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Research Article

In Vitro Anti-Inflammatory and Free Radical Scavenging Activities of Morus alba (Mulberry) Seed Extracts

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ABSTRACT

Fresh seeds of *Morus alba* were collected, dried, and coarsely powdered. The powdered material was subjected to Soxhlet extraction followed by successive fractionation using solvents of increasing polarity, namely petroleum ether, chloroform, ethyl acetate, and ethanol. Preliminary phytochemical screening was performed to identify major bioactive constituents. The anti-inflammatory activity was evaluated using the protein denaturation (egg albumin) assay, while antioxidant activity was assessed using the DPPH assay. The percentage inhibition was calculated and compared with standard drugs such as Diclofenac and Ascorbic acid. Result: In the anti-inflammatory assay, the ethyl acetate extract showed the highest inhibition of protein denaturation (96.67%), followed by petroleum ether (77.22%), ethanol (64.44%), and chloroform (25.56%). In the antioxidant study, the ethyl acetate extract exhibited maximum free radical scavenging activity (89.43%), followed by ethanol (83.76%), petroleum ether (80.41%), and chloroform (60.82%), while the standard ascorbic acid showed 97.68% inhibition. Discussion: The significant biological activities observed in the ethyl acetate extract may be attributed to the presence of phenolic and flavonoid compounds. The results demonstrate a correlation between antioxidant and anti-inflammatory activities, indicating that reduction of oxidative stress contributes to anti-inflammatory effects. The variation in activity among different extracts is due to the difference in polarity and solubility of phytoconstituents. Conclusion: The study concludes that *Morus alba* seeds possess potent anti-inflammatory and antioxidant properties. Among all extracts, the ethyl acetate fraction showed the highest activity, suggesting its potential as a natural therapeutic agent. Further studies are required to isolate active compounds and validate their efficacy through in vivo models.

INTRODUCTION

1.1 GENERAL INTRODUCTION

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Inflammation is a complex biological response of vascular tissues to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain. Inflammation is either acute or chronic inflammation. Acute inflammation, with exudation of fluid and plasma proteins as its main features, occurs very rapidly, and the process can last for few or several minutes to several days. Chronic inflammation occurs when the acute inflammatory process occurs repeatedly or continuously, with the process lasting for several weeks to months and even years.¹

Anti-inflammation is the reaction that occurs to minimize inflammation. Anti-inflammatory drugs, Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are used to treat pain and other inflammatory responses. These NSAIDs are known as anti-inflammatory agents, an anti-inflammatory agent is a chemical compound along with a medication that can decrease inflammation²

Antioxidants from spices are a large group of bioactive compounds which consist of flavonoids, phenolic compounds, sulphur-containing compounds, tannins, alkaloids, phenolic

diterpenes, and vitamins. Natural antioxidants contained in spices help to reduce oxidative stress. Oxidative stress, which is caused by high concentration of free radicals in cells and tissues, can be induced by various negative factors, such as gamma, UV, and X-ray radiation, psycho-emotional stress, polluted food, adverse environmental conditions, intensive physical exertion, smoking, alcoholism, and drug addiction. Chronic oxidative stress has been reported to lead to a variety of diseases, including cancer, heart related diseases, and the acceleration of aging.³

The role of the antioxidants is to neutralise the free radicals in biological cells, the free radicals having a negative impact on living organisms. A special role in neutralising the effects of the oxidative stress related to the presence of free radicals is played by the enzyme called superoxide dismutase (SOD).⁴

1.1.1 *Morus alba*

Morus alba L. also known as Tut in India belongs to the *Moraceae* family and is well recognized for its medicinal qualities.⁵



Fig. 1.1 Leaves



Fig. 1.2 Fruits



Fig.1.3Seeds



Fig.1.4 Plants

1.1.2 Available species

1. *Morus alba* L.
2. *Morus rubra*
3. *Morus nigra*
4. *Morus australis*
5. *Morus atropurpurea*
6. *Morus cathayana*
7. *Morus notabilis*
8. *Morus mesozygi*⁶

1.1.3 Vernacular Names

Sanskrit: Toola, Tula

Hindi: Chinni, Tut, Tutri

Bengali: Tut

Marathi: Tut, Ambat

Gujarati: Shetur

Telegu: Reshme chettu, Pippalipandu chettu

Tamil: Kambli chedi

Kannada: Bili uppu nerale

Punjabi: Tut, Tutri

Oriya: Tuto, Tuticoli

English: Mulberry, White mulberry⁷

1.1.4 Distribution

In plain areas in India *Morus alba* species is extensively cultivated. In Himalayan hills up to 3300 meters height for its foliage which is used as a food source for silkworms. In Europe and Asia *Morus alba* is cultivated and is occasionally naturalized.⁸

1.1.5 Botanical Description

Bark-*Morus alba* L., a fast-growing shrub or medium-sized tree, has a straight, cylindrical trunk of 1.8 m lacking buttresses. The bark is dark greyish brown with longitudinal cracks and a rough surface, and latex is usually white or slightly yellowed white.

Leaves-The lateral, scaly coral stem has a simple trilobal palm with three veins at the base and two rows of oval or practically oval leaves. Four-

petaled, greenish blooms resemble scales. Catkin-like racemes of free bloom with four stamens and a pistil. Male blossoms.

Flowers- The female flowers exhibited ovary blockage, one or two chambers, one ovule, two styles, and a fan-shaped ovary with one ovule. The spikes may be long or short. Ovary-containing one-ovule fan. This ovarian syncarpous fruit has fleshy perianths around several drupes up to 5 cm long.

Seed-The seed is light yellow or brown in color, oval shaped with a nearly flat surface at the micropylar region. The seed coat contains two layers; the outer hard and brittle layer called the testa and the inner thin papery and slightly brownish layer called the tegmen. Inside the seed coat, there is the kernel, which contains outer endosperm and inner embryo⁹

1.1.6 Taxonomic Classification

Kingdom- Plantae

Subkingdom -Tracheobionta

Super division-Spermatophyta

Division-Magnoliophyta

Class- Magnoliopsida

Subclass- Hamamelididae

Order- Urticales

Family- Moraceae

Genus- *Morus* L

Species -*Morus alba* L

Scientific Name *Morus alba*¹⁰

1.1.7 Morphological characters¹¹

Table 1.1

Morphological characters	<i>M. alba</i>
Bud color	Brown
Bud size (mm ²)	16.50–39.90
Branch color	Gray or greyish yellow
Branching	Erect
Leaf lobation	Lobed to unlobed
Leaf color	Pale green
Leaf surface	Smooth
Leaf margin	Larger round serration
Leaf length (cm)	10–15
Inflorescence length (cm)	3–4
Fruit color	White-red
Petiole groove	Present
Lenticel size (mm ²)	0.41–2.00

1.1.8 Cultivation

Mulberry trees can be propagated by seeds, cuttings, or grafting's. Seeds should be treated with camphor water before sowing to ward off disease. Thin layer of soil and ashes spread over seed after sowing. Beds are kept moist. Seeds germinate in 9 to 14 days, depending on the season. When seedlings are about 7.5 cm tall, they are thinned and weeded. For bush mulberries, seedlings 10 to 15 cm tall are used as transplants; for trees, seedlings are allowed to grow 1.3 m and trained before transplanting. Branches are cut into pieces 22 to 30 cm long with 3 buds and planted immediately.

Mulberry plants from seedlings are more expensive, but give better plants than those from cuttings. Root grafting is usually practiced in India. Rooted cuttings are planted in pits or furrows. When irrigation is used, cuttings are planted in furrows in April or May, 10 cm apart, the furrows being 22 cm apart. With this very close planting, 110,000 to 200,000 cuttings/ha are required. Grafted plants develop a better root system than those from seedlings, cuttings, or layering, and are used exclusively in Japan. Grafted trees are planted 1.6 m apart each way,



about 4,000/ha, and are especially suitable for irrigated areas. After each pruning, the field is cultivated and manured.¹²

1.1.9 Nutritional Assessment

Fats, carbohydrates, proteins, fibers, vitamins, and minerals are present in *Morus alba* and their precursors are present in significant amount. The fruits of *Morus alba* contain moisture about 71.5% and the weight of the fruit is about 3.49grams. *Morus alba* have lower moisture quantity and have more fat contents (1.10%).

Morus alba contains palmitoleic acid, behenic acid and ascorbic acid. The fresh leaves of *Morus alba* contain carbohydrate 8.01-13.42%, proteins 4.72-9.96%, fats 0.64-1.51% and contain moisture from 71.13-76.68% while the dried leaves of *Morus alba* contain carbohydrates 9.70-29.64%, proteins 15.31-30.91%, fats 2.09-4.93% and the moisture quantity decreases up to 5.11-7.24%.

The quantity of ascorbic acid in fresh leaves range from 160-280mg/100g while in dried leaves this quantity decreases up to 100-200mg/100g. The

quantity of β -carotene in dried leaves ranges from 8.438-13.125mg/100g while in case of fresh leaves 10.00-14.688mg/100g.¹³

1.1.10 Bioactive Compound

There are various types of chemicals present in *Morus alba* viz. folic acid, carotene, vitamins, flavonoids, tannins, saponins, ascorbic acid and antioxidants, bioflavonoids, moracetin, rutin, isoquercetin and quercetin 3-triglucoside, sterols, β -Sitosterol, amino acid and organic acid, triterpenes, volatile oil, alkaloids, 1 deoxynojirimycin, prostaglandin E2, nitric acid and cytokinin, calystegin, Albanol, Albafuran, Kuwanol, Murasin, Hydroximorasin and Moranoline, phytosterols, triterpenes, sitosterols, benzofuran derivatives, morusimic acid, anthraquinones, glycosides, oleanolic acid and anthocyanins are present as a main active principles.¹⁴

1.1.11 Ethnomedical importance of *Morus alba*¹⁵

Activities	Plant Part Used	Activity Model	Reference
Anti-inflammatory activity	Twig, root	Carrageenan in mice	Chen et al., 2013; Chung et al., 2003
Antioxidant activity	Twig, leaves, fruit	Ferrous ion chelating activity, ferric reducing power	Chang et al., 2001; Yang, 2011; Yea et al., 2016; Lye, 2012
Anti-cancer activity	Leaves, root	Hepatocellular carcinoma cells, hepatoma cells	Dat, 2010; Naowaratwattana, 2010; Chan et al., 2016
Antihyperlipidemic activity	Leaves, root	High-cholesterol diet treated hyperlipidemic rats	Zeni and Dall Molin, 2010; Jo et al., 2014
Antimicrobial activity	Leaves, root	<i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> , <i>Bacillus subtilis</i> , <i>Streptococcus mutans</i> , <i>Streptococcus sanguis</i> , <i>Streptococcus sobrinus</i>	Omidiran et al., 2012; Park et al., 2003
Neuroprotective activity	Leaves	Foot shock-induced aggression, Water maze test	Yadav and Nade, 2008; Kaewkaen et al., 2012
Antidiabetic activity	Twig, leaves, root, fruit	Alloxan-induced diabetes, brain-derived neurotropic factor, Zucker diabetic fatty rats	Liu et al., 2015; Shukla et al., 2016; Vichasilp et al., 2012; Mohammadi and Naik, 2012;

			Kumar, 2012; Yea et al., 2016; Sarikaphuti et al., 2013
Anti-atherosclerotic activity	Leaves, fruit	Human endothelial cells	Rynkoa et al., 2016; Harauma et al., 2007; Chen et al., 2005
Anti-obesity activity	Leaves, fruit	Diet-induced obese mice, Obese mice	Oh et al., 2009; Valacchi et al., 2014
Tyrosinase inhibitory activity / skin whitening activity	Twig, leaves	Melanin formation in melan A cells	Zhang et al., 2016; Lee et al., 2002
Hepatoprotective activity	Fruit, leaves	Carbon tetrachloride in rats	Hogade et al., 2010; Hsu et al., 2012
Cardioprotective activity	Leaves	Cardiac markers	Madhumitha and Indhuleka, 2012

- Inflammation and oxidative stress are major contributors to chronic diseases such as arthritis, diabetes, cardiovascular disorders, cancer, and neurodegenerative conditions.
- Current synthetic anti-inflammatory and antioxidant drugs cause serious long-term adverse effects, increasing the demand for safer, natural therapeutic agents.
- Although *Morus alba* is widely used in traditional medicine, most research has focused on leaves and fruits, while seeds remain under-studied despite their rich phytochemical content.
- Seeds of *Morus alba* contain potent bioactive compounds such as phenolics, flavonoids, fatty acids, resveratrol-like molecules, and antioxidants that may exhibit strong pharmacological properties.
- Protein denaturation is one of the key mechanisms involved in inflammation, while free radicals are responsible for cellular damage and disease progression. Therefore, inhibition of protein denaturation is considered a useful approach for screening anti-inflammatory agents.
- Oxidative stress plays a crucial role in aging and the development of several degenerative diseases. Hence, evaluation of free radical scavenging activity is essential to identify potential antioxidant compounds.
- Scientific evaluation of *Morus alba* seed extract will validate its traditional usage and uncover its therapeutic value, contributing to evidence-based herbal medicine.
- Since seeds are often discarded as waste, exploring their bioactivity can promote value addition and sustainable utilization of plant resources.
- Identifying the antioxidant and anti-inflammatory potential of seeds may help in developing new herbal formulations, supplements, or nutraceuticals.

1.4 AIM & OBJECTIVE

AIM: To evaluate the in vitro anti-inflammatory and antioxidant activities of different extracts (petroleum ether, ethyl acetate, chloroform, ethanol) prepared from *Morus alba* seeds.

OBJECTIVE-

1.4.1 Extraction and Fractionation:



To prepare crude extracts of *Morus alba* seeds and subject them to successive solvent fractionation using solvents of increasing polarity, namely petroleum ether, chloroform, ethyl acetate, and ethanol, to separate and isolate different classes of bioactive compounds.

1.4.2 Evaluation of Anti-inflammatory Activity:

To evaluate the in vitro anti-inflammatory potential of the prepared fractions by assessing their ability to inhibit protein denaturation using the egg albumin assay method.

1.4.3 Evaluation of Free Radical Scavenging Activity:

To determine the in vitro antioxidant activity of the fractions by performing free radical scavenging assays such as the DPPH assay to estimate their radical scavenging capacity.

1.4.4 Phytochemical Screening:

To carry out preliminary phytochemical screening of different fractions to identify the presence of major bioactive constituents such as phenolics, flavonoids, alkaloids, and tannins responsible for biological activities.

1.4.5 Comparative Study:

To compare the anti-inflammatory and antioxidant activities of different fractions with standard drugs such as Diclofenac (for anti-inflammatory activity) and Ascorbic acid (for antioxidant activity) to evaluate their relative effectiveness.

1.5 PLAN OF WORK

1.5.1 Collection and Preparation of Plant Material:

Mature seeds of *Morus alba* will be collected and authenticated. The seeds will be cleaned to remove impurities, shade-dried, and coarsely powdered to ensure uniformity for extraction.

1.5.2 Extraction

The powdered seed material will be subjected to extraction using suitable methods such as Soxhlet extraction. The extract will be further fractionated successively using solvents of increasing polarity, namely petroleum ether, chloroform, ethyl acetate, and ethanol, to obtain different fractions containing diverse phytoconstituents.

1.5.3 Preliminary Phytochemical Screening

The obtained extracts/fractions will be subjected to qualitative phytochemical screening to detect the presence of major bioactive constituents such as flavonoids, phenolic compounds, alkaloids, tannins, saponins, and glycosides using standard procedures.

1.5.4 In Vitro Anti-inflammatory Activity:

- The anti-inflammatory activity will be evaluated using the protein denaturation (egg albumin) assay. Reaction mixtures containing egg albumin, phosphate buffer, and different extract fractions will be prepared and incubated. The mixtures will then be heated, and the absorbance will be measured (around 660 nm) to determine the percentage inhibition of protein denaturation.
- A standard drug, Diclofenac, will be used for comparison.

1.5.5 Antioxidant Assessment

- Assess antioxidant activity via free-radical scavenging assays:



- DPPH assay: Mix extract with DPPH solution, incubate, and measure decrease in absorbance at 517 nm.
- Compare extract activity to a standard antioxidant (e.g., ascorbic acid).

1.5.6 Data Analysis

- Calculate percentage inhibition (for protein denaturation and radical scavenging).
- Analyse results statistically using appropriate methods.

1.5.7 Interpretation and Reporting: The results obtained will be analysed and compared among different solvent fractions to identify the most active extract. The findings will be systematically interpreted and presented in the form of tables, graphs, and discussion.

2. MATERIAL AND METHOD

2.1 Materials

2.1.1 Plant Material:

Fresh seeds of *Morus alba* were collected, authenticated, cleaned, shade-dried, and coarsely powdered for further experimental work.

2.1.2 Solvents:

- Petroleum ether
- Chloroform
- Ethyl acetate
- Ethanol
- Methanol

2.1.3 Glassware: (All glassware was properly cleaned and dried before use.)

- Beakers
- Test tubes
- Pipettes
- Conical flasks
- Measuring cylinder

2.1.4 Equipment:

- Soxhlet apparatus (for extraction)
- Heating mantle
- Hot air oven (for drying)
- Water bath (for incubation and heating)
- Rotary evaporator (for solvent evaporation and concentration of extracts)
- UV-Visible spectrophotometer (for absorbance measurement)

2.1.5 Phytochemical Reagent

Preliminary phytochemical screening was carried out using standard reagents such as:

- Dragendorff's reagent (for alkaloids)
- Barfoed's reagent (for carbohydrates)
- Concentrated sulfuric acid
- Dilute iodine solution (for starch)
- 10% sodium hydroxide solution (for flavonoids)
- Chloroform



2.1.6 Drug:

- Diclofenac (used as standard for anti-inflammatory activity)

2.1.7 Chemicals:

- DPPH
- Phosphate buffer
- Egg albumin

2.1.8 Other Materials:

- Filter papers
- Micropipettes
- Spatula
- Mortar and pestle

2.2. Methodology

2.2.1 Collection, Preparation, and Authentication of Plant Material

Fresh seeds of *Morus alba* were collected from the local market of Itaunja, Lucknow (Uttar Pradesh), India. The collected plant material was properly cleaned to remove dust and other extraneous matter and then shade-dried.

The plant material was authenticated by the Botanical Survey of India, Central Regional

Centre, Allahabad, with authentication reference number 1504260014102.



Fig.2.1 *Morus alba* Seed

2.2.2 Grinding and Powdering

The dried seeds were triturated using a mortar and pestle to reduce their size. The material was further processed to obtain a coarse powder, which was stored in an airtight container for further experimental use.



Fig.2.2 *Morus alba* Seed (Coarse powder)

2.2.3 Method of Extraction

A Soxhlet extractor was filled with 14 g of coarsely powdered seeds of *Morus alba*. Extraction was carried out successively using 140 ml of solvents in increasing order of polarity, namely petroleum ether, chloroform, ethyl acetate, and ethanol. Each solvent was used separately for extraction.

The extraction process was continued for approximately 6–8 hours or until the solvent in the siphon tube became colourless, indicating complete extraction of phytoconstituents. The temperature was maintained between 40–50°C throughout the process.

During extraction, the solvent repeatedly vaporized, condensed, and percolated through the

plant material, allowing the active constituents to dissolve and accumulate in the extraction flask.

After completion, the extracts were collected and the solvents were evaporated using a rotary evaporator. The concentrated extracts were then stored in airtight containers under refrigerated conditions for further analysis.⁴¹



Fig.2.3 Soxhlet apparatus



Fig.2.4 Petroleum Ether Extract



Fig. 2.4 Chloroform Extract



Fig.2.6 Ethyl Acetate Extract



Fig.2.7 Ethanol Extract

2.2.4 Phytochemical Testing

a) Test for Alkaloids (Dragendroff's test)

Add 1ml of filtrate and 1ml of Dragendroff's reagents.

The presence of alkaloids will result in the formation of a reddish-brown precipitate.

b) Test for Carbohydrates (Barfoed's test)

Add 1ml of filtrate and 1ml of Barfoed's reagents then heated for 2min. A red precipitate indicates the presence of Carbohydrates.

c) Test for Flavonoid (Conc. sulfuric acid test)

Add Plant extract and add few drops of conc. Sulfuric acid. An orange colour indicates the presence of Flavonoid.

d) Test for Phenolic Compound (Iodine test)

Add 1 ml of extract and few drops of dil. Iodine solution. A transient red colour indicates the presence of Phenolic Compound.

e) Test for Tannins (10%NaOH test)

Add 0.4 ml plant extract and 4ml of 10% NaOH and shaker well. The presence of Tannins will result in the formation of emulsion precipitate.

f) Test for Phytosterols (Salkowski's test)

Add 1ml of filtrate and few drops of conc. Sulfuric acid (Shaken well and allowed to stand).

Red color (In lower layer) indicates the presence of Phytosterols.

g) Test for Fixed Oils and Fat (Spot/Stain test)

Little quantity of plant extract is pressed in between to filter papers. Oil stain on filter paper indicates the presence of Fixed oil and fats.

h) Test for Terpenoids

Add 2ml of chloroform and 5ml of plant extract then evaporated on water bath and add 3ml conc. Sulfuric acid then boiled on water bath. A gray-colored solution indicates the presence of terpenoids.⁴²

2.2.5 Determination of DPPH Activity

Preparation of DPPH Solution

- 3.9 mg of DPPH was accurately weighed and dissolved in 100 mL of methanol to obtain a 0.004% w/v solution.

- The solution was freshly prepared and stored in dark conditions to prevent degradation.

Preparation of Standard (Ascorbic Acid)

- A stock solution of Ascorbic acid was prepared by dissolving 10 mg in 10 mL of solvent (methanol/distilled water) to obtain a concentration of 1 mg/mL.
- Further dilutions were prepared as required.

Procedure

- 60 μ L of extract/standard solution was taken and diluted with 1440 μ L of distilled water.
- To this, 1.5 mL of DPPH solution was added.
- The reaction mixture was shaken well and incubated in the dark for 30 minutes at room temperature.
- After incubation, the absorbance was measured at 517 nm using a UV-Visible spectrophotometer.⁴³

2.2.6 Measurement of Anti-Inflammatory Activity

a) Preparation of 1% Egg Albumin Solution

A 1% egg albumin solution was prepared using a fresh hen's egg. The egg was carefully cracked, and the clear (translucent) portion, known as egg albumin, was separated from the yolk.

Approximately 1 ml of egg albumin was transferred into a measuring cylinder and diluted with distilled water to make up the volume to 100 ml (w/v). The solution was mixed thoroughly to obtain a uniform preparation. Cold distilled water was used during preparation to prevent coagulation of proteins, as heating may lead to denaturation of egg albumin.



b) Egg Albumin Assay

The anti-inflammatory activity of unknown crude extracts can be determined *in vitro* for inhibition of the denaturation of egg albumin (protein).

- 0.2 mL of 1-2% egg albumin solution (from fresh hen's egg), 2mL of plant extracts, 2.8 ml Phosphate buffer solution (pH 7.4) for standard 2ml Diclofenac sodium, 0.2 mL of 1-2% egg albumin solution, 2.8 ml Phosphate buffer solution (pH 7.4) was mixed to form a reaction mixture of a total volume of 5 mL.
- A total volume of 5 mL of the control was created by combining 2 mL of water + DMSO, 0.2 mL of 1-2% egg albumin solution, and 2.8 mL of phosphate-buffered saline.
- The reaction mixtures were then incubated at $37\pm 2^{\circ}\text{C}$ for 30 min and will be heated in a water bath at $70\pm 2^{\circ}\text{C}$ for 5 min.

- After cooling, the absorbance was measured at 660 nm by a suitable UV/Vis spectrophotometer using distilled water as the blank.
- The following equation was used to determine the % inhibition of protein denaturation.⁴⁴

Percentage inhibition=

$$\frac{\text{Absorbance of control} - \text{Absorbance of test sample} \times 100}{\text{Absorbance of control}}$$

Then plant extract/positive control concentration for 50% inhibition (IC₅₀) was determined by plotting percentage inhibition concerning control against concentration.

3. RESULT AND DISCUSSION

3.1 RESULT

3.1.1 PHYTOCHEMICAL TESTING

Table 3.1 Phytochemical Testing of *Morus alba* extract

Sr. No	Phytochemical	Observations	<i>Morus alba</i> Seed Extract			
			Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol
1.	Alkaloids	A reddish-brown precipitate	Positive	Positive	Positive	Negative
2.	Carbohydrates	A red precipitate	Negative	Negative	Positive	Positive
3.	Flavonoids	An orange colour	Positive	Negative	Positive	Negative
4.	Phenolic compound	A transient red colour	Negative	Negative	Negative	Positive
5.	Tannins	Formation of emulsion	Negative	Negative	Negative	Positive
6.	Phytosterols	Red colour (in lower layer)	Positive	Positive	Negative	Positive
7.	Fixed oils and fat	Oil stain on the paper	Positive	Positive	Negative	Negative
8.	Terpenoids	A gray-coloured solution	Negative	Positive	Positive	Positive

(+) Present, (-) Absent

Test for Fixed Oils and Fat



Fig.3.1

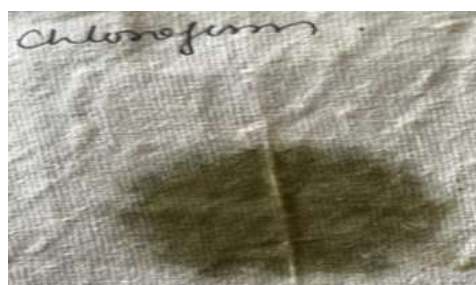


Fig.3.2



Fig.3.3 Ethyl Acetate



Fig.3.4 Ethanol



Fig.3.5 Phytochemical testing of *Morus alba* Petroleum Ether Extract



Fig.3.6 Phytochemical testing of *Morus alba* Chloroform Extract



Fig.3.7 Phytochemical testing of *Morus alba* Ethyl Acetate Extract



Fig.3.8 Phytochemical testing of *Morus alba* Ethanol Extract

3.1.2 Percentage Inhibition of Antioxidant Activity

The antioxidant activity of the extracts was evaluated using the DPPH assay. The percentage inhibition was calculated using the following formula:

Formula used:

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Control Absorbance = 0.388

1. Standard (Ascorbic acid):

$$\% \text{ Inhibition} = \frac{(0.388 - 0.009)}{0.388} \times 100 = 97.68\%$$

2. Ethyl acetate:

$$\% \text{ Inhibition} = \frac{(0.388 - 0.041)}{0.388} \times 100 = 89.43\%$$

3. Ethanol:

$$\% \text{ Inhibition} = \frac{(0.388 - 0.063)}{0.388} \times 100 = 83.76\%$$

4. Petroleum ether:

$$\% \text{ Inhibition} = \frac{(0.388 - 0.076)}{0.388} \times 100 = 80.41\%$$

5. Chloroform:

$$\% \text{ Inhibition} = \frac{(0.388 - 0.152)}{0.388} \times 100 = 60.82\%$$

Table. Absorbance and % Inhibition of different extract in DPPH Assay

Sample	Absorbance	% Inhibition
Control	0.388	0%
Standard	0.009	97.68%
Ethyl acetate	0.041	89.43%
Ethanol	0.063	83.76%
Petroleum ether	0.076	80.41%
Chloroform	0.152	60.82%



Fig.3.9 UV-Visible Spectrophotometric Measurement of Absorbance of different extract at 517 nm in DPPH assay

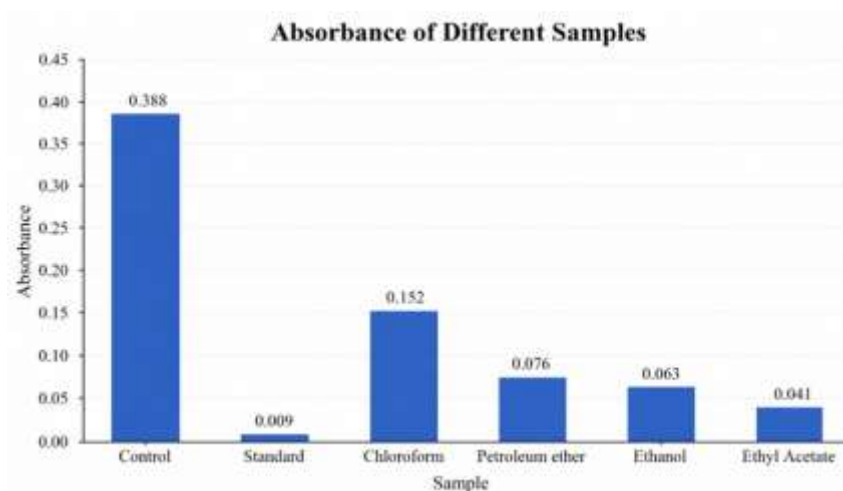


Fig. 3.10 Comparative Absorbance of Different Extracts in DPPH Assay

- Control (0.388) → highest absorbance → no antioxidant activity (baseline)
- Standard (0.009) → very low absorbance → very high % inhibition (strong antioxidant)
- Chloroform (0.152) → moderate inhibition
- Petroleum ether (0.076) → higher inhibition than chloroform
- Ethanol (0.063) → even stronger
- Ethyl acetate (0.041) → strongest among extracts

Standard > Ethyl acetate > Ethanol > Petroleum ether > Chloroform > Control

3.1.3 Percentage Inhibition of Anti-inflammatory Activity

The anti-inflammatory activity was evaluated by calculating the percentage inhibition of protein denaturation using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Control Absorbance = 0.180

Calculations-

6. Standard (Diclofenac)

$$\begin{aligned} \% \text{ Inhibition} &= \frac{(0.180 - 0.041)}{0.180} \times 100 \\ &= 77.22\% \end{aligned}$$

7. Pet ether

$$\begin{aligned} \% \text{ Inhibition} &= \frac{(0.180 - 0.041)}{0.180} \times 100 \\ &= 77.22\% \end{aligned}$$

8. Ethanol

$$\begin{aligned} \% \text{ Inhibition} &= \frac{(0.180 - 0.064)}{0.180} \times 100 \\ &= 64.44\% \end{aligned}$$

9. Chloroform

$$\begin{aligned} \% \text{ Inhibition} &= \frac{(0.180 - 0.134)}{0.180} \times 100 \\ &= 25.56\% \end{aligned}$$

10. Ethyl acetate



$$\% \text{ Inhibition} = \frac{(0.180 - 0.006)}{0.180} \times 100$$

$$= 96.67\%$$

Table 3.3 Absorbance and % Inhibition of different extracts in Protein denaturation Assay

Sample	Absorbance	% inhibition
Control	0.180	0%
Diclofenac	0.041	77.22%
Pet ether	0.041	77.22%
Ethanol	0.064	64.44%
Chloroform	0.134	25.56%
Ethyl acetate	0.006	96.67%

**Fig.3.11 UV-Visible Spectrophotometric Measurement of Absorbance at 660 nm in Protein denaturation Assay****Fig.3.12 UV-Visible Spectrophotometric Measurement of Absorbance of different extracts at 660 nm in Protein Denaturation Assay**

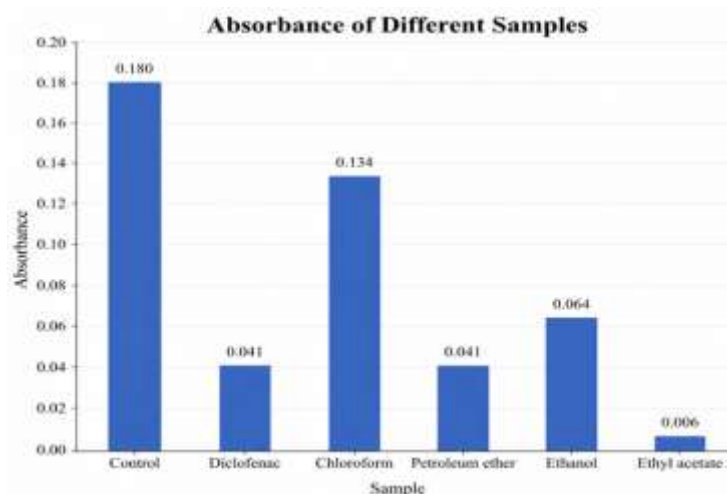


Fig. 3.13 Comparative Absorbance of Different Extracts in Protein Denaturation Assay

- **Control (0.180)** → highest absorbance → no inhibition (baseline inflammation)
- **Diclofenac (0.041)** → strong inhibition → reference standard drug
- **Ethyl acetate (0.006)** → extremely low absorbance → highest anti-inflammatory activity
- **Petroleum ether (0.041)** → strong activity (similar to diclofenac)
- **Ethanol (0.064)** → moderate activity
- **Chloroform (0.134)** → weak activity

Ethyl acetate > Diclofenac ≈ Petroleum ether > Ethanol > Chloroform > Control

4. DISCUSSION

The present study was carried out to evaluate the in vitro anti-inflammatory and antioxidant activities of different solvent extracts of *Morus alba* seeds. The extracts were prepared using solvents of increasing polarity, namely petroleum ether, chloroform, ethyl acetate, and ethanol, to ensure the extraction of a wide range of phytoconstituents.

The phytochemical screening revealed the presence of important bioactive compounds such as flavonoids, phenolic compounds, alkaloids, tannins, and terpenoids in different extracts. These compounds are well known for their pharmacological activities, particularly antioxidant and anti-inflammatory effects.

In the DPPH free radical scavenging assay, all extracts exhibited varying degrees of antioxidant activity. The ethyl acetate extract showed the highest percentage inhibition (89.43%), followed by ethanol (83.76%), petroleum ether (80.41%), and chloroform (60.82%), while the standard Ascorbic acid exhibited maximum inhibition (97.68%). The superior activity of the ethyl acetate extract may be attributed to its ability to extract semi-polar compounds such as phenolics and flavonoids, which are potent hydrogen donors and play a crucial role in neutralizing free radicals.

Similarly, in the protein denaturation assay, which is a widely accepted method for screening anti-inflammatory activity, the ethyl acetate extract demonstrated the highest inhibition (96.67%), surpassing even the standard Diclofenac (77.22%). Petroleum ether extracts also showed comparable activity to the standard, while ethanol exhibited moderate activity and chloroform showed the least

effect. The inhibition of protein denaturation indicates the ability of the extract to stabilize proteins and prevent inflammatory processes.

The correlation between antioxidant and anti-inflammatory activities observed in this study suggests that oxidative stress plays a significant role in inflammation. Extracts rich in phenolic and flavonoid content are capable of scavenging free radicals and inhibiting inflammatory mediators, thereby exhibiting dual biological activity.

Overall, the results indicate that the ethyl acetate fraction is the most bioactive, likely due to the presence of a higher concentration of pharmacologically active phytoconstituents. These findings are in agreement with previous studies, which report that medium-polar solvents are more effective in extracting bioactive compounds responsible for therapeutic effects.

5. CONCLUSION

The present study concludes that *Morus alba* seeds possess significant anti-inflammatory and antioxidant activities when evaluated using in vitro models.

Among all the extracts, the ethyl acetate extract exhibited the highest activity in both DPPH free radical scavenging assay and protein denaturation assay, indicating its strong potential as a natural therapeutic agent. The presence of bioactive compounds such as flavonoids and phenolics is likely responsible for these effects.

The results support the traditional use of *Morus alba* and highlight the importance of its seeds as a valuable source of natural antioxidants and anti-inflammatory agents. Furthermore, this study suggests that plant-based extracts may serve as safer alternatives to synthetic drugs, which are often associated with adverse effects.

However, further studies, including isolation of active constituents, in vivo studies, and clinical investigations, are required to validate these findings and to explore their potential for pharmaceutical applications.

REFERENCES

1. Adetutu, O. A., & Olukorede, A. O. (2021). Evaluation of in vitro anti-inflammatory Potential of Aqueous Solanum aethiopicum (Garden Egg) Leaf Extract. J. Biomed. Biosens., 1, 1-14.
2. Ranaweera CB, Senadeera NN, Samaraweera T, Samaraweera T. In vitro anti-inflammatory activity of leaves of Jeffreyia zeylanica using the egg albumin denaturation method and human red blood cell stabilization method.
3. Yashin A, Yashin Y, Xia X, Nemzer B. Antioxidant activity of spices and their impact on human health: A review. Antioxidants. 2017 Sep 15;6(3):70.
4. Sarveswaran RJ, Jayasuriya WJ, Suresh TS. In vitro assays to investigate the anti-inflammatory activity of herbal extracts a review.
5. Ramandeep Singh, R. S., Anindya Bagachi, A. B., Alok Semwal, A. S., Satinder Kaur, S. K., & Abhishek Bharadwaj, A. B. (2013). Traditional uses, phytochemistry and pharmacology of Morus alba Linn.: a review.
6. Porasar P, Gibo R, Gogoi B, Sharma D, Bharadwaj A, Gam S, Hazarika D, Dutta KN. A Systematic review on the phytochemistry, isolated compounds, nutritional benefits, pharmacology and toxicology of the plant species Morus alba L. Discover Plants. 2025 Jan 17;2(1):7.
7. Fatima, M., Dar, M. A., Dhanavade, M. J., Abbas, S. Z., Bukhari, M. N., Arsalan, A., & Ouyang, Z. (2024). Biosynthesis and pharmacological activities of the bioactive



- compounds of white mulberry (*Morus alba*): Current Paradigms and Future Challenges. *Biology*, 13(7), 506.
8. Batiha GE, Al-Snafi AE, Thuwaini MM, Teibo JO, Shaheen HM, Akomolafe AP, Teibo TK, Al-Kuraishy HM, Al-Garbeeb AI, Alexiou A, Papadakis M. *Morus alba*: a comprehensive phytochemical and pharmacological review. *Naunyn-schmiedeberg's Archives of Pharmacology*. 2023 Jul;396(7):1399-413.
 9. Sanghi, S. B., & Mushtaq, S. (2017). Phytopharmacological activity of *Morus alba* Linn. Extracts—a review. *Asian J Pharm Sci*, 6(4), 10-19.
 10. Vijayan K, Tikader A, Weiguo Z, Nair CV, Ercisli S, Tsou CH. *Morus*. In *Wild crop relatives: genomic and breeding resources: tropical and subtropical fruits* 2011 Jun 8 (pp. 75-95). Berlin, Heidelberg: Springer Berlin Heidelberg.
 11. Bajirao, K. D., & Pallavi, J. (2022). The properties and nutritional uses of mulberry (*Morus Alba*): A Review. *Int. J. Creat. Res. Thoughts*, 10(6), 2320-2882.
 12. Sethuramani, A., Devi, P., Jaslin, E., Meera, R., & Kameswari, B. (2010). Pharmacognostical and preliminary phytochemical investigation on the leaves of *Morus alba* Linn. *Microscopy*, 7(8), 9-10.
 13. Škovranová, G., Čulenová, M., Treml, J., Dzurická, L., Marova, I., & Sychrová, A. (2022). Prenylated phenolics from *Morus alba* against MRSA infections as a strategy for wound healing. *Frontiers in Pharmacology*, 13, 1068371.
 14. Adetutu OA, Olukorede AO. Evaluation of in vitro anti-inflammatory Potential of Aqueous *Solanum aethiopicum* (Garden Egg) Leaf Extract. *J Biomed Biosens*. 2021;1.
 15. Chan EW, Phui-Yan LY, Siu-Kuin WO. Phytochemistry, pharmacology, and clinical trials of *Morus alba*. *Chinese journal of natural medicines*. 2016 Jan 1;14(1):17-30.
 16. Butt MS, Nazir A, Sultan MT, Schroën K. *Morus alba* L. nature's functional tonic. *Trends in food science & technology*. 2008 Oct 1;19(10):505-12.
 17. Hsu JH, Yang CS, Chen JJ. Antioxidant, anti- α -glucosidase, antityrosinase, and anti-inflammatory activities of bioactive components from *Morus alba*. *Antioxidants*. 2022 Nov 11;11(11):2222.
 18. Ranaweera CB, Senadeera NN, Samaraweera T, Samaraweera T. In vitro anti-inflammatory activity of leaves of *Jeffreyia zeylanica* using the egg albumin denaturation method and human red blood cell stabilization method.
 19. Manubolu K, Peeriga R, Bonthu MG, Raju B, Vatchavai NK, Gope ER, Kadirvel D. Evaluation of anti-inflammatory activity of *Millingtonia hortensis* leaf extract. *Int J Chem Biochem Sci*. 2023;24(6):380-5.
 20. Hussen EM, Endalew SA. In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. *BMC complementary medicine and therapies*. 2023 May 4;23(1):146.
 21. Yongram C, Panyatip P, Siriparu P, Ratha J, Sungthong B, Tadtong S, Puthongking P. Influence of maturity stage on tryptophan, phenolic, flavonoid, and anthocyanin content, and antioxidant activity of *Morus alba* L. fruit. *Rasayan J. Chem*. 2022 Jul 1;15(3):1693-701.
 22. Saensouk S, Senavongse R, Papayrata C, Chumroenphat T. Evaluation of color, phytochemical compounds and antioxidant activities of mulberry fruit (*Morus alba* L.) during ripening. *Horticulturae*. 2022 Dec 5;8(12):1146.
 23. Chaiya P, Senarat S, Phaechamud T, Narakornwit W. In vitro anti-inflammatory activity using thermally inhibiting protein denaturation of egg albumin and antimicrobial

- activities of some organic solvents. *Materials Today: Proceedings*. 2022 Jan 1; 65:2290-5.
24. Polumackanycz M, Wesolowski M, Viapiana A. *Morus alba* L. and *Morus nigra* L. leaves as a promising food source of phenolic compounds with antioxidant activity. *Plant Foods for Human Nutrition*. 2021 Dec;76(4):458-65.
 25. Olasunkanmi AA, Afuye. Evaluation of In vitro Anti-Inflammatory Potential of Aqueous *Solanum Aethiopicum* (Garden Egg) Leaf Extract. *Further Biomedicine*. 2021;1(1):1-4.
 26. Hussain A, Aslam B, Muhammad F, Faisal MN. In vitro antioxidant activity and in vivo anti-inflammatory effect of *Ricinus communis* (L.) and *Withania somnifera* (L.) hydroalcoholic extracts in rats. *Brazilian Archives of Biology and Technology*. 2021;64:e21200783.
 27. Marano S, Minelli C, Ripani L, Marcaccio M, Laudadio E, Mobbili G, Amici A, Armeni T, Stipa P. Insights into the antioxidant mechanism of newly synthesized benzoxazinic nitrones: in vitro and in silico studies with DPPH model radical. *Antioxidants*. 2021 Jul 29;10(8):1224.
 28. Mssillou I, Agour A, Hamamouch N, Lyoussi B, Derwich E. Chemical Composition and In Vitro Antioxidant and Antimicrobial Activities of *Marrubium vulgare* L. *The Scientific World Journal*. 2021;2021(1):7011493.
 29. Xu X, Huang Y, Xu J, He X, Wang Y. Anti-neuroinflammatory and antioxidant phenols from mulberry fruit (*Morus alba* L.). *Journal of Functional Foods*. 2020 May 1; 68:103914.
 30. Zeng Y, Song J, Zhang M, Wang H, Zhang Y, Suo H. Comparison of in vitro and in vivo antioxidant activities of six flavonoids with similar structures. *Antioxidants*. 2020 Aug 11;9(8):732.
 31. Boomi P, Ganesan R, Prabu Poorani G, Jegatheeswaran S, Balakumar C, Gurumallesh Prabu H, Anand K, Marimuthu Prabhu N, Jeyakanthan J, Saravanan M. Phyto-engineered gold nanoparticles (AuNPs) with potential antibacterial, antioxidant, and wound healing activities under in vitro and in vivo conditions. *International journal of nanomedicine*. 2020 Oct 7:7553-68.
 32. Shahinuzzaman M, Yaakob Z, Anuar FH, Akhtar P, Kadir NH, Hasan AM, Sobayel K, Nour M, Sindi H, Amin N, Sopian K. In vitro antioxidant activity of *Ficus carica* L. latex from 18 different cultivars. *Scientific reports*. 2020 Jul 2;10(1):10852.
 33. Dharmadeva S, Galgamuwa LS, Prasadine C, Kumarasinghe N. In vitro anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. *AYU (An international quarterly journal of research in Ayurveda)*. 2018 Oct 1;39(4):239-42.
 34. Yiemwattana I, Chaisomboon N, Jamdee K. Antibacterial and anti-inflammatory potential of *Morus alba* stem extract. *The open dentistry journal*. 2018 Mar 30;12:265.
 35. Soonthornsit N, Pitaksutheepong C, Hemstapat W, Utaisinchaoen P, Pitaksutheepong T. In vitro anti-inflammatory activity of *Morus alba* L. stem extract in LPS-stimulated RAW 264.7 cells. *Evidence-based Complementary and Alternative Medicine*. 2017;2017(1):3928956.
 36. Kariawasam KW, Pathirana R, Ratnasooriya W, Handunnetti S, Abeysekera WP. Phytochemical profile and in vitro anti-inflammatory activity of aqueous leaf extract of Sri Lankan variety of *Psidium guajava* L. *Journal of Pharmacognosy and Phytochemistry*. 2017;6(4):22-6.
 37. Sangeetha G, Vidhya R. In vitro anti-inflammatory activity of different parts of

- Pedaliium murex (L.). Inflammation. 2016;4(3):31-6.
38. Rahman H, Eswaraiiah MC, Dutta AM. In-vitro anti-inflammatory and anti-arthritic activity of Oryza Sativa Var. joha rice (an aromatic indigenous rice of Assam). *Am. Eurasian J. Agric. Environ. Sci.* 2015;15(1):115-21.
 39. Thabti I, Marzougui N, Elfalleh W, Ferchichi A. Antioxidant composition and antioxidant activity of white (*Morus alba* L.), black (*Morus nigra* L.) and red (*Morus rubra* L.) mulberry leaves. *Acta botanica gallica*. 2011 Jan 1;158(2):205-14.
 40. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. *Asian Pacific Journal of Tropical Biomedicine*. 2012 Jan 1;2(1): S178-80.
 41. Evans, W. C. (2002). *Trease and Evans pharmacognosy* (15th ed.). W.B. Saunders.
 42. Shahinuzzaman, M., Yaakob, Z., Anuar, F. H., Akhtar, P., Kadir, N. H. A., Hasan, A. M., ... & Akhtaruzzaman, M. (2020). In vitro antioxidant activity of *Ficus carica* L. latex from 18 different cultivars. *Scientific reports*, 10(1), 10852.
 43. Hsu, J. H., Yang, C. S., & Chen, J. J. (2022). Antioxidant, anti- α -glucosidase, antityrosinase, and anti-inflammatory activities of bioactive components from *Morus alba*. *Antioxidants*, 11(11), 2222.
 44. HDT, M. (2023). In vitro anti-inflammatory egg albumin denaturation assay: an enhanced approach. *Journal of Natural & Ayurvedic Medicine*, 7(3), 1-6.

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