

Assessment of In-Vitro Hemolytic Activity of "*Myristica Fragrans Houtt*"  
Amol V. Pore, Sanjay K. Bais , Gopika D. Dongare, Pranali B. Bandgar , Meher S. Sutar  
Fabtech College of pharmacy, Sangola Dist – Solapur, Maharashtra.  
Corresponding Author: pranalibandgar362@gmail.com

## **ABSTRACT**

*Myristica fragrans* is the source of javettri, or mace, help for its therapeutic qualities and contains a number of bioactive substances. Study is designed to use spectrophotometric analysis hemolytic activity of Javettri extract. Key components like alkaloids, flavonoids, tannins, and saponins, the extract was first put through a preliminary phytochemical screening process using an appropriate solvent extraction technique Red blood cells were exposed to varying concentrations of the extract in a lab setting for the hemolytic assay. Using a spectrophotometer set ideal wavelength; liberated hemoglobin was measured to ascertain hemolysis. The experimental results were validated by using standard compounds as references. Statistical techniques were used to calculate and interpret the percentage of hemolysis. According to the results, Javettri extract exhibits significant hemolytic activity, which could be related the inclusion of particular phytochemicals. The current analysis enriches the available scientific information of Javettri's biological effects and highlights the need for additional research to assess its safety and possible therapeutic uses.

**KEYWORDS:** *Myristica fragrans*, Organic extract, phytochemical screening, Hemolytic activity, Therapeutic plants.

## INTRODUCTION

*Myristica fragrans* (nutmeg), especially its aril known as Javetri (mace), is highly enriched with essential oils and bioactive phenolic constituents and terpenoids. These constituents are known produce various medicinal effects, including antimicrobial, antioxidant, and membrane-active properties.<sup>[1]</sup>

An essential biological metric for assessing how substances affect the integrity of red blood cell (RBC) membranes is hemolytic activity. Because the erythrocyte membrane resembles other biological membranes, it provides a straightforward and sensitive model for studying membrane stability. Hemolysis is the term for the release of hemoglobin when this membrane is disrupted by exposure to specific phytochemicals. Thus, measuring hemolytic activity offers important insights into the cytotoxic potential and safety of extracts derived from plants.<sup>[2]</sup>

A number of substances found in Javetri engage with cell membrane proteins and lipid bilayers alter permeability of membranes. At higher concentrations, these interactions may result in unwanted cell damage, contribute to therapeutic effects. These effects are frequently assessed in controlled laboratory settings using *in vitro* hemolytic assays, which provide a quick and accurate way to identify dose-dependent responses.<sup>[3,4]</sup>

To balance the medicinal benefits of *Myristica fragrans* extract with safety concerns, it is crucial to comprehend its hemolytic behavior. Its *in vitro* hemolytic activity is the main focus.<sup>[5]</sup>

### Definition of Hemolysis

Hemolysis represents the breakdown or destruction of red corpuscles due to disruption of cellular boundary that leads to intracellular components, mainly hemoglobin, to be released into the surrounding fluid. Numerous physical, chemical, or biological elements that jeopardize membrane integrity may cause this process.<sup>[6]</sup>

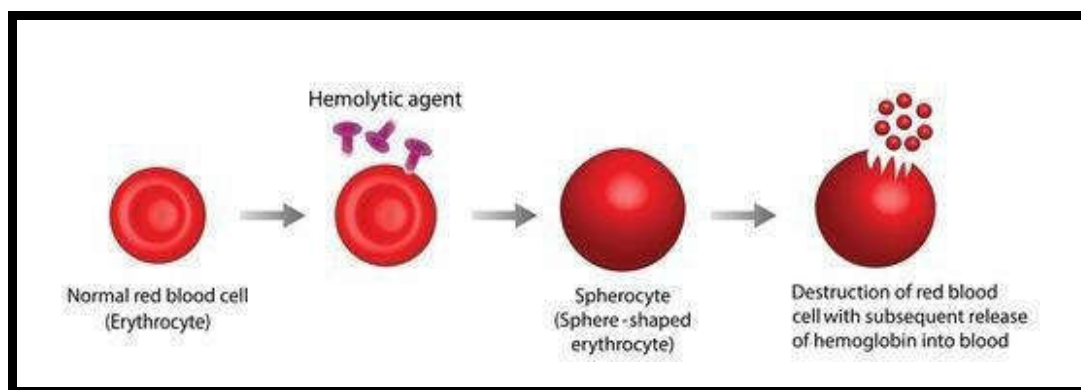


*Figure 1: Hemolysis.*

### **Physiology of Red Blood Cells**

Red blood cells (erythrocytes) play a vital role in transporting oxygen throughout the body tissues and carrying carbon dioxide back to the lungs. This function is performed by hemoglobin, an iron-containing protein present in RBCs. They have a biconcave disc shape, thereby improving surface availability for efficient gas exchange and allow flexibility to pass through narrow capillaries. Mature RBCs lack a nucleus and other organelles, providing more space for hemoglobin and enhancing their oxygen-carrying capacity. Their cell membrane plays an important role in maintaining shape, flexibility, and osmotic balance. <sup>[7]</sup>

### **Types of Hemolysis**

#### **Intravascular Hemolysis**

RBCs are destroyed within arteries and veins rather than in organs like the spleen or liver. Leads to direct release of hemoglobin in systemic circulation <sup>[8]</sup>

#### **Extravascular Hemolysis**

Is a process that primarily occurs in organs like the splenic and hepatic organs and includes destruction of red blood cells. RBCs become less flexible and can readily be identified and eliminated by reticuloendothelial system macrophages when they get old, damaged, or have an irregular shape. Instead of being released straight into the bloodstream in type of hemolytic, hemoglobin is lysed inside these cells into components like bilirubin, which is subsequently sent to the liver for additional processing. Because of this, extravascular hemolysis frequently results in elevated levels of indirect (unconjugated) bilirubin, which can occasionally cause jaundice. <sup>[9]</sup>

#### **Osmotic Hemolysis**

Consequently, water passes into the red blood cells through osmosis, causing them to swell and ultimately rupture (lysis). Hemoglobin is released into the surrounding fluid. It is frequently employed in lab experiments to evaluate RBC osmotic fragility and investigate membrane stability. <sup>[10, 11, 12]</sup>

#### **Immune Hemolysis**

When antibodies mistakenly identify red blood cells (RBCs) as foreign, the body's immune system destroys them, a process as immune hemolysis. When these antibodies bind to the surface of red blood cells, they either cause intravascular hemolysis in blood vessels or extravascular hemolysis in organs such as the spleen. Hemolytic disease of the newborn, incompatible blood transfusions, Anemia, jaundice, and other associated complications are possible outcomes. <sup>[13, 14]</sup>

#### **Mechanical Hemolysis**

The breakdown of red blood cells leads of trauma or physical forces is known as mechanical hemolysis. This can happen in mechanical heart valves and dialysis devices or during intense physical activity when red blood cells encounter to shear stress, turbulence, or compression. Because of the damage to the cell membrane caused by these forces, the cells burst and release hemoglobin into the blood. Mechanical hemolysis is frequently linked to medical devices or conditions that change normal blood flow and it can result in anemia. <sup>[15, 16]</sup>

### **Laboratory Diagnosis**

#### **Diagnosing Hemolysis involves various pathological examinations**

CBC: Decreased hemoglobin and hematocrit (anemia)

Reticulocyte Count: Increased (bone marrow compensation)

Peripheral Blood Smear: Abnormal RBC shapes Lactate Dehydrogenate (LDH): Increased

Indirect (Unconjugated) Bilirubin: Increased

Haptoglobin Level: Decreased

Direct Coombs Test: Positive in immune hemolysis

Urine Examination: Hemoglobinuria (in intravascular hemolysis)

Serum Hemoglobin: Increased (in intravascular hemolysis)<sup>[17]</sup>

## MATERIAL AND METHODOLOGY

### 1. Plant Collection and Authentication

The best time for collection of *Myristica fragrance* Houtt when the fruit is fully ripe and naturally splits open were collected from sangola, solapure, Maharashtra, India. The plant was authenticated by Mr. Tebhurne R.R.M.Sc.B.Ed Botony plant physiology.

### Plant profile



*Figure 2: Myristica fragrance Houtt*

**Synonym:** Jaatishasya, Maalatiphala (seed kernel).

**Biological source:** *Myristica fragrans* Houtt. (Nutmeg) is obtained from the dried seed and aril (mace) of the fruit of the evergreen tree.

**Family:** Myristicaceae

**Color:** Dark brown (seed), bright red when fresh and yellowish-orange to reddish-brown when dried (aril/mace).

**Odour:** Strong, aromatic, and characteristic spicy fragrance.

**Taste:** Warm, slightly sweet, and strongly aromatic with a pungent spicy taste.<sup>[18]</sup>

**Description:** *Myristica fragrans* Houtt It is a medium-sized aromatic tree that produces a fleshy yellow fruit containing a single seed known as nutmeg, surrounded by a lacy red aril called

mace. The seed is hard, oval, and dark brown in color, while the aril is reddish when fresh and turns yellowish-brown on drying. Both nutmeg and mace are highly aromatic and widely used as spices and in traditional medicine for their carminative, antioxidant, and antimicrobial properties.

[19]

**Seedling care** – Young seedlings are

#### **Geographical Distribution**

**Indonesia** – Native and largest producer (Maluku/Spice Islands)

**India** – Mainly Kerala, Karnataka, Tamil Nadu

**Sri Lanka** – Cultivated in humid tropical regions

**Malaysia** – Grown in small to moderate scale plantations

**Grenada** – One of the major global exporters (“Spice Island”)

**Caribbean Islands** – Including Trinidad and Tobago, Jamaica

**Brazil** – Limited cultivation in tropical zones

**Papua New Guinea** – Small-scale production in suitable climates

#### **Cultivation and cultivation**

**Climate requirement** – *Myristica fragrans* grows well in a tropical climate that is warm, humid, and an average temperature of 20–30°C and high rainfall throughout the year.

**Soil requirement** – It prefers deep, well-drained, fertile loamy soil rich in organic matter. Waterlogging conditions should be avoided.

**Nursery preparation** – Seeds are shielded from the sun using shade nets or partial shade and are watered regularly to maintain moisture.

**Transplantation** – When seedlings reach sufficient height (about 20–30 cm), they are transplanted into the main field at proper spacing.

**Field planting** – Pits are prepared in advance and filled with topsoil and organic manure before transplanting. Proper spacing ensures good growth and aeration.

**Shade management** – Since it is a shade-loving plant in early stages, intercrops or shade trees are used to protect it from direct sunlight.

**Irrigation** – Regular irrigation is essential, especially during dry seasons, to maintain soil moisture and healthy growth.

**Manuring and fertilization** – Organic manure and balanced fertilizers are applied periodically to improve growth, flowering, and fruit yield.

**Weeding and maintenance** – Regular weeding, mulching, and pest control are done to maintain plant health.

**Flowering and fruiting** – The tree begins flowering after about 6–8 years, and fruits develop over several months.

**Harvesting** – Harvesting fruits occurs when it turns yellow and splits open naturally on the tree.

**Storage** – Stored in airtight containers somewhere dry and cool, far from moisture and light to preserve aroma and active constituents. [20]

#### **Chemical constituent**

It contains essential oils (such as myristicin, safrole, eugenol, and elemicin), fixed oils, phenolic compounds, flavonoids, lignans, and starch.

#### **Uses**

Flavoring agent in food.

Carminative (relieves gas and bloating).

Improves digestion and appetite.

Applied asaaromatic stimulant.

Possesses antioxidant activity.

Shows antimicrobial properties.

Has an anti-inflammatory effect.

Used in traditional medicine for mild pain relief and relaxation.<sup>[21]</sup>

### **2. Preparation of Plant extracts Myristica fragrance Houtt**

#### **Collection and Plant Material**

Javetri (mace) is harvested from ripe *Myristica fragrans* fruits that have naturally split open, exposing the bright red aril, indicate maximum maturity and active constituents. The fruits are either plucked or gathered right after they fall, and the mace is carefully separated from the seed. To avoid moisture contamination, collection should be done in dry conditions in the morning or later afternoon.

#### **Cleaning and Drying**

*Myristica fragrans* Houtt. is cleaned and dried by removing the outer fleshy pericarp after harvesting and carefully separating the mace (aril) from the seed, then washing to remove dirt and discarding any damaged or infected material. The mace and seeds are then dried separately, usually in the sun; mace dries in 10-14 days until crisp, while seeds take 4-8 weeks until the kernel shrinks within the shell. The material is turned frequently during the drying process to ensure uniform moisture removal, and proper drying is required prior to storage to prevent fungal growth and spoilage.

#### **Grinding and Crushing**

occurs after proper drying, in which the hard shells of the seeds are first broken to obtain the kernels, which are then crushed or ground into a fine powder using mechanical grinders. This process increases surface area, aiding in the extraction of active constituents and enhancing flavor and aroma; however, it must be done carefully under controlled conditions to avoid volatile oil loss and overheating, which can reduce final product quality.<sup>[22]</sup>

### **3. Preparation of Ethanolic Extract**

Can be prepared by placing the dried powder in a Soxhlet apparatus and extracting it with ethanol as the solvent; the system is then maintained at around 60°C and allowed to run continuously for about 6-8 hours to ensure efficient extraction of active constituents; the obtained extract is then filtered and concentrated to yield the final ethanolic extract.<sup>[23]</sup>

### **4. Preparation of Phosphate buffer Solution**

A phosphate buffer solution is made by dissolving suitable amounts of potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) and disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) in distilled water to achieve the required pH (typically 6.8–7.4). Distilled water is used to make up the final volume after the solutions are combined in the appropriate amounts and the pH is measured with a pH meter. For later use in the extraction or analysis of *Myristica fragrans* Houtt, the prepared buffer is filtered if needed and kept in a clean, airtight container.<sup>[24]</sup>

### **5. Calculate the Proportions**

Ascertain the buffer solution's ideal pH, which is typically between 6.8 and 7.4. Determine the necessary amounts of dibasic sodium phosphate ( $\text{Na}_2\text{HPO}_4$ ) and monobasic potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) using the Henderson–Hasselbalch equation or standard buffer tables.

#### **Prepare stock solution**

Make stock solutions of  $\text{Na}_2\text{HPO}_4$  (0.2 M) and  $\text{KH}_2\text{PO}_4$  (0.2 M) by dissolving precisely weighed amounts in distilled water. Make sure it dissolves completely.<sup>[25]</sup>

#### **Mixing**

To achieve the required pH, gradually add the calculated volumes of both stock solutions into a clean container while stirring constantly.

#### **Adjust the pH**

by measuring it with an indicator paper or pH meter. Adjust by adding tiny amounts of either solution if necessary.<sup>[26]</sup>

#### **Final Dilution (if needed)**

Once the pH of the buffer is adjusted to the desired range, make up the volume with purified water and combine thoroughly.

#### **Filtering (optional)**

If required, filter the buffer solution using a sterile filter to remove any particulate matter or impurities.<sup>[27]</sup>

#### **Sterilization (optional)**

For biological applications, sterilize the buffer by autoclaving at 121°C for 15 minutes or by passing via a 0.22  $\mu\text{m}$  membrane filter.

#### **Storage**

Store the prepared phosphate buffer solution in a clean, airtight container. It can be kept at room temperature for short-term use or refrigerated for longer storage.

### **6. Preparation of erythrocyte cells**

Samples of blood are taken from healthy volunteers. The cells are washed using PBS by centrifugation. After centrifuging about 5 mL of blood, the pellet is washed and re-suspended to obtain a 0.5% erythrocyte suspension.<sup>[28]</sup>

### **7. Perform the in-vitro hemolytic activity test**

Prepare different concentrations of Javetri extract and mix with erythrocyte suspension. Incubate and centrifuge the samples, then collect the supernatant. Measure absorbance using a UV-Visible spectrophotometer and calculate the percentage hemolysis.<sup>[29]</sup>

### **Phytochemical Investigation**<sup>[30]</sup>

| Sr. No | Name of Test                                                                                                                                                                                       | Observation                                                                                     | Inference           |
|--------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|---------------------|
| 1      | <b>Test for Phenol:</b><br>Extract Mixed with 2ml of 2% of Solution of $\text{FeCl}_3$                                                                                                             | Blue /green color                                                                               | Phenol Present      |
| 2      | <b>Test for Saponin:</b><br>A portion of the extract was taken in a test tube and agitated strongly with water.                                                                                    | formation of stable foam                                                                        | Saponin present     |
| 3      | <b>Test for Tannins:</b><br>Extract Mixed with 2% of $\text{FeCl}_3$                                                                                                                               | Black color                                                                                     | Tannins Present     |
| 4      | <b>Test for Terpenoids:</b><br>The extract Mixed with chloroform, then 2ml of conc. sulphuric acid added gently and shaken gently                                                                  | Reddish brown color observed in the inerphase                                                   | Terpenoids Present  |
| 5      | <b>Test for Flavonoids:</b><br>The extract was subjected to treatment with sodium hydroxide solution.                                                                                              | Observation of strong yellow coloration. Which becomes colorless on the addition of dilute acid | Flavonoids Present  |
| 6      | <b>Test for Glycoside:</b><br>A mixture of the extract and glacial acetic acid with 2% $\text{FeCl}_3$ was prepared and subsequently added to concentrated sulphuric acid in a separate test tube. | visible brown ring developed at the interface                                                   | Carbohydrate Absent |
| 7      | <b>Test for Protein:</b><br>The extract was carefully mixed with a 'few drops of conc. Nitric acid                                                                                                 | Formation of yellow color                                                                       | Protein Absent      |
| 8      | <b>Test for Alkaloids:</b><br><b>A) Dragandroff's test:</b><br>A few ml of filtrate, 1 or 2ml Dragandroff 's reagent                                                                               | Brown colored ppt                                                                               | Alkaloids Present   |
|        | <b>B) Mayer's test:</b><br>To few ml of extract, 2 drops of Mayer's reagents                                                                                                                       | Cream colored ppt                                                                               | Alkaloids Present   |
|        | <b>C) Hager's test:</b><br>To few ml of extract 1 or 2ml of Hager's reagent add                                                                                                                    | Yellow colored ppt.                                                                             | Alkaloids Present   |
|        | <b>D) Wagner's test:</b><br>To few ml extract, a minimal volume of Wagner's reagent                                                                                                                | Reddish brown colored ppt                                                                       | Alkaloids Present   |

*Table no. 1: Phytochemical screening of Myristica fragrance Hout.*

## EXPERIMENTAL WROK

## Requirement

### Chemical used

Ethanol, PBS buffer Solution, Distilled water Triton x-100

### Apparatus used:

Test Tube, Heparin Tube, Beaker, Stirrer. 5ml Syringe. Soxhlet Extractor.

### Equipment used:

Centrifugation machine, Incubator, UV- Vis spectrophotometer

## Procedure:

### 1. Procedure of phytoextract *Myristica fragrans* Houtt:

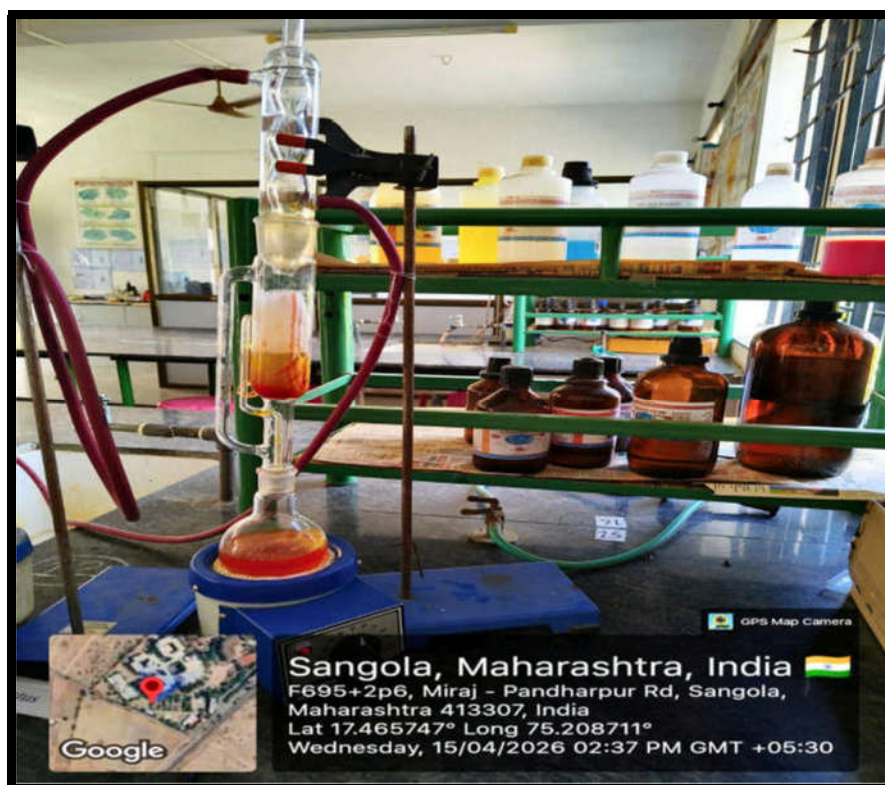


*Figure 3: Preparation of Plant Extract.*

25 g of dried, coarsely powdered sample is placed in a thimble inside the Soxhlet extractor, which is assembled with a round bottom flask containing 200–250 mL ethanol at the bottom and a condenser on top with continuous water flow. The setup is heated at about 60°C so ethanol evaporates, condenses, and repeatedly siphons through the sample for 6–8 hours until extraction is complete; the solvent is then evaporated to obtain the crude extract.<sup>[31]</sup>

### 2. Preliminary Phytochemical screening of Extraction



*Figure 4: Phytochemical screening.*

### 3. Preparation of phosphate buffer solution

To prepare a phosphate buffer solution, you need to mix monobasic sodium phosphate and dibasic phosphate in appropriate proportions to achieve the desired PH. <sup>[32]</sup>

#### 1. Calculate buffer ratio

Use the Henderson-Hasselbalch equation to calculate the ratio of monobasic to dibasic sodium phosphate needed to achieve the desired PH. The equation is  $\text{pH} = \text{pK}_a + \log \left( \frac{[\text{A}^-]}{[\text{HA}]} \right)$ . <sup>[33]</sup>

#### 2. Prepare stock solution

Prepare separate stock solutions of monobasic sodium phosphate (0.1 M) and dibasic sodium phosphate (0.2 M) by dissolving the appropriate amounts of each salt in distilled water. Ensure complete dissolution of both solutions before use. <sup>[34]</sup>

#### 4. Adjust pH

Check the pH of the buffer using a pH meter and adjust it to the desired value by adding small amounts of acid or base as required.

#### 5. Storage

Store the prepared buffer solution in a clean, airtight container at room temperature or as required for further use. <sup>[35]</sup>

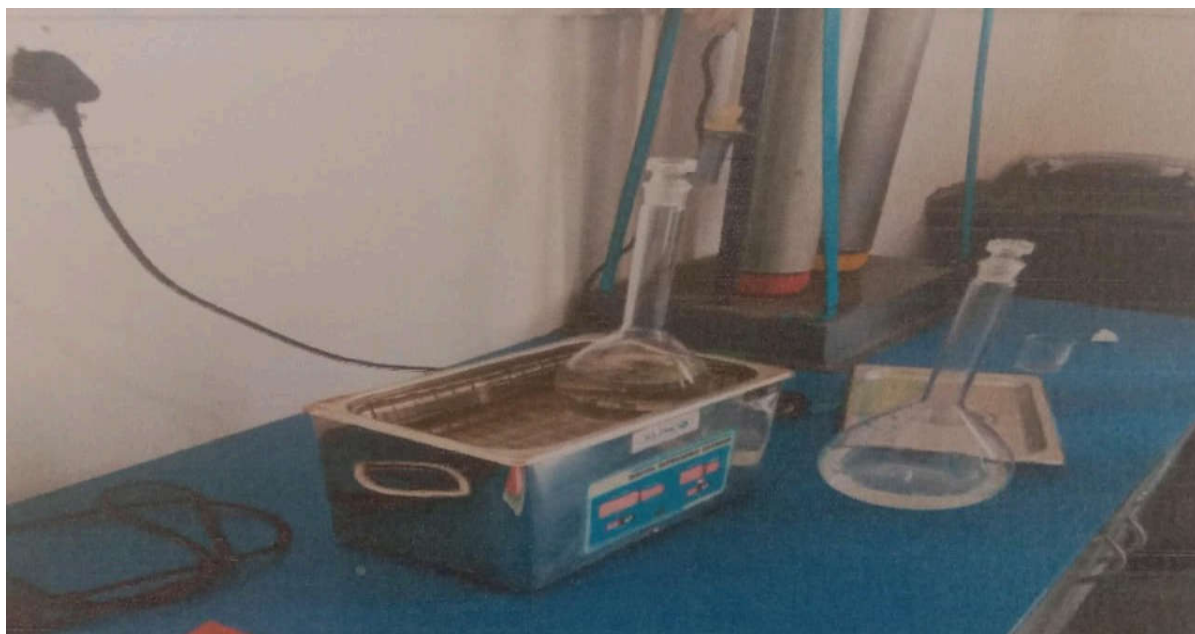


*Figure 5: Preparation of phosphate buffer.*

#### 4. Preparation of erythrocyte cell

##### 1. Collection of Blood

Draw fresh blood a tube containing an anticoagulant such as EDTA or heparin and process it within 1–2 hours.

##### 2. Centrifugation

Spin the sample at about 3000 rpm over 10 minutes to separate the red blood cells from plasma.

##### 3. Washing

Remove the plasma and flush the packed blood cells with isotonic saline or phosphate buffer. Repeat this step 2–3 times, centrifuging at 3000 rpm for 5 minutes each time.

##### 4. Preparation of Suspension

Dilute the washed erythrocytes with phosphate buffer (pH 7.4) to obtain a 2% v/v cell suspension.

##### 5. Storage

Keep the prepared erythrocyte suspension at 4°C and use it within 24 hours for accurate results.  
[36]



*Figure 6: separation of Serum and RBCs.*

#### 5. Hemolytic Activity Test

### **Preparation of RBCs**

Separating erythrocytes from a blood sample by centrifugation, washing them with normal saline, and resuspending the cells to obtain a clean sample for laboratory analysis.

### **Washing of RBCs**

Washing of red blood cells is carried out by repeatedly rinsing erythrocytes with an isotonic saline solution after centrifugation to remove plasma, proteins, and impurities, resulting in a purified cell sample for laboratory use.

### **Preparation of Test sample**

Test samples are prepared by dissolving the required substance in saline phosphate buffer at different concentrations (25, 50, 75, and 100 g/ml). The material used may be a natural extract, synthetic compound, or pharmaceutical formulation.

### **Incubation with RBCs**

RBC suspension is mixed with test samples and kept under physiological conditions (around 37°C, pH 7.4) for 1–2 hours.<sup>[37]</sup>

### **Centrifugation**

After incubation, the mixture is centrifuged to separate intact cells (pellet) from lysed cells present in the supernatant.

### **Measurement of Hemolysis**

Hemolysis is determined by measuring the absorbance of the supernatant at around 540 nm using a UV-visible spectrophotometer. The extent of red cell lysis is calculated as a percentage by comparing the test sample absorbance with positive and negative controls.<sup>[38, 39]</sup>

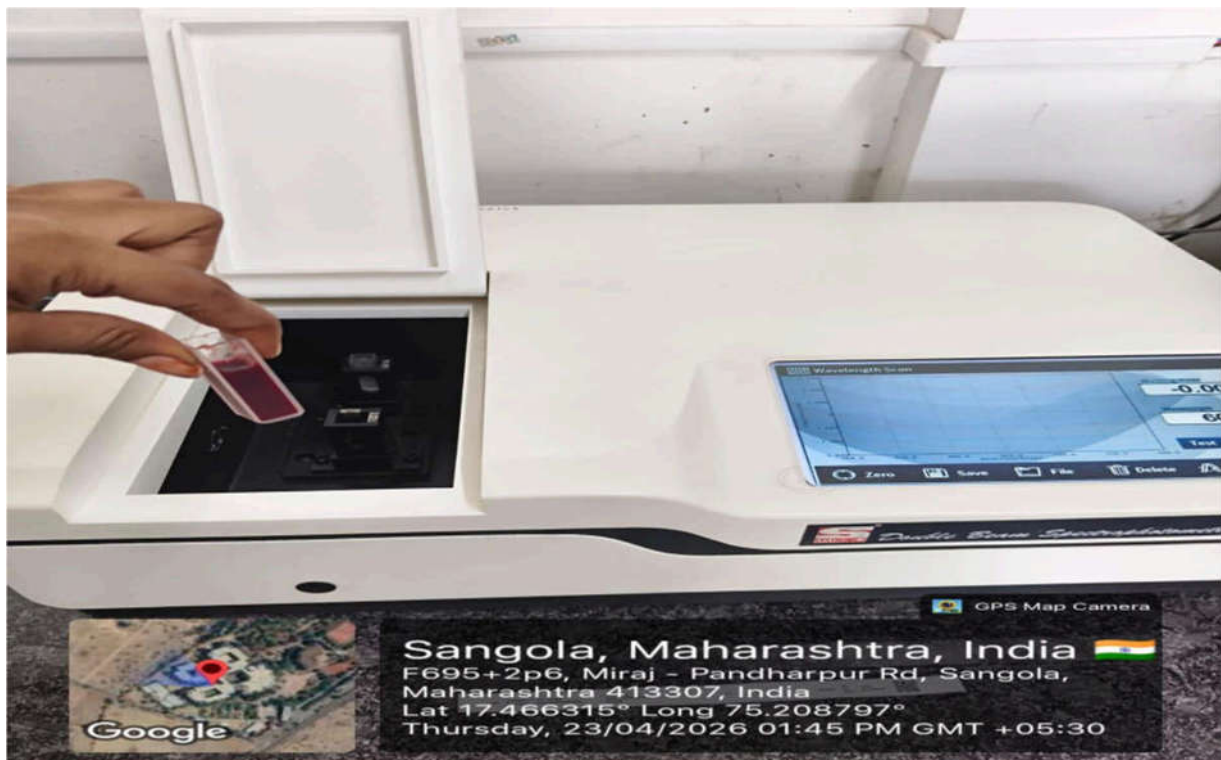


*Figure 7: Checking Absorbance of Sample.*

## RESULT

The following formula used for assess proportion of Hemolysis.

$$\% \text{ Hemolysis} = [(A_t - A_n) / (A_c - A_n)] .100$$

Where,

$A_t$ : Absorbance of the test sample

$A_n$ : Minimum control absorbance (PBS solution)

Ac: Maximum control absorbance (Distilled water)

### Observation

| Sr.No | Concentration | Absorbance | Hemolysis | Protection |
|-------|---------------|------------|-----------|------------|
| 1     | 25 ml         | 0.042      | 72.14%    | 0.27       |
| 2     | 50ml          | 0.118      | 46.82%    | 0.53       |
| 3     | 75ml          | 0.205      | 18.64%    | 0.51       |
| 4     | 100ml         | 0.332      | 7.25%     | 0.92       |

*Table no. 2: Absorbance of Sample.*

Preliminary phytochemical evaluation of the ethanolic extract of *Myristica fragrans* confirmed the presence of several bioactive secondary metabolites. Compounds such as saponins, phenolic compounds, tannins, terpenoids, flavonoids, and alkaloids were detected in the extract, while proteins and carbohydrates were absent. These phytoconstituents are widely associated with antioxidant and protective biological activities, suggesting the therapeutic potential of the plant extract.

| Sr. No | Constituents      | Observation |
|--------|-------------------|-------------|
|        | Ethanolic Extract | +           |
| 1      | Saponins          | +           |
| 2      | Phenols           | +           |
| 3      | Tannins           | +           |
| 4      | Terpenoids        | +           |
| 5      | Flavonoids        | +           |
| 6      | Proteins          | -           |
| 7      | Carbohydrates     | -           |
| 8      | Alkaloids         | +           |

*Table No. 3 : Result of preliminary phytochemical screening of Myrestica Fragarns Hoult.*

(-) indicates absence of compound

(+) indicates presence of compound

The hemolytic behavior of the ethanolic extract of *Myristica fragrans* was investigated using human erythrocytes, and the results were represented in terms of percentage hemolysis. The experimental findings revealed that the extract produced varying hemolytic effects depending on its concentration. A minimal hemolytic response of 7.25% was detected at 100 g/ml, whereas the extract at 75 g/ml produced 18.64% hemolysis. At 50 g/ml, a moderate erythrocyte lysis of 46.82% was recorded, while the maximum hemolytic effect of 72.14% was observed at 25 g/ml.

These results suggest that higher concentrations of the extract provided greater protection against erythrocyte membrane disruption. The biological activity of the extract may be associated with the presence of several phytoconstituents such as flavonoids, phenolic compounds, tannins, terpenoids, and alkaloids. Evaluation of hemolytic activity serves as an important parameter for determining the cytotoxic influence of plant extracts on normal red blood cells and for analyzing their membrane protective or membrane damaging potential. The intermediate hemolytic pattern observed during the investigation indicates that the extract possesses moderate cytotoxic properties toward human erythrocytes.

| Sr. no | Concentration | Hemolysis % |
|--------|---------------|-------------|
| 1      | 25ml          | 72.14%      |
| 2      | 50ml          | 46.82%      |
| 3      | 75ml          | 18.64%      |
| 4      | 100ml         | 7.25%       |

*Table no. 4: Percentage of Hemolysis.*

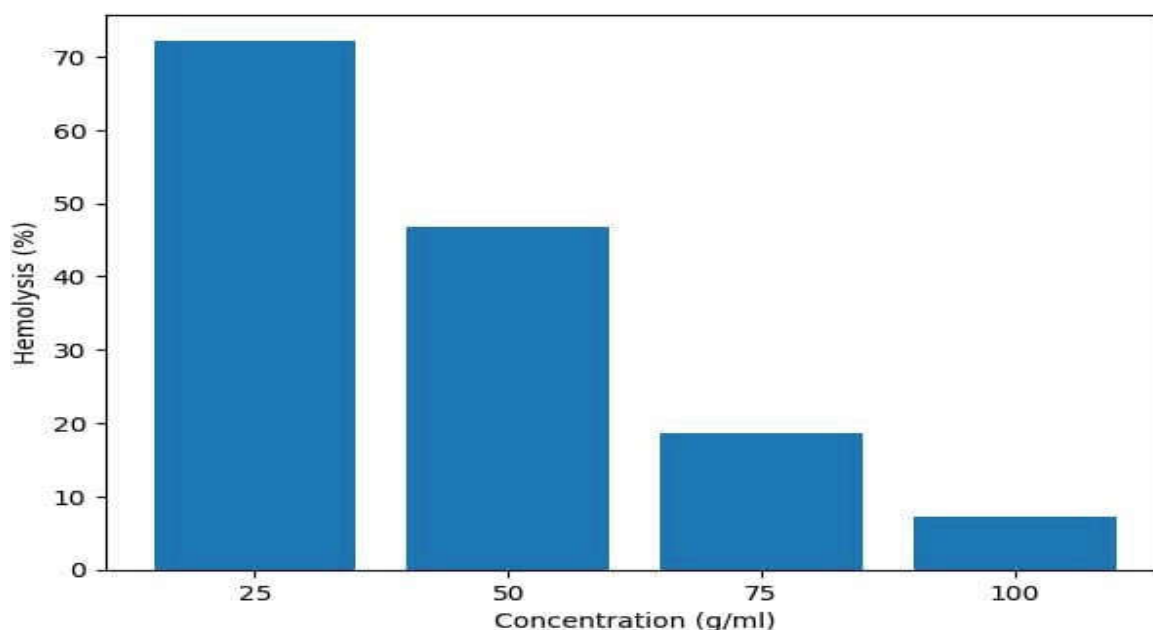


*Figure 8: Bar Graph of Hemolytic Activity of Javetri*

## DISCUSSION

The current investigation was conducted to study phytochemical profile and in-vitro erythrocyte hemolytic effect of the ethanolic extract of *Myristica fragrans* Houtt. The phytochemical evaluation revealed the existence of several biologically constituents, tannins, phenolic compounds, terpenoids, and saponins, whereas proteins and carbohydrates were not detected. Are widely recognized for their antioxidant, protective, and therapeutic activities.

The hemolytic study demonstrated that the extract exhibited a concentration-related effect on red blood cell membranes. A greater percentage of hemolysis was observed at lower concentration levels, while higher concentrations produced reduced erythrocyte damage. The extract showed maximum hemolysis at 25 µg/mL and minimum hemolysis at 100µg/mL, indicating possible membrane eprotective behavior at elevated concentrations. This effect may be associated with the interaction of phytoconstituents with membrane proteins and phospholipid layers of erythrocytes.

The findings present investigation align with earlier reports suggesting that compounds present in *Myristica fragrans* possess the ability to alter membrane permeability and influence cellular integrity. Phenolic and flavonoid components may contribute to antioxidant defense, thereby minimizing oxidative stress-induced membrane injury. The moderate hemolytic response observed during the experiment indicates that the extract possesses limited cytotoxicity under controlled conditions.

## CONCLUSION

That demonstrated that thehydro alcoholic extract of *Myristica fragrans* Houtt. (Javetri) contains various bioactive phytoconstituents including alkaloids, flavonoids, tannins, terpenoids, phenols, and saponins. Screening of plant constituents confirmed the therapeutic significance plant extract and supported its traditional medicinal use.

The in-vitro hemolytic assay revealed concentration-dependent hemolytic action of the extract on red blood cells. Higher hemolysis appeared at lower concentrations, while reduced hemolysis was found at higher concentrations, indicating possible membrane protective activity. Overall, the study suggests that *Myristica fragrans* Houtt. Further detailed pharmacological and toxicological studies are needed to evaluate its safety and therapeutic applications.

## REFERENCES

1. Gupta, A. D., Bansal, V. K., Babu, V., Maithil, N. Chemistry, antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans* Houtt.). Journal of Genetic Engineering and Biotechnology, 2013;11(1): 25–31.

2. Lippi, G. Plebani, M. Hemolysis: An overview of the leading cause of unsuitable specimens in clinical laboratories. *Clinical Chemistry and Laboratory Medicine*, 2015: 53(1): 1–6.
3. Sharma, P., & Sharma, J. D. In vitro hemolysis of human erythrocytes by plant extracts with antiplasmodial activity. *Journal of Ethnopharmacology*, 2001: 74(3): 239–243.
4. Afsar, T., Razak, S., Khan, M. R., Mawash, S., Almajwal, A., Shabir, M. Evaluation of antioxidant, anti-hemolytic and anticancer activity of plant extracts. *BMC Complementary and Alternative Medicine*, 2016:16(4) :258.
5. Tlili, M. L., Laib, I., Salemi, K., Chetehouna, I. Ben Moussa, I. Phytochemical analysis and evaluation of hemolytic activity of plant extracts. *Acta Chimica Slovenica*, 2024: 71(3): 492–499.
6. Lippi, G.,Plebani, M. Hemolysis: An overview of the leading cause of unsuitable specimens in clinical laboratories. *Clinical Chemistry and Laboratory Medicine*, 2015: 53(1):1–6.
7. D'Alessandro, A., Mohandas, N., Zolla, L. Red blood cell storage: The story so far. *Blood Transfusion*, 2015: 13(2):178–186.
8. Qadri, S. M., Dever, L. L. Wolfe, J. D. Intravascular hemolysis: mechanisms and clinical implications. *Blood Reviews*, 2017: 31(3):113–120.
9. Sharma, R. K., Mehta, P. L., Verma, S., Patil, N. Extravascular hemolysis: Mechanism and clinical implications. *International Journal of Hematology*, 2021:113(3): 321–330.
10. Kumar, S., Singh, R. P., Patel, A. Deshmukh, P. Osmotic hemolysis: Mechanism and laboratory applications. *Journal of Hematology and Blood Disorders*, 2020:8(2):78–84.
11. Sharma, V., Gupta, N., Mehra, R., Kulkarni, S. Study of osmotic fragility and hemolysis of red blood cells. *International Journal of Medical Research*, 2019:7(1): 45–50.
12. Patel, D. K., Joshi, M. A., Verma, P., Nair, R. Evaluation of osmotic hemolysis in human erythrocytes. *Asian Journal of Biomedical Sciences*, 2021:10(3):112–118.
13. Kaur, H., Malhotra, P., Singh, S., Arora, D. Immune hemolysis: Pathophysiology and diagnostic approach. *Asian Journal of Hematology*, 2018:6(1): 21–27.
14. Reddy, K. V., Nair, P. S., Thomas, A., Iyer, R. Autoimmune hemolytic anemia and immune hemolysis mechanisms. *Journal of Blood Disorders and Transfusion*, 2019:10(4):150–156.
15. Deshmukh, P., Nair, A., Kulkarni, R., Patil, S. Evaluation of mechanical hemolysis in blood-contacting medical devices. *Asian Journal of Health Sciences*, 2020:10(1):33–39.
16. Rao, V. K., Thomas, L., Verma, A.,Singh, M. Mechanical trauma and hemolysis in extracorporeal circulation systems. *Journal of Blood Disorders*, 2018:6(4):200–206.
17. Kaur, H., Malhotra, P., Singh, S. Arora, D. Role of biochemical markers in the diagnosis of hemolysis. *Asian Journal of Hematology*, 2019:7(1):25–32.
18. Singh, G., Kapoor, I. P. S., Singh, P., Heluani, C. S. Chemical composition and antioxidant activity of nutmeg (*Myristicafragrans*Houtt.). *Journal of Agricultural and Food Chemistry*, 2008:56(14):5353–5360.

19. Rao, P. K., Nair, A., Verma, S., Thomas, L. Morphological and pharmacognostic evaluation of *Myristica fragrans* Houtt. *Journal of Pharmacognosy and Phytochemistry*, 2019;8(4):2150–2155.
20. Mathew, P. J., George, V., Nair, G. M., Joseph, A. Cultivation practices and geographical distribution of *Myristica fragrans* Houtt. *Journal of Spices and Aromatic Crops*, 2016;25(2):95–102.
21. Joshi, M., Verma, P., Thomas, L., Nair, R. Traditional uses and phytochemical constituents of nutmeg (*Myristica fragrans* Houtt.). *World Journal of Pharmaceutical Sciences*, 2021;9(5):145–151.
22. Sharma, V., Gupta, N., Mehra, R., Kulkarni, S. Post-harvest handling and drying techniques of nutmeg and mace (*Myristica fragrans* Houtt.). *International Journal of Herbal Medicine*, 2018;6(4): 88–93.
23. Thongdon-AJ, Udomsangpetch R, Sueblinvong T, Chotivanish K, Antimalarial Activity of *Acacia pennata* Extracts Against *Plasmodium falciparum*, *Southeast Asian Journal of Tropical Medicine and Public Health*, 2011;42(4):784–789.
24. Pujenjob N, Choomwattana S, Mitrevej A, Sinchaipanid N, Chemical Composition and Antibacterial Activity of *Acacia pennata* (L.) Willd, Essential Oil against Multidrug-Resistant Pathogenic Bacteria, *Journal of Natural Remedies*, 2016;16(1):11–17.
25. Sookkongwaree K, Geitmann M, Roengsumran S, Antioxidant Activity and Cytotoxicity of Selected Thai Medicinal Plants, *Journal of Ethnopharmacology*, 2006;107(3):280–285.
26. Kamkaen N, Wilkinson J. M, Antibacterial Activity of Thai Medicinal Plants against Methicillin-Resistant *Staphylococcus aureus* Fitoterapia, *Journal of Natural Remedies*, 2009;80(2):102–104.
27. Kooltheat N, Sranujit R. P, Chumark P, Antimicrobial and Antioxidant Activities of Selected Thai Medicinal Plants, *Journal of Health Research*, 2007;21(3):145–150.
28. Boonsri P, Karalai C, Ponglimanont C, Chantrapromma K, Prengueurm C, Fun H. K, Cytotoxic Hemolytic Activities of Triterpenoid Saponins from the Flowers of *Acacia pennata*, *Planta Medica*, 2007;73(5):503–509.
29. Powell, W. A., Catranis, C. M., Maynard, C. A. Design of self-processing antimicrobial peptides for plant protection and evaluation by hemolytic assay. *Plant Cell Reports*, 2000;19(2):111–114.
30. Dr. K. R. Khandelwal, Dr. Varuna Sheti, *Practical Pharmacognosy Textbook*, Nirali Prakashan, 2020;31(1):25.pp 1–25.9.
31. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., Yoga Latha, L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 2011;8(1):1–10.
32. Good, N. E., Winget, G. D., Winter, W., Connolly, T. N., Izawa, S., Singh, R. M. Hydrogen ion buffers for biological research and preparation of phosphate buffer systems. *Biochemistry*, 1966;5(2): 467–477.

33. Henderson, L. J. The theory of neutrality regulation in the animal organism and the Henderson–Hasselbalch equation. *American Journal of Physiology*, 1908;21(2):173–179.
34. Bates, R. G. Preparation and standardization of phosphate buffer solutions for analytical chemistry applications. *Analytical Chemistry*, 1964;36(5):871–877.
35. Stoll, V. S., Blanchard, J. S. Buffers: principles and practice in biological research. *Methods in Enzymology*, 1990;(18)2: 24–38.
36. Collee, J. G., Fraser, A. G., Marmion, B. P., Simmons, A. Preparation of blood samples and erythrocyte suspensions for biological studies. *Mackie and McCartney Practical Medical Microbiology*, 1996;14(3): 842–845.
37. Amol V Pore, Sanjay K Bais, Kazi, S. M., Nikte, A. A. Assessment of In-Anti Urolithiatic Activity *Epiphyllumoxypetalum*. *International Journal of Pharmacy and Herbal Technology*, 2023;1(2):72–76.
38. Pore, A. V., Bais, S. K., Dhumal, S. S., Kazi, S. M. Assessment of red blood cell hemolysis by centrifugation and absorbance measurement at 540 nm. *International Journal of Pharmacy and Herbal Technology*, 2023;1(2):77–80.
39. Zade, N., Sanjay K Bais, Kolekar, Y. B. Assessment of In-Vitro Hemolytic Activity of *Selenicereusundatus*. *International Journal of Pharmacy and Herbal Technology*, 2024;2(3):1718–1727.