the initial phytochemical examination of the Chloroform extract of leaf of the plant of *Mussaenda philippica*. Table 6: Screening for phytochemicals.

Table 6: Phytochemical Screening of Chloroform Extract of *Mussaenda philippica*

S. No	Phytochemical test	Chloroform Extract
1	Alkaloids	-
2	Carbohydrates	-
3	Glycosides	-
4	Phytosterols	+
5	Flavanoids	+
6	Phenolic compounds	+
7	Tannins	+
8	Protein	-
9	Amino acids	-
10	Saponins	-
11	Fixed oils	+

Note

(+ indicates the presence of constituents)
(- indicates the absence of constituents)

4. *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY

1. MUSSAENDA PHILIPPICA BENZENE EXTRACT

IC_{50} value of *Mussaenda philippica* benzene extract: 14.40 mg
IC_{50} value of Diclofenac sodium: 11.40 mg

Table 7: *Mussaenda philippica* Benzene Extract *In-vitro* Anti-Inflammatory Activity

S.NO	Concentration (mg)	COD	SOD	% inhibition	Average	$IC_{50\ (mg)}$
1	5 mg	0.28	0.07	75%	69.6 ± 3.75	14.40 mg
2		0.28	0.08	71%		
3		0.28	0.08	71%		
4		0.28	0.09	67%		
5		0.28	0.09	67%		
6		0.28	0.10	64%		
1	10 mg	0.28	0.09	67%	62.5 ± 3.75	
2		0.28	0.10	64%		
3		0.28	0.10	64%		
4		0.28	0.11	60%		
5		0.28	0.11	60%		
6		0.28	0.12	57%		
1	15 mg	0.28	0.13	53%	48.2 ± 3.75	
2		0.28	0.14	50%		
3		0.28	0.14	50%		
4		0.28	0.15	46%		
5		0.28	0.15	46%		

6		0.28	0.16	42%		
***In-vitro* Standard Diclofenac sodium**						
1	10 mg	0.28	0.06	78%	80.6 ± 2.30	
2		0.28	0.05	82%		
3		0.28	0.05	82%		

Table 8: *Mussaenda philippica* Benzene Extract Anti-Inflammatory Activity IC_{50} Value (mg) Compared to Standard Diclofenac Sodium IC_{50} Value (mg)

S. NO	Concentration (mg)	Average (%)	$IC_{50\ (mg)}$
1	5 mg	70%	14.40 mg
2	10 mg	63%	
3	15 mg	48%	
Standard Diclofenac Sodium			
1	5 mg	75%	11.40 mg
2	10 mg	71%	
3	15 mg	65%	

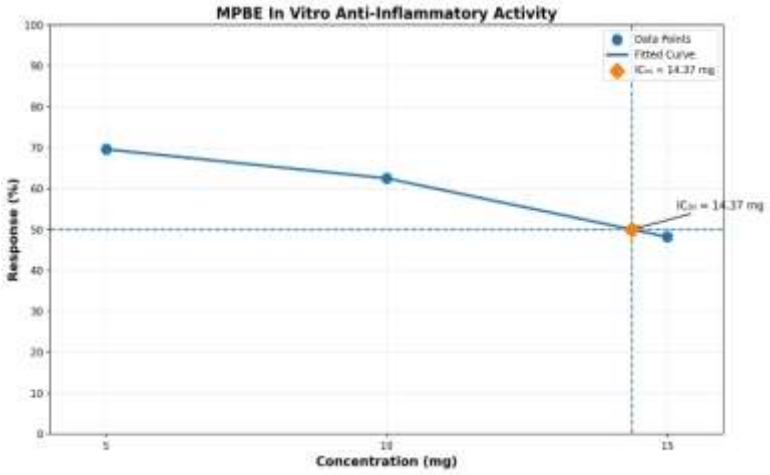

Fig. 5: *In-vitro* Anti-Inflammatory Activity of *Mussaenda philippica* Benzene Extract

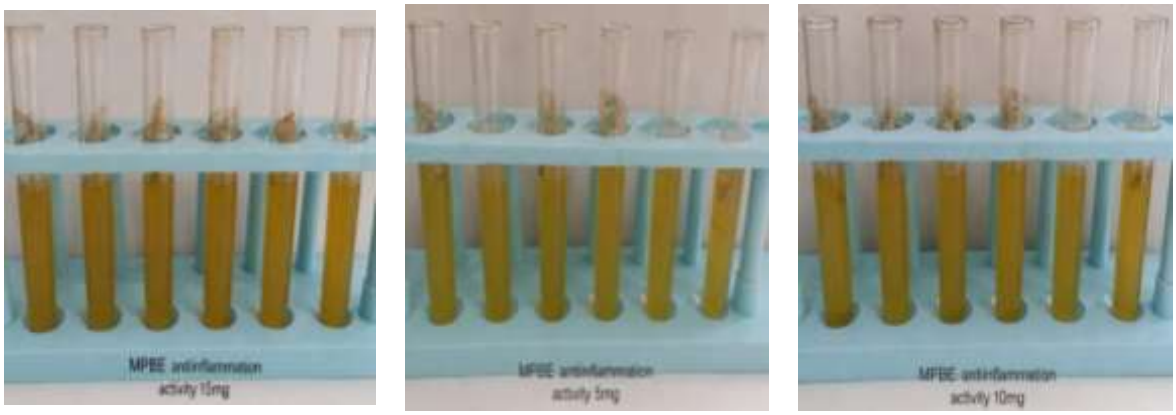

Fig. 6: Anti-Inflammatory Assay of *Mussaenda philippica* Benzene Extract

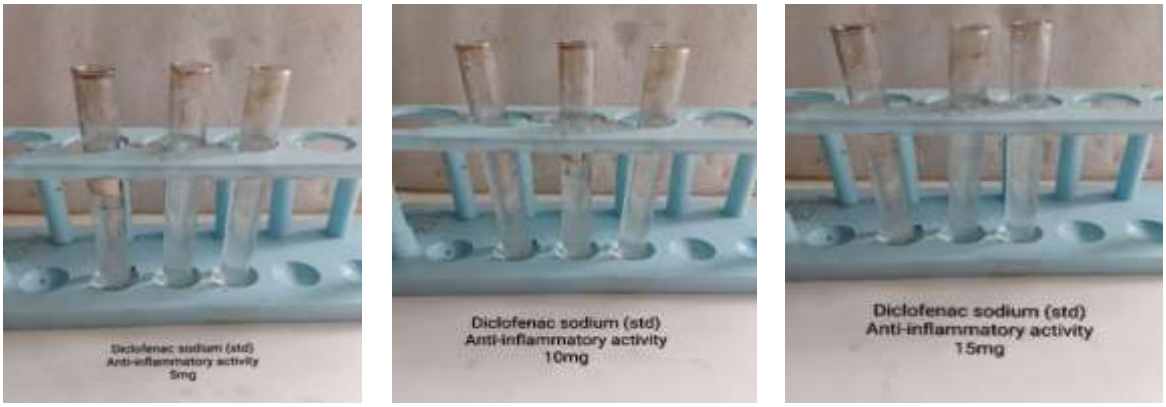

Fig. 7: Anti-Inflammatory Assay of Standard Drug Diclofenac Sodium

To assess anti-inflammation activity *in-vitro*, the albumin denaturation method was utilized. At 5 mg, demonstrated a 70% inhibition and an IC_{50} value of 14.40 mg, compared to the standard diclofenac sodium, which had an IC_{50} value of 11.40 mg. During our investigation of various concentrations in a dose-dependent manner, we found that many concentrations exhibited antioxidant and anti-inflammation properties of MPBE. When compared to diclofenac sodium, MPBE showed significant anti-inflammation effects.

6.4.2 *MUSSAENDA PHILIPPICA* DIETHYL ETHER EXTRACT

IC_{50} value of *Mussaenda philippica* benzene extract: 13.3 mg
IC_{50} value of Diclofenac sodium: 11.40 mg

Table 9: *Mussaenda philippica* Diethyl Ether Extract *In-vitro* Anti-Inflammatory Activity

S.NO	Concentration (mg)	COD	SOD	% inhibition	Average	IC_{50} (mg)
1	5 mg	0.28	0.08	71%	67.8± 3.19	13.3 mg
2		0.28	0.08	71%		
3		0.28	0.09	67%		
4		0.28	0.09	67%		
5		0.28	0.10	64%		
6		0.28	0.10	64%		
1	10 mg	0.28	0.10	64%	60.7±3.19	
2		0.28	0.10	64%		
3		0.28	0.11	60%		
4		0.28	0.11	60%		
5		0.28	0.12	57%		
6		0.28	0.12	57%		
1	15 mg	0.28	0.14	50%	44.4 ± 3.75	
2		0.28	0.15	46%		
3		0.28	0.15	46%		
4		0.28	0.16	42%		
5		0.28	0.16	42%		
6		0.28	0.17	39%		
***In vitro* Standard Diclofenac Sodium**						
1	10 mg	0.28	0.06	78%	80.6 ± 2.30	

2		0.28	0.05	82%	
3		0.28	0.05	82%	

Table 10: *Mussaenda philippica* Diethyl Ether Extract Anti-Inflammatory Activity IC_{50} Value (mg) Compared to Standard Diclofenac Sodium IC_{50} Value (mg)

S. NO	Concentration (mg)	Average (%)	IC_{50} (mg)
1	5 mg	68%	13.3 mg
2	10 mg	61%	
3	15 mg	44%	
Standard Diclofenac sodium			
1	5 mg	75%	11.40 mg
2	10 mg	71%	
3	15 mg	65%	

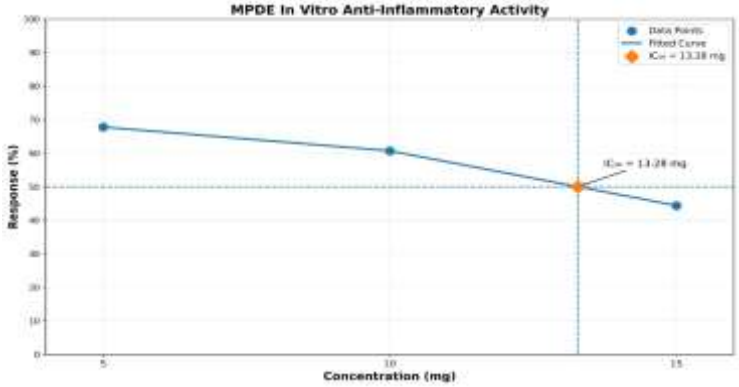

Fig. 8: *In-vitro* Anti-Inflammation Activity of *Mussaenda philippica* Diethyl Ether Extract

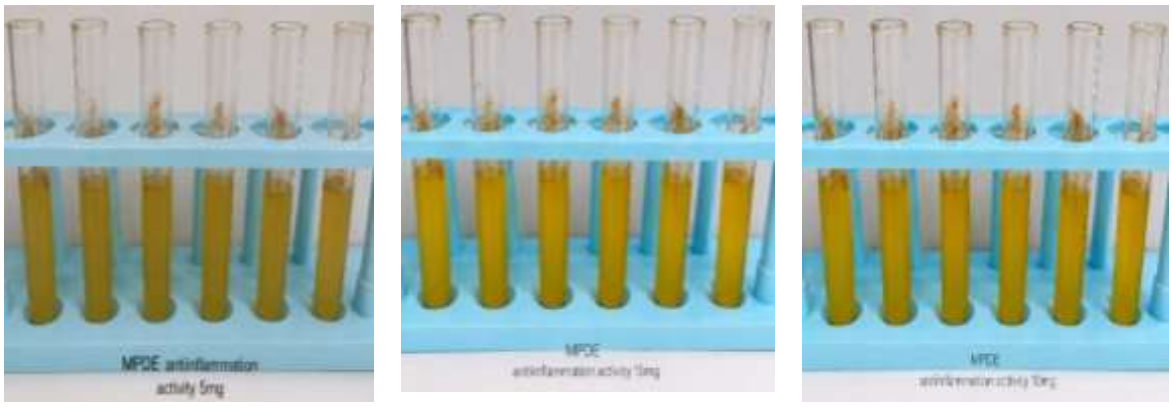

Fig. 9: Anti-Inflammatory Assay of *Mussaenda philippica* Diethyl Ether Extract

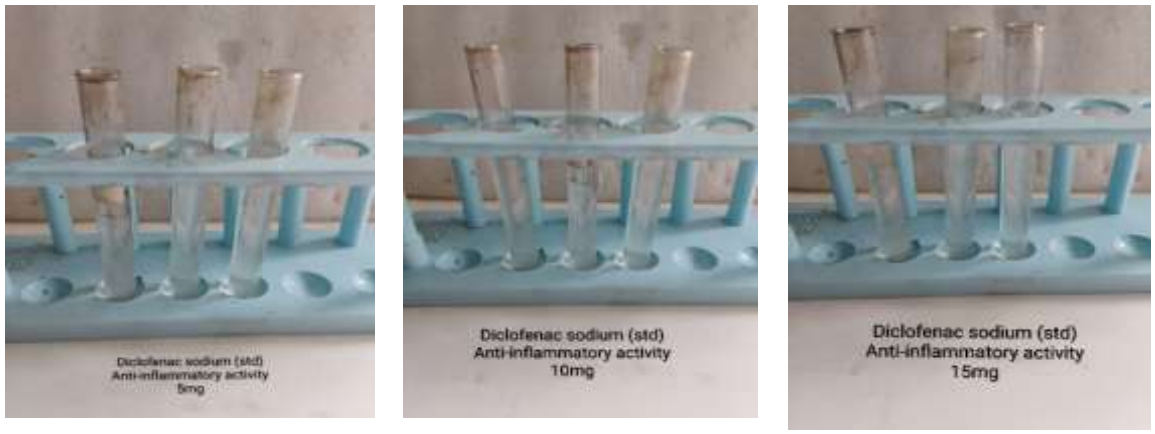

Fig. 10: Anti-Inflammatory Assay of Standard Drug Diclofenac Sodium

To assess anti-inflammation activity in vitro, the albumin denaturation method was utilized. At 5 mg, demonstrated a 68% inhibition and an IC_{50} value of 13.3 mg, compared to the standard diclofenac sodium, which had an IC_{50} value of 11.40 mg. During our investigation of various concentrations in a dose-dependent manner, we found that many concentrations exhibited antioxidant and anti-inflammation properties of MPDE. When compared to diclofenac sodium, MPDE showed significant anti-inflammation effects.

3. *MUSSAENDA PHILIPPICA* CHLOROFORM EXTRACT

IC_{50} value of *Mussaenda philippica* Chloroform extract: 9.89 mg
IC_{50} value of Diclofenac sodium: 11.40 mg

Table 11: *Mussaenda philippica* Chloroform Extract *In Vitro* Anti-Inflammatory Activity

S.NO	Concentration(mg)	COD	SOD	% inhibition	Average	$IC_{50\ (mg)}$
1	5 mg	0.28	0.08	71%	66.1 ± 5.23	9.89 mg
2		0.28	0.09	67%		
3		0.28	0.09	67%		
4		0.28	0.08	71%		
5		0.28	0.10	64%		
6		0.28	0.12	57%		
1	10 mg	0.28	0.13	53%	53.1 ± 4.95	
2		0.28	0.15	46%		
3		0.28	0.14	50%		
4		0.28	0.13	53%		
5		0.28	0.12	57%		
6		0.28	0.11	60%		
1	15 mg	0.28	0.16	42%	41.6 ± 4.22	
2		0.28	0.15	46%		
3		0.28	0.18	35%		
4		0.28	0.17	39%		
5		0.28	0.16	42%		
6		0.28	0.15	46%		
In vitro Standard Diclofenac sodium						
1	10 mg	0.28	0.06	78%	80.6 ± 2.30	
2		0.28	0.05	82%		
3		0.28	0.05	82%		

Table 12: *Mussaenda philippica* Chloroform Extract Anti-Inflammation Activity IC_{50} Value (mg) Compared to Standard Diclofenac Sodium IC_{50} Value (mg)

S.NO	Concentration (mg)	Average (%)	$IC_{50\ (mg)}$
1	5 mg	66%	9.89 mg
2	10 mg	53%	
3	15 mg	42%	
Standard Diclofenac sodium			
1	5 mg	75%	11.40 mg
2	10 mg	71%	
3	15 mg	65%	

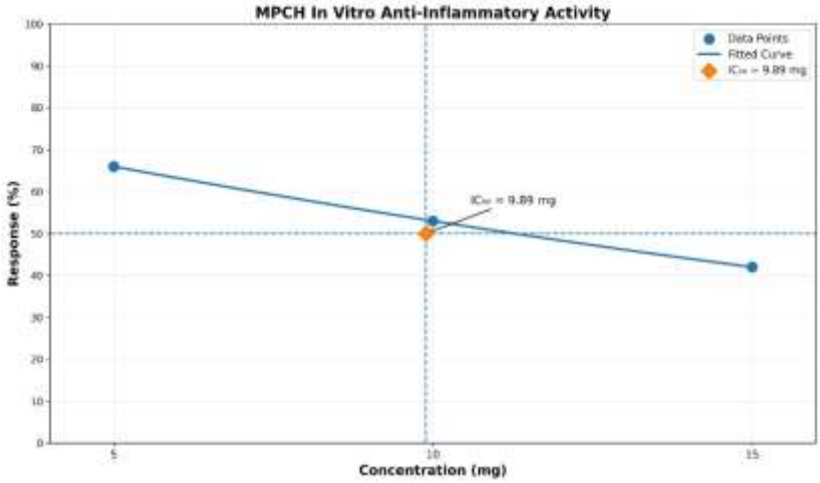

Fig. 11: *In-vitro* Anti-Inflammatory Activity of *Mussaenda philippica* Chloroform Extract

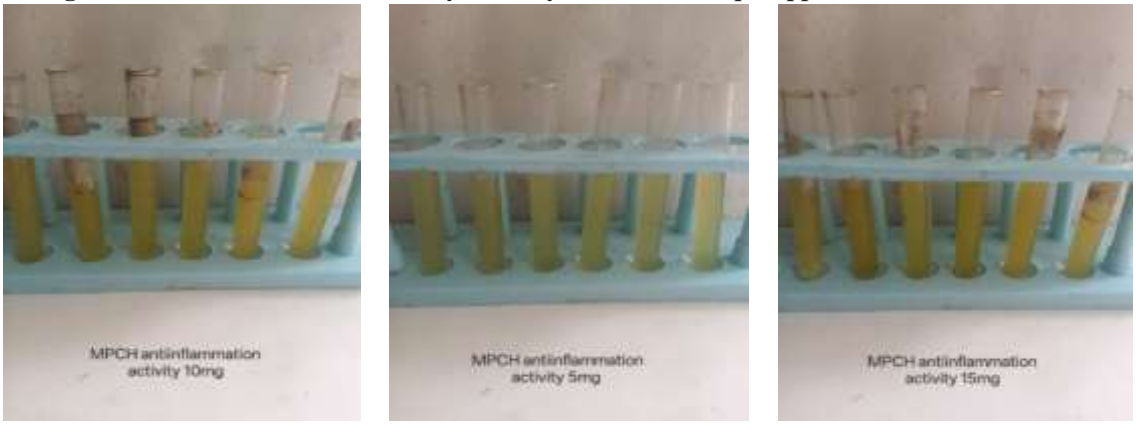

Fig. 12: Anti-Inflammatory Assay of *Mussaenda philippica* Chloroform Extract

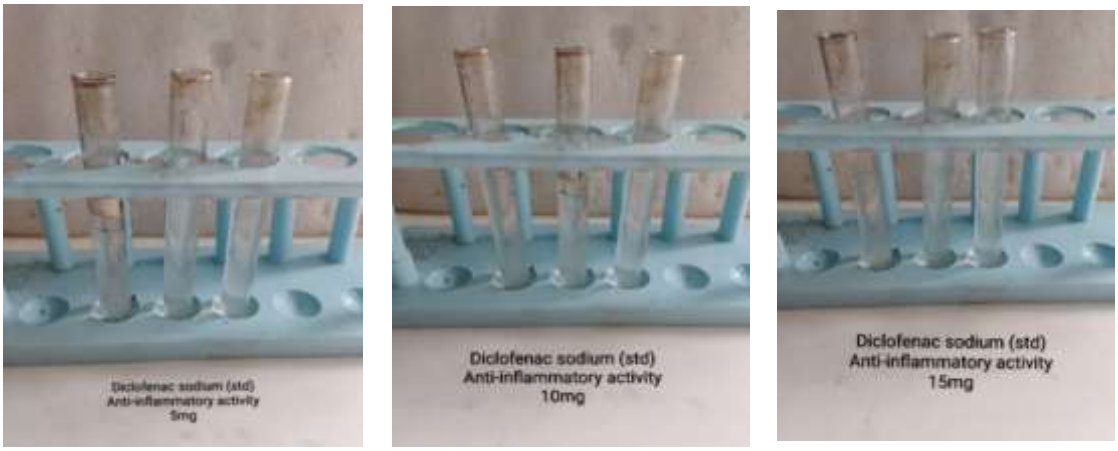

Fig. 13: Anti-Inflammatory Assay of Standard Drug Diclofenac Sodium

To assess anti-inflammation activity in vitro, the albumin denaturation method was utilized. At 5 mg, demonstrated a 66% inhibition and an IC50 value of 9.89 mg, compared to the standard diclofenac sodium, which had an IC50 value of 11.40 mg. During our investigation of various concentrations in a dose-dependent manner, we found that many concentrations exhibited antioxidant and anti-inflammation properties of MPCH. When compared to diclofenac sodium, MPCH showed significant anti-inflammation effects

CONCLUSION

The present study demonstrated that the different extracts of *Mussaenda philippica* possess *in-vitro* anti-inflammatory activity by inhibiting albumin denaturation. Among the three extracts, the chloroform extract showed the highest anti-inflammatory potency with the lowest IC_{50} value of 9.89 mg. It exhibited comparatively greater activity than the standard drug diclofenac sodium, which showed an IC_{50} value of 11.40 mg under the experimental conditions.

The diethyl ether and benzene extracts showed comparatively lower activity, with IC_{50} values of 13.30 mg and 14.40 mg, respectively. Therefore, the chloroform extract can be considered the most promising extract among the extracts investigated for *in-vitro* anti-inflammatory activity.

Overall, the findings suggest that *Mussaenda philippica*, particularly its chloroform extract, may contain bioactive phytoconstituents responsible for the observed anti-inflammatory effect. Further studies are required for the isolation and identification of the active constituents and to confirm the anti-inflammatory potential through additional *in-vitro* and *in-vivo* studies.

The present study indicates that the different solvent extracts of *Mussaenda philippica* leaves possess phytoconstituents and exhibit *in-vitro* anti-inflammatory activity. The findings suggest that *Mussaenda philippica* may be a potential source of natural anti-inflammatory agents and warrants further investigation to identify the active constituents responsible for the activity.

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