IJMPR 2023, 7(9), 53-60

International Journal of Modern Pharmaceutical Research

www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article

SJIF Impact Factor: 5.273

# PHARMACOLOGICAL INVESTIGATION ON *MELASTOMA MALABATHRICUM LINN*

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**Received on: 06/07/2023**
**Revised on: 26/07/2023**
**Accepted on: 16/08/2023**

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## ABSTRACT

Melastoma malabathricum is a highly medicinal herb. Our research focuses on several pharmacological activities such as antimicrobial, anticoagulant, and anti- inflammatory properties. We are primarily interested in anticoagulant activity in order to develop a unique treatment for the rising number of cardiac illnesses in our society. We used extracts of Melastoma malabathricum in various solvents such as hot water, hexane, ethyl acetate, and ethanol in this investigation. Finally, we discovered that hot water extract has stronger anticoagulant and anti-inflammatory properties than other extracts. Furthermore, ethanol extract has higher antimicrobialactivity than other extracts.

**KEYWORDS:** Polyphenolic polysaccharides, anti coagulant, anti microbial, anti inflammatory.

## INTRODUCTION

Melastoma malabathricum L. (Melastoma) is a Melastomataceae genus. The family contains approximately 100 species and is found on all continents. They were formerly found in India, Japan, Australia, and the Pacific Islands. Melastoma has now been naturalized in the United States. Because of its quick growth and reproduction, it has now become an invasive species. They can be found in grasslands and woodlands. Melastoma does not grow tall, although it does become bushy with time. The plant's blossoms, which are brilliant purple in color,are its main draw.

In this work, the crude extract of M. malabathricum Linn. Leaf was refined bysolid phase extraction and tested for bioactivechemical constituents on blood coagulation, antibacterial response, and anti-inflammatoryactivity.

The chemical ingredients characterized included acidic polysaccharides (rhamnogalacturonan, homogalacturonan, and rhamnose hexose-pectic type polysaccharide) and anticoagulant polyphenolics. The primary volatile chemicals with antibacterial action were squalene (25%), hexadecanoic acid ethyl ester (18.95%), linolenic acid ethyl ester (17.35%), and phytol (11.89%). Polyphenols, which are found in Melastoma plants, have the potential to be anti-inflammatory drugs or enzyme inhibitors.However, current research has revealed that Melastoma possesses numerous therapeutic benefits. Plant extracts can be used to treat a wide range of ailments and medical issues. Melastoma is a type of cancer.

According to traditional beliefs of communities/tribes, M. malabathricum has numerous medical qualities, and the entire plant could be utilized as herbal medicine. Itis also a well-known herb in Malaysia, where its leaves, shoots, and roots are prepared in avariety of methods for the treatment of various ailment.

Many reviews on medical usage have appeared in the literature. However, no one has completely defined the chemical and pharmacological properties of this essential ethnomedicinal plant.As a result, we set out to create an up-to-date and complete assessment of M. malabthricum, including ethnomedicinal usage phytochemical content, and scientifically established pharmacological effec

The leaves, shoots, barks, seeds, and roots of M. malabathricum have been used ethnopharmacologically to cure diarrhea, dysentery, hemorrhoids, cuts and wounds, toothache, and stomachache. Scientific discoveries further shown that various components of M. malabthricum have a wide range of pharmacological properties, including antinociceptive, anti-inflammatory, wound healing, antidiarrheal, cytotoxic, and antioxidant activity. Phytochemical components of various types have also been isolated and identified from various areas of M.Malabathricum.The use ofwater and organic solvent in combination may aid in the extraction of phytochemicals that are soluble in both water and organic solvent.

### Anticoagulant properties

M. malabathricum Linn. has anticoagulant effects. The anticoagulant activity of hot water extract is exceptional. To the best of our knowledge, no esearch has been published on the chemical contents of M. malabathricum Linn.hot water extract, which shows anticoagulant

activity. Previous research has also shown that acidic polysaccharides and polyphenolic chemicalscontribute to the anticoagulant activity ofLythrum salicaria and Porana volubilis. This type of discovery is critical since it provides hints for potential treatment of blood coagulant problems. In the intrinsic pathway, it helped to extend blood coagulation

**Antimicrobial properties**
Melastoma's antibacterial qualities are critical in eliminating germs and disorders from the body. It fights yeast infections and aids in the inhibition of the growth of dangerous microorganisms in the body.

**Anti-inflammatory properties**
Melastoma is reported to have anti- inflammatory characteristics that can help reduce swelling throughout the body. Furthermore, it might alleviate the uncomfortable symptoms of allergies. Melastoma can be used to alleviate the symptoms of sore throat, bronchitis, and other inflammatory disorders.

***Melastoma malabathricum Linn.***

## MATERIALS AND METHODS

**Sample preparation**
*M. Malabthricum* leaves were collected. Thefresh leaves of *M.Malabthricum* were

**Chemical reagents**
- Ethyl acetate -300ml,Ethanol-300ml,Hexane-300 ml ,DMSO solution
- Coagulating agent,Spirit,anticoagulation agent
- Nacl,agar,beef extract,peptone,distilled water, cleaned and dried at 50°C. The samples wereground into fine powder (500 µm) by blender.
- 0.06 mg trypsin, 1 mL of 20 mM Tris-HCl buffer (pH 7.4), and 1 mL test sample, 1 mL of 0.8% (*w*/*v*) casein , 2 mL of 70% perchloric acid,phosphate buffer

**Materials**
- Syringe, Filter paper (no.1)
- Egg, needle, Black ink, cotton

| EQUIPMENT | MANUFACTURER |
|---|---|
| SOXHLET APPATATUS | ROTEK |
| INCUBATOR | KEMI |
| CENTRIFUGAL MACHINE | LABTECH |

**Methods**
**Extraction (soxhlet extraction)**
- Approximately 250 gram of dried powder *M. Malabthricum* leaves were weighed and packed into thimblewith 300 ml of the extracting solvent.
- The sample were extracted using soxhlet extraction method with different types of solvents including water (H2O),ethanol (EtOH), ethyl acetate (EA) and hexane (Hex).
- Temperature of extraction process was based on solvents used.
- The extract from *M. Malabathricum* was filtered through filter paper (Whatman No. 1) under vacuum. However, the extract from EtOH, EAand Hex were recovered using evaporation process.
- The evaporation process was conducted at room temperature to minimize any possible degradation of the phytochemicals in the samples.
- Extraction yield from both water andorganic solvent were calculated usingfollowing equation,

$$Y = \frac{W_d}{V_e} \; x \; R_{ss} \; x \; 100 \; (1)$$

- Ve = volume of aqueous filtered(mL)
- Rss = ratio of solvent to solid (mL g-1).
- Wd = weight of dried extract (g)

**In-OVO Method**
**Anticoagulant effect in chickchoriallantoic membrane model**
Fertile chicken eggs were obtained from poultry farm and were incubated at 38° C in humidified environment in horizontal position.
- The window was then closed with a sterile transparent tape and the eggs were returned to the incubator.
- By day 8 of post incubation,
- 36 Eggs were selected for experiment and divided

into 6 groups of 6 each.

- Group 6 eggs were left untreated
- In group 6 eggs were treated withvehicle control
- Group 6 eggs were treated with standard anticoagulant drug.
- Group 6 eggs were received with vtamin K injection and extract of *melastoma* in different solvents like hexane, ethyl acetate, ethanol and hotwater.
- On day 12 of incubation anticoagulant activity was observed.
- A small hole drilled at the narrow endof the eggs and the holes were filled with plaster.
- The upper surface of the shell was then cut to make a window with a sterile forceps under laminar airflow.
- Observe the anticoagulant activity in different solvent extract of *Melastoma malabathricum*

**Anti Microbial Study**
**Agar well diffusion method**

- Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts
- In this method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface.
- A hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antimicrobial agent or extract solution at desire concentration is introduced into the well.
- Agar plates are incubated under suitable conditions depending upon the testmicroorganism.
- The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

**Anti-InflammatoryActivity**
**Proteinase inhibitory activity**

- Proteinase inhibitory activity of the leaf extracts was performed according to the method of Sakat et al. which is modified by Gunathilake et al.
- Briefly, the reaction solution (2 mL) consisted of 0.06 mg trypsin, 1 mL of20 mM Tris-HCl buffer (pH 7.4), and 1 mL test sample (0.02 mL extract 0.980 mL methanol).
- The solution was incubated (37 °C for 5 min), and then 1 mL of 0.8% (*w*/*v*) casein was added, and the mixture was further incubated for an additional 20 min.
- At the end of incubation, 2 mL of 70% perchloric acid was added toterminate the reaction.
- The mixture was centrifuged, and the absorbance of the supernatant was measured at 210 nm against buffer asthe blank.[9]
- Phosphate buffer solution was used asthe control. The percentage inhibition of protein denaturation was calculated by using the following formula:
- % inhibition of denaturation = 100 × (1 − A2/A1)
- Where
- A1 = absorption of the control sample
- A2= absorption of the test sample

**Extraction**
**Collection**: *Melastoma malabathricum* was collected near wet lands.

**Drying:** Shade drying process and powderedby blending process

**Figure 01**

**Figure 02**

**Figure 03**


**Figure 04** **Figure 05**

**Figure 01: Collected *Melastoma malabathricum* leaf. Figure 02: Powdered leaf. Figure 03: Soxhlet apparatus Figure .04: Decoction process. Figure 05: Extracts of solvents.**

## RESULT AND DISCUSSION

**In OVO Method**

**Anticoagulant effect in chick embryo choriallantoic membrane model**

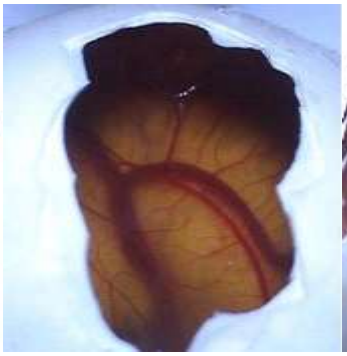
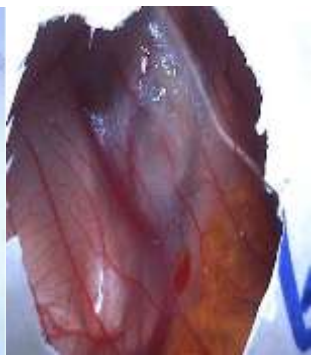




**Fig. 06: Group 1 normal (untreated)showing normalvasculature.**
**Fig. 07: Group 2 vehicle controlshowing normal vasculature.**
**Fig. 08: Group 3 aqeous extract of M*elastoma malabathricum linn.*showingblood thinning.**

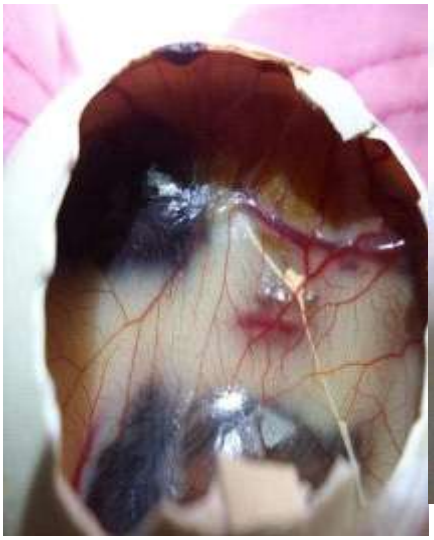
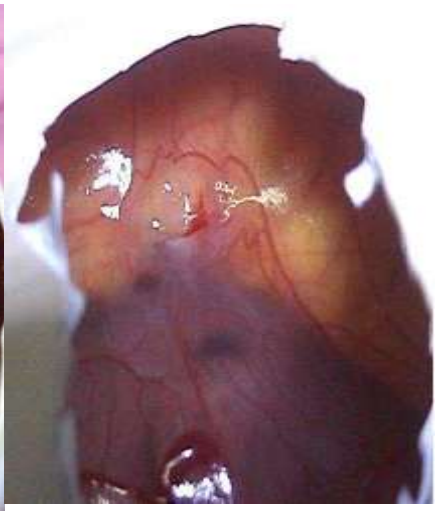



**Fig. 09: Group 4 hexane extract of M*elastoma malabathricum linn.* showing vascular defect of neumorous new blood vessels.**
**Fig. 10: Group 5 ethanolic extract of M*elastoma malabathricum linn.* Showingblood thinning effect less than.**

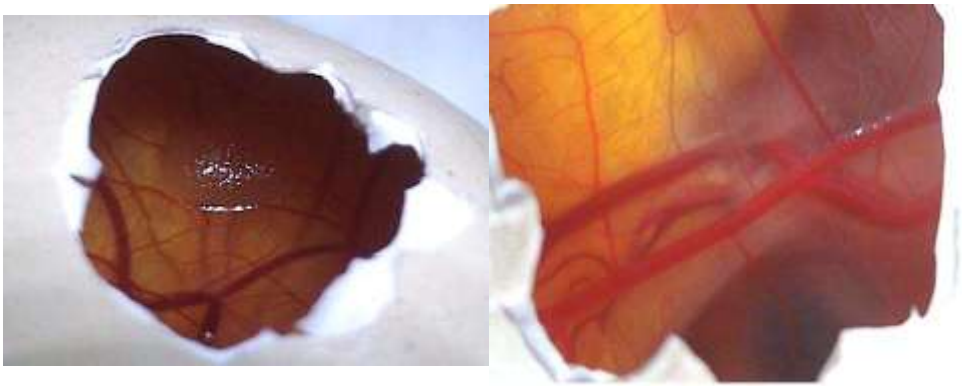


**Fig. 11: Group 6 ethyl acetateextract of M*elastoma malabathricum linn.* Showing.**
**Fig. 12: Group 7 standard drug (heparin).**

**Antimicrobial Activity**

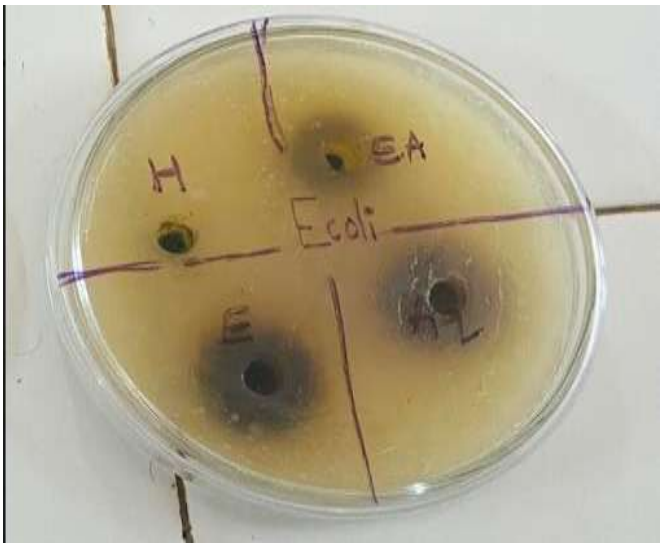



**Figure 1: Antibacterial activity of *Melastoma malabathricum* leaf extractagainst gram-ve bacterial species. (Zone of inhibition: Ethanolic extract=19, Aqeous extract=16mm Ethyl acetate=11mm,hexane extract=0mm)**

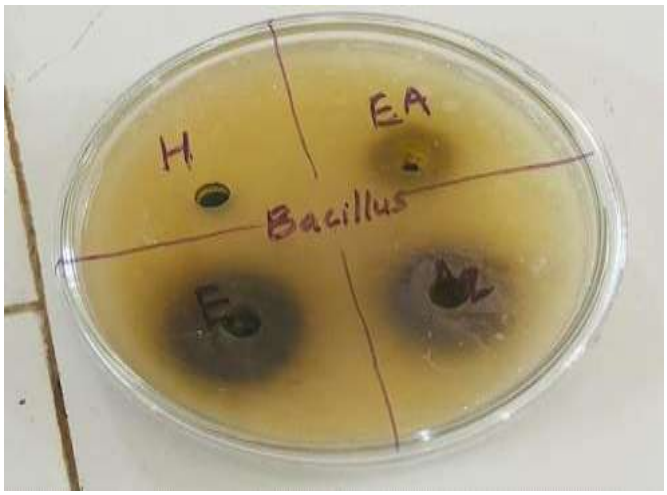
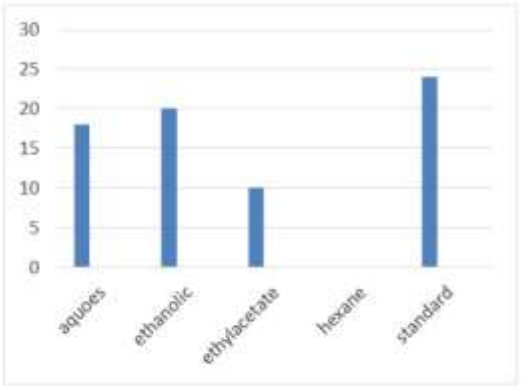
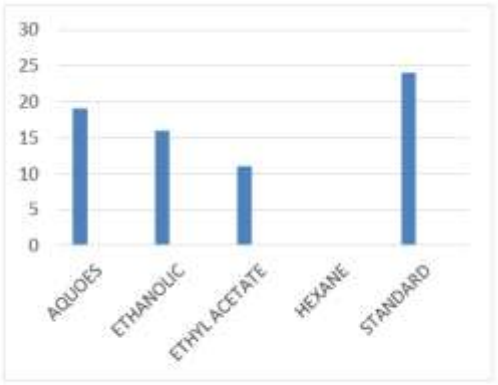



**Figure 2: Antibacterial activity of *Melastomamalabathricum* leaf extract against gram+ve bacterial species. Zone of inhibition: Ethanolic extract=20mm, aqeous extract=18mm, Ethyl acetate extract=10mm, Hexane extract=0mm.**

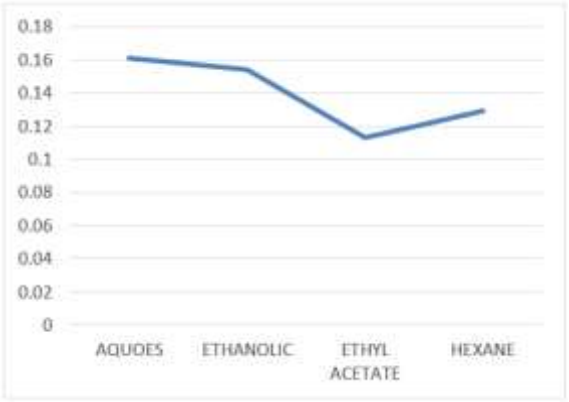



BACILLUS




ECOLI

**Figure 1: Zone of inhibition shown by*Melastoma malabathricum* extract against gram –ve bacteria E.coli.**
**Figure 2: Zone of inhibition shown by *Melastoma malabathricum* against gram +vebacteria bacillus.**




**Anti InflamatoryActivity**

Proteinase inhibitory activity of *Melastoma malabathricum* is shown in Figure, and the inhibition levels were within the range of 0.16%. aqueous extract of *Melastoma malabathricum* have shown more proteinase inhibition level compared with other organic solvents

## DISCUSSION

**Anticoagulant activities of leaf extracts:** A previous study by Manicam et al. showed that *malabathricum Linn.* leaf hot water extract exhibited an excellent anticoagulant activity. Therefore, in this study, the hot water extract of *M. malabathricum Linn.* leaf was fractionated, while the anticoagulant activity of each fraction was evaluated.Whenvitamin k added to the chick plasma and develop a clot in the blood vessels at $38^0$c over 24 hrs.this was an expected as egg yolkWas known to be rich in phospholipids,some of which promote clotting effect.The effect was not due to removal of ca ions by material in the yolk,as addition of quantity of a extract of *Melastoma malabathricum* failed to induce clotting.Anticoagulant activity was determined for albumin and egg yolk from several fresh egg.In view of the previous report that polyphenols inhibit the blood coagulation.In this a hot aqeous extract of *M.malabathricum* shows more anticoagulant effect when comparing with solvents like ethanol,ethyl acetate, hexane. however ethanol extract also shows blood thinning effect.

**Antimicrobial activitie of leaf extracts:** Antimicrobial activities of *M.malabathricum* hexane,ethyacetate,eth anol and aqueous extracts were evaluatedagainst Gram-positive (*Basillus subtilis*) and Gram-negative (*E. coli* ) bacteria.Under the conditions tested, antibacterial activities weredetected in all concentration for both *M. malabathricum* aqueos extract,ethyl acetate,ethanol extracts, indicating the presence of antibacteriala activity in plant species. Statistical comparison between *M.malabathricum* hexane,ethylacet ate ,ethanol,aqueos extracts showed the means of zone of inhibition were significantly different between the two extracts for *Basillus subtilis* and *E. coli*, This suggests that the ethanol extracts might have differential effectiveness against *Basillus subtilis* and *E. coli*. However, this needs to be interpreted with caution because although the size of the inhibition zone could indicate the antimicrobial potency, the test itself is considered as a qualitative technique. The observed antibacterial activities could possibly be due to the presence of phytol and tocopherol that were identified in the ethanol extracts, as these 2 compounds have been reported to possess antimicrobial properties. However, other compounds have also been

reported. For example, a few phytochemicals namely ursolic acid, 2α-hydroxyursolic acid, asiatic acid, β-sitosterol 3-O-β-D- glucopyranoside, kaempferol, quercetin and ellagic acid, which were found either in the chloroform or ethyl acetate fraction of *M.malabathricum* had exhibited antimicrobialactivities.

**Anti inflammatory activity:** Proteinases have been associated with arthritic reactions. Neutrophils, in their lysosomal granules, carry many serine proteinases. Proteinases of leukocytes play a significant role in the development of tissue damage during inflammatory processes. ,a significant level of protection was provided by proteinase inhibitors. Various recent studies have shown that many flavonoids contributed significantly to the antioxidant and anti- inflammatory activities of many plants. Therefore, the presence of bioactives present in these leaves may contribute to their anti- inflammatory activity. Our previous studies have shown that these leafy vegetables are rich in polyphenols, flavonoids, and carotenoids. According to our previous study, total flavonoid and polyphenolic contents of soluble and bound phenolic fractions of leafy vegetables were within the range of rutin and the β-carotene and lutein contents of these studied leafy extracts. In this studies, aqueos extracts of *Melastoma malabathricum*, have exhibited significant antiproteinase (trypsin) activity.

## CONCLUSION

**Anticoagulant activity:** All in all, the presence of negative charged polyphenolic- polysaccharides were suggested to have played a role in the anticoagulant activity, especially prolonging blood coagulation in the intrinsic pathway, as exhibited by the purified *M. malabathricum* Linn. hot water leaf extract. This discovery opens up the avenue that the simple preparation of fractions and the abundant availability of *M. malabathricum* Linn. plant materials couldlead to the development of *M. malabathricum* Linn. active anticoagulant fractions as safe and cheap natural anticoagulant agents in chick choriallantoic membrane model.

**Antimicrobial activity*:*** *Melastoma malabathricum* ethanol leaf crude extracts have almost similar phytochemical constituents and yet both extracts were still relatively different, strongly implying the close relatedness and yet the uniqueness of the plant. Extracts exhibited antibacterial activities against *b. Subtilis, e. Coli* excepthexane.

**Anti-inflammatory activity:** In conclusion, results indicate that the aquoeus extracts of Melastoma malabathricum leaves extract of antiinflammatory properties. Leaves of *Melastoma malabathricum* possess good proteinase inhibition properties. Pearson's correlation studies showed that there were significant correlations between estimated bioactives and anti-inflammatory properties. Results indicate that these anti-inflammatory activities may be due to the occurrence of bioactive compounds, such as polyphenols.

## ACKNOWLEDGEMENT

First and foremost, We would like to thank Almighty for giving us the courage, knowledge, ability to undertake this researchand complete it satisfactorily. We express my heartfelt gratitude and respectful thanks to MS .Anju M P and college athourities for their guidance.

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