



## ***In vitro* study of biological activity in essential oil of *Curcuma amada* Roxb**

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### **Abstract**

Plants contribute to the sustenance of all life on earth. Plants are in fact the only major sources of active biological substances and consequently are in great use in the pharmaceutical formulations. The research endeavours in search of therapeutically worthy plants are hiking every day on account of man's initiative for finding out newer compounds to benefit health and life span. Plants are the base for home remedies, dietary supplements and drugs. Phytocompounds in the plants can play an important role in pharmacology. The essential oil of *Curcuma amada* was the study material in this study to determine their antifungal and anti-inflammatory properties.

**Keywords:** antifungal activity, anti-inflammatory, phytocompounds, *Curcuma amada*

### **Introduction**

Nature has proved to be an enigmatic bounty of medicinal resources since the inception of human culture and many modern drugs can be traced back to traditional herbal formulations. Investigations on the bio-chemical properties of plants in the past two centuries resulted in the discovery of compounds that enabled the discovery of novel and effective therapeutic compositions. Essential oils from aromatic medicinal plants are economically valuable products. They are composed of hydrocarbons that may have or lack Oxygen (monoterpenes, sesquiterpenes, and phenylpropanoids). gingeraceae- the ginger family is a vital group of rhizomatous therapeutic and aromatic plants known for the presence of volatile oils and oleoresins. Many other tuberous *Curcuma* species with aromatic rhizomes are rich in essential oils with diverse chemical constituents. However, they remain unexplored for their pharmacological potential. Essential oil from *Curcuma amada* Roxb. could find use as a significant source of antioxidants which can be used in food and pharmaceutical industry. The objectives of this study is to throw light on the antifungal and anti-inflammatory properties of the essential oil of *Curcuma amada* rhizome.

### **Materials and methods**

#### **Plant material collection**

*Curcuma amada* was obtained from the places namely Kottayam and Poonjar (Kerala, India). They were formally identified and authenticated by Dr. S. John Britto, the Director and Head of The Rapinat Herbarium and Centre for Molecular Systematics at St. Joseph's College (Autonomous), Tiruchirappalli (Tamil Nadu, India). The voucher specimen numbered RHT 65181 was deposited at Rapinat Herbarium.

#### **Essential oil extraction**

Fresh *C.amada* rhizomes were subjected to hydro-distillation for 3 hours with a Clevenger type apparatus. The essential oil thus collected was dried over anhydrous sodium

sulphate (Na<sub>2</sub>SO<sub>4</sub>) and preserved in an airtight and sealed vial at 4°C until further examination.

#### **Fungal organisms for antimycotic assay**

The pathogenic fungal species, *Aspergillus flavus* and *Mucor indicus* were obtained from Tropical Institute of Ecological Sciences based at Kottayam in Kerala.

#### **Anti-mycotic study**

The antifungal activities of essential oil of *C. amada* rhizomes were gauged by agar-well diffusion method (Murray *et al.*, 1995), modified by Olurinola (1996). Melted agar was poured into sterile petri dishes seeded with 100 µl each of fungal strains under consideration. Wells were made at equal distance on the inoculated plates. Each well was occupied up with 10 µl oregano oil prepared in varying concentrations (diluted with DMSO) and were tested with antibiotics. After letting it stay at 4°C for 2 hours, all petri dishes were again incubated at 37°C for 48 hours.

Antifungal activity was assessed by measuring the radius of the inhibition zones to the nearest millimetre. All experimentations were done in triplicate.

#### **Evaluation of anti-inflammatory activity *in vitro***

##### **Albumin denaturation inhibition**

The anti-inflammatory potential of essential oil is studied by a technique that uses inhibition of albumin denaturation. The reaction mixture involves test extracts at diverse concentrations (100-500 µg/ml) and 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was attuned with little amount of 1N HCl. The extracts were incubated at 37 °C for about 20 minutes and then heated at 51° C for 20 minutes. After cooling the samples, the turbidity was calculated at 660 nm. The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as follows:

Percentage of inhibition is equal to: (Abs Control – Abs Sample) X 100/ Abs control.

### Anti-proteinase action

2 ml of reaction mixture with 0.06 mg trypsin, 1 ml of 20 mM Tris HCl buffer at pH 7.4 and 1-ml test sample at different concentrations (100-500 µg/ml) was prepared. The mixture was then incubated at 37°C for 5 minutes and then 1 ml of 0.8% (w/v) casein was added. The combination was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to stop the reaction. The resulting suspension (cloudy) underwent centrifugation and the absorbance at 210 nm was read against buffer as blank. The experiment was duplicated thrice and the percentage inhibition of proteinase inhibitory activity was found.

Percentage of inhibition is equal to:

$(\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$ .

### Red blood cells (RBCs) suspension preparation

The blood collected from healthy human volunteers were taken. It was ascertained that the volunteers have not been exposed to any NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) knowingly or unknowingly for 2 weeks before the experiment and taken in the centrifuge tubes and centrifuged at 3000 rpm for about 10 minutes. It was then washed thrice with normal saline (equal volume) (11; 13). The volume of blood was noted and reconstituted into 10% volume to volume suspension using normal saline.

### Haemolysis induced by heat

The reaction mixture having a total volume of 2ml was constituted with 1 ml of test sample at varying concentrations (50-250 µg per ml) together with 10% RBCs suspension (1 ml). Saline was added to the control test tube instead of test sample. The standard drug used was Aspirin. Every centrifuging tube with reaction mixture was incubated in water bath at a temperature of 56 °C for atleast 30 minutes. Towards the end of the incubation the tubes were let to cool under tap water. Centrifugation of reaction mixture at 2500 rpm was carried out for 5 minutes. Absorbance of the supernatants at 560 nm was measured. The experiment was duplicated thrice for all the test samples. The percentage inhibition of haemolysis was estimated by using the following equation:

$\text{Percentage inhibition} = (\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$ .

### Haemolysis induced by hypotonicity

Varying concentrations of extract (100 -500 µg per ml), were separately mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension along with reference sample, and control. The standard drug used was Diclofenac sodium (100µg/ml). Incubation at 37°C for 30 minutes was done for all the assay mixtures and centrifuged at a speed of 3000 rpm. After decantation of the supernatant liquid, the estimation of haemoglobin content by a spectrophotometer was done at 560 nm. The percentage hemolysis was measured by assuming that 100 % is the haemolysis produced in the control.

“The percentage inhibition =  $100 - ((A_1 - A_2) / A_0) \times 100$ ”

Where,  $A_1$  is the absorbance of the sample,  $A_2$  is the absorbance of the product control and  $A_0$  is the absorbance of the positive control.” (Saranya & Prema, 2020)

## Results and Discussion

Plants synthesize essential oils which are concentrated volatile aromatic compounds. Essential oils easily evaporate and their essence is responsible for aroma. Essential oils are therapeutically valuable and used for their flavour and cosmetics. Essential oils of aromatic plants are rich sources of a myriad of antimicrobial and antifungal agents. Essential oils have surprising potential as anticancer agents.

### Antifungal potential

Plants based bioactive molecules are healthier and safer than synthetic counterparts. Numerous herbal medicines are used to treat or pacify various fungal diseases. It's important to understand essential oils better to incorporate them into fundamental research prospects, especially the anti-microbial activity. Essential oils are usually effective antimicrobials by themselves.

The antifungal potential of essential oil was determined quantitatively by monitoring presence and/or absence of inhibition zone and by measuring the inhibition zone. Clear inhibition zones were found after 24 hours of incubation at 37°C. Table 1(Fig1&2) shows the results of antifungal activity of the plant extracts. Essential oil extracted from the rhizome displayed prominent activity against both fungal strains and the highest activity against *Mucor indicus* (6.8±0.83) and showed high inhibition zone against *Aspergillus flavus* (6±0.7).

Antifungal therapy plays a significant role in health care sector. The screening of traditional medicinal plants to discover novel antifungals is now frequently done (Motsei *et al.*, 2003).

### Anti-inflammatory action

The phytomedicine holds a significant position in the inflammation treatment and related ailments. Many medicinal plants exhibit potent anti-inflammatory effect in the treatment of inflammation. Anti-inflammatory and wound healing activity of *Curcuma aromatica* extract and its formulation has been described by Kumar *et al.* The present work was designed to analyse the anti-inflammation studies on essential oil of *C. amada*.

### Inhibition of albumin denaturation

Mechanism of the anti-inflammatory activity in essential oils was studied.

The essential oils were effective in stopping albumin denaturation induced by heat. In *C. amada*, the highest inhibition was seen in the essential oil of rhizomes (80.9±0.87) at 500 µg per ml and the IC<sub>50</sub> value of essential oil is 296 µg. Standard drug used was acetyl salicylic acid which showed the 86.14±0.89 inhibition in 500 µg/ ml and IC<sub>50</sub> value was 182 µg (Table-2, Fig-3).

### Anti-proteinase action

High source of serine proteinase is localized in lysosome of neutrophils.

Leukocytes proteinase plays a key role in the tissue damage in the course of inflammatory reactions and a considerable extent of protection was offered by inhibitors of proteinase. Essential oils reflected significant activity as anti-proteinase in a dose influenced manner.

The highest inhibition was seen from essential oil 67.14±1.15 percentage and IC<sub>50</sub> value of essential oil is 345 µg (Table-3, Fig-4).

**Haemolysis induced by heat**

*C.amada* essential oil was operative in inhibition of the heat-induced hemolysis at different concentrations. The results displayed that concentrations of 100 and 500 µg/ml effectively protect the erythrocyte membrane from lysis due to heat. The percentage of inhibition by essential oil was at maximum (61.11±1.14) and IC<sub>50</sub> values of essential oil were 367µg/ml (Table-4, Fig-5).

**Haemolysis induced by hypotonicity**

It was revealed from the study that at a concentration range of 100-500 µg/ml, essential oil shows significant protection of the erythrocyte membrane against hypotonic solution induced lysis (Table 5.3.5.10, Fig 5.3.5.10). At a concentration of 500 (µg/ml), the test sample exhibited a maximum of (73.2±1.15) percentage of protection, IC<sub>50</sub> values of essential oil were 256 µg/ml (Table-5, Fig-6).

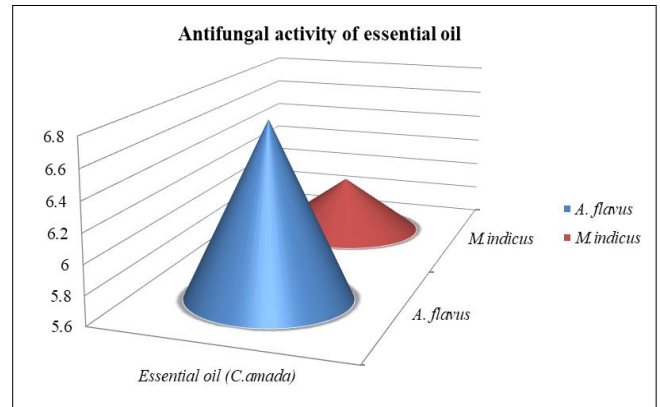
The method of action of the essential oil and anti-inflammatory drugs is by binding to the erythrocyte membranes with associated change in the surface charges of erythrocytes. Reportedly, certain saponins and flavanoids exerts stabilizing effect on lysosomal membrane in both *vivo* and *in-vitro* conditions while tannin and saponins has the ability to bind cations, thus stabilizing membranes of erythrocytes and certain bio-macromolecules.

Zerumbone is a monocyclic sesquiterpene obtained from the rhizome of *Zingiber zerumbet* which possess anti-inflammatory effects. Curcuminoids are one of the

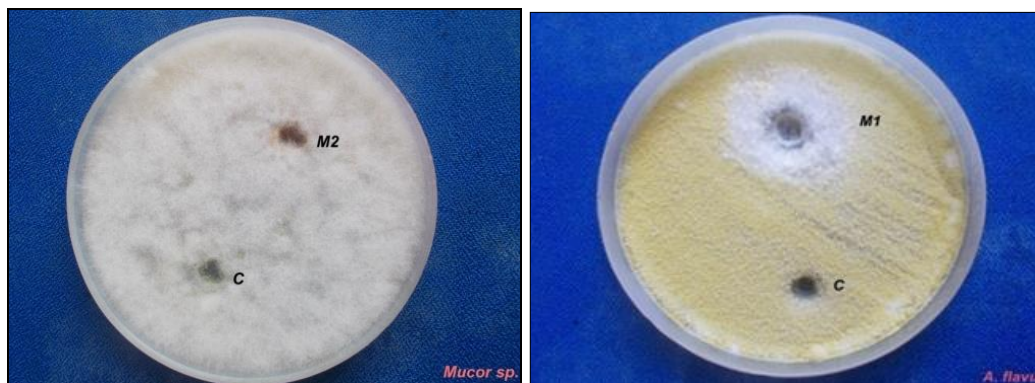
important active ingredients of many *Curcuma* species which has high anti-inflammatory activity. The anti-inflammatory effect of the ethanol extracts of ginger (*Zingiber officinale*) was studied by Anosike *et al.* Lu *et al.* reported anti-inflammatory activity and phytochemical composition of essential oil from *Hedychium coronarium*.

**Table 1:** Antifungal activity of essential oil

S.no	Extracts (Antifungal drug)	Inhibition zone (mm)	
		<i>A. flavus</i>	<i>M.indicus</i>
1	Essential oil ( <i>C. amada</i> )	6.8±0.83	6±0.7



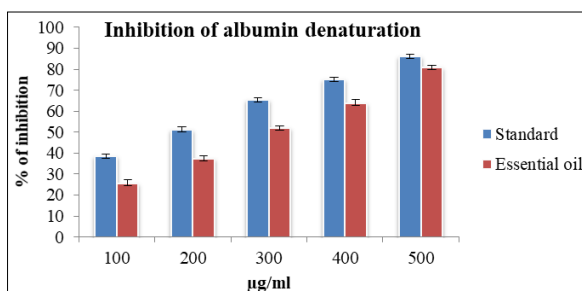
**Fig 1:** Antifungal activity of essential oil



**Fig 2:** Antifungal activity of essential oil against *A. flavus* and *M. indicus*

**Table 2:** Inhibition of albumin denaturation of *C. amada*

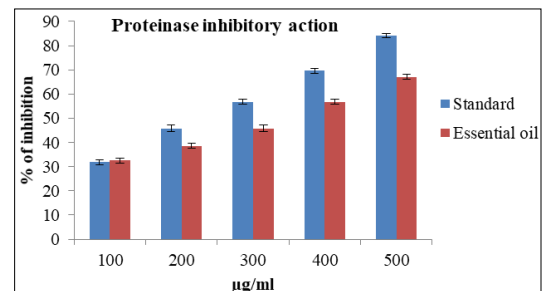
S.no	Concentration (µg/ml)	% of inhibition	
		Standard	Essential oil
1	100	38.6±1.15	25.47±1.8
2	200	51.2±1.52	37.16±1.6
3	300	65.3±1.17	51.8±1.15
4	400	75.14±0.97	63.74±1.67
5	500	86.14±0.89	80.9±0.87
IC <sub>50</sub> (µg/ml)		197	296



**Fig 3:** Inhibition of albumin denaturation of *C. amada*

**Table 3:** Proteinase inhibitory action of *C. amada*

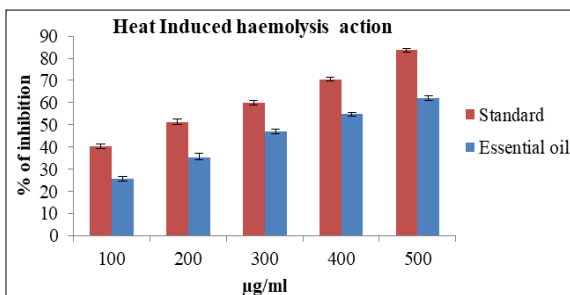
S.No	Concentration (µg/ml)	% of inhibition	
		Standard	Essential oil
1	100	31.8±1.15	32.6±0.81
2	200	45.6±1.52	38.54±1.15
3	300	56.8±1.17	45.6±1.54
4	400	69.7±0.97	56.7±1.10
5	500	84.14±0.89	67.14±1.15
IC <sub>50</sub> (µg/ml)		256	354



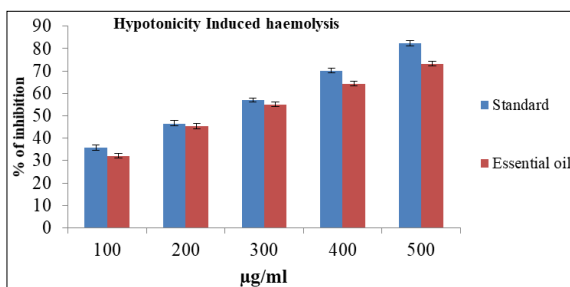
**Fig 4:** Proteinase inhibitory action of *C. amada*

**Table 4:** Heat Induced haemolysis action of *C. amada*

S.no	Concentration (µg/ml)	% of inhibition	
		Standard	Essential oil
1	100	40.12±1.15	25.8±1.02
2	200	51.23±1.52	35.4±1.7
3	300	59.8±1.17	46.9±1.15
4	400	70.6±0.97	54.7±0.87
5	500	83.67±0.89	62.12±0.91
IC <sub>50</sub> (µg/ml)		196	346

**Fig 5:** Heat Induced haemolysis action of *C. amada***Table 5:** Haemolysis induced by hypotonicity of *C. amada*

S.No	Concentration (microgram per ml)	% of inhibition	
		Standard	Essential oil
1	100	35.64±1.21	32.16±1.2
2	200	46.51±1.3	45.26±1.36
3	300	57.14±0.89	55.04±1.09
4	400	70.21±1.12	64.21±1.06
5	500	82.3±1.36	73.2±1.15
IC <sub>50</sub> (µg/ml)		252	254

**Fig 6:** Hypotonicity Induced haemolysis of *C. amada*

## Conclusion

The essential oil of *C. amada*, traditionally used in treatment of numerous diseases could be of use in arresting fungal infections and could be a potent source of new, safe and biodegradable antifungal drugs. Inflammation is a very complex process mediated via a wide variety of mechanisms.

Here, in this study, it is indicative from the results that essential oil of *C. amada* has potential against inflammations and also antifungal properties. This attribute is the result of notable presence of polyphenolics such as alkaloids, phenols flavonoids, tannin and steroids.

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