



## ***In vitro* Anti-inflammatory Properties in Various Extracts (Ethanol, Chloroform and Aqueous) of *Kaempferia galanga* Linn Rhizome**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Author HLA literature search, survey, data collection, analysis, manuscript writing. Author GS Study design, data verification, manuscript drafting. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Introduction:** *Kaempferia galanga* is a medicinal plant belonging to the family Zingiberaceae: ginger family. It is treated as a folk traditional herb. Anti Inflammatory property refers to the ability of a substance to reduce inflammation or any of its 5 cardinal signs.

**Aim:** To assess and compare in vitro the anti-inflammatory properties of various extracts (ethanol, chloroform and aqueous) of *Kaempferia galanga* L Rhizome.

**Materials and Methods:** Protein Denaturation Inhibition was carried out in vitro and statistical analysis was done using ONE WAY -ANOVA and Duncan Multiple Range Tests. The test was done in triplicates.

**Results:** Chloroform extract of *Kaempferia galanga* rhizome has the best anti-inflammatory potential followed by Ethanol and Aqueous extracts of the rhizome.

**Conclusion:** With further in vivo and clinical research, the chloroform, ethanolic and aqueous extracts of *Kaempferia galanga* can be recommended as a novel, innovative and potent anti-inflammatory drug in the market as it's natural and doesn't have side effects.

**Keywords:** Innovative; anti-Inflammatory; flavonoids; *Kaempferia galanga*; rhizomes.

## 1. INTRODUCTION

The response of the immune system to pathogens, damaged cells or toxic compounds is called Inflammation. (1) and acts by removing injurious stimuli and initiating the healing process (2). The inflammatory response is the synchronized activation of signalling paths which regulate inflammatory mediator levels in resident tissue cells and inflammatory cells recruited from blood (3) Anti-inflammatory property refers to the ability of a substance to reduce inflammation or any of its 5 cardinal signs (4).

*Kaempferia galanga* is a medicinal plant of the family Zingiberaceae used as a folk herb in medicine. It is commonly known as kencur, sand ginger, aromatic ginger, or cutcherry. They have a sharp and bitter taste with a strong smell. The plants are available in Kerala, Andhra Pradesh and other few regions in India. The plant shows vegetative propagation and regeneration through rhizomes(5)(6). The rhizome has been extensively grown in Southeast Asia(7). In Indonesia it has been used in the preparation of a herbal drink Jamu (8)(9). In India it has been used increasingly in the treatment of cough, cold and other ailments.(10)In China it has been used in Traditional Chinese Medicine since time immemorial(11,12).

*Kaempferia galanga* has compounds like flavonoids-kaempferol, kaempferide, camphene, triterpenoids-borneol, cineol(eucalyptol), diarylheptanoids, polyphenols, camphene and other compounds which can be discovered by gas chromatography mass spectrometry (13).

Through years of ingenious syntheses, many non-steroidal anti-inflammatory agents (NSAIDS) have been prepared and marketed .However, These drugs are known to provoke adverse effects such as gastrointestinal irritations.(14)Their prolonged use often leads to gastric intolerance, bone marrow depression, water and salt retention(15) .Hence, the search for alternative anti-inflammatory drugs mainly from natural herbs is required as they don't have side effects are cost-effective and are easily accessible (16).

Previously, in-vivo studies on mice have proven the anti-inflammatory potentials of ethanol, chloroform and aqueous extracts of *Kaempferia galanga* Linn. (17) rhizome, but this is the first time that the anti-inflammatory potentials of ethanol, chloroform and aqueous extracts of *Kaempferia galanga* Linn. rhizomes have been compared to each other as well as the standard drug aspirin. (18)The objective of the study is to determine and compare the anti-inflammatory potentials of ethanol, chloroform and aqueous extracts of *Kaempferia galanga* Linn. rhizome. Our team has extensive knowledge and research experience that has translate into high quality publications[1–5] [6–10].

Different solvents were used because each solvent could be capable of extracting the bioactive components in the plant.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

All reagents and chemicals were procured from Invitrogen USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; Sigma Chemical Company St., USA; New England Biolabs (NEB), USA; Promega, USA.

### 2.2 Inhibition of Albumin Denaturation

The plant extract's anti-inflammatory activity was explained by inhibition of albumin denaturation technique which was examined by ways of Mizushima and Kobayash, 1968 and Sakat et al (2010) followed with minor modifications. The reaction mixture comprised of 1% aqueous solution of bovine serum albumin and the test extracts, pH of the reaction mixture was adjusted with a small amount of 1N HCl to dissolve the mixture. The plant extract with increase in concentration (100 to 500 µg/ml) were incubated at 37 °C for 20 minutes and later heated to 51 °C for 20 min, after the samples were let to cool down, The turbidity had been measured at 660nm.(UV Visible Spectrophotometer Model 371, Elico India Ltd) The experiment was

performed in triplicate. In this study, Aspirin was used as a standard anti-inflammatory drug.

### 2.3 Calculation

$$\% \text{ Inhibition} = 100 - ((A1 - A2) / A0) * 100$$

### 2.4 Statistical Analysis

The data were analysed statistically using one way analysis of variance (ONE-WAY ANOVA). Duncan Multiple range test was used to analyze the statistical significance between groups. The levels of significance were considered at the levels of  $p < 0.05$ .

## 3. RESULTS

The Protein Denaturation Inhibition by the chloroform extract of *Kaempferia galanga*

rhizome happens in a dose dependent manner ranging from 30% to 85% in a dose dependent manner as the concentration of the chloroform extract of *Kaempferia galanga* rhizome increases from 100 ug/ml to 500ug/ml. The Protein Denaturation Inhibition by the Ethanolic extract of *Kaempferia galanga* rhizome happens in a dose dependent manner ranging from 25% to 81% in a dose dependent manner as the concentration of the Ethanolic extract of *Kaempferia galanga* rhizome increases from 100 ug/ml to 500ug/ml. The Protein Denaturation Inhibition by the Aqueous extract of *Kaempferia galanga* rhizome happens in a dose dependent manner ranging from 20% to 65% in a dose dependent manner as the concentration of the Aqueous extract of *Kaempferia galanga* rhizome increases from 100 ug/ml to 500ug/ml.

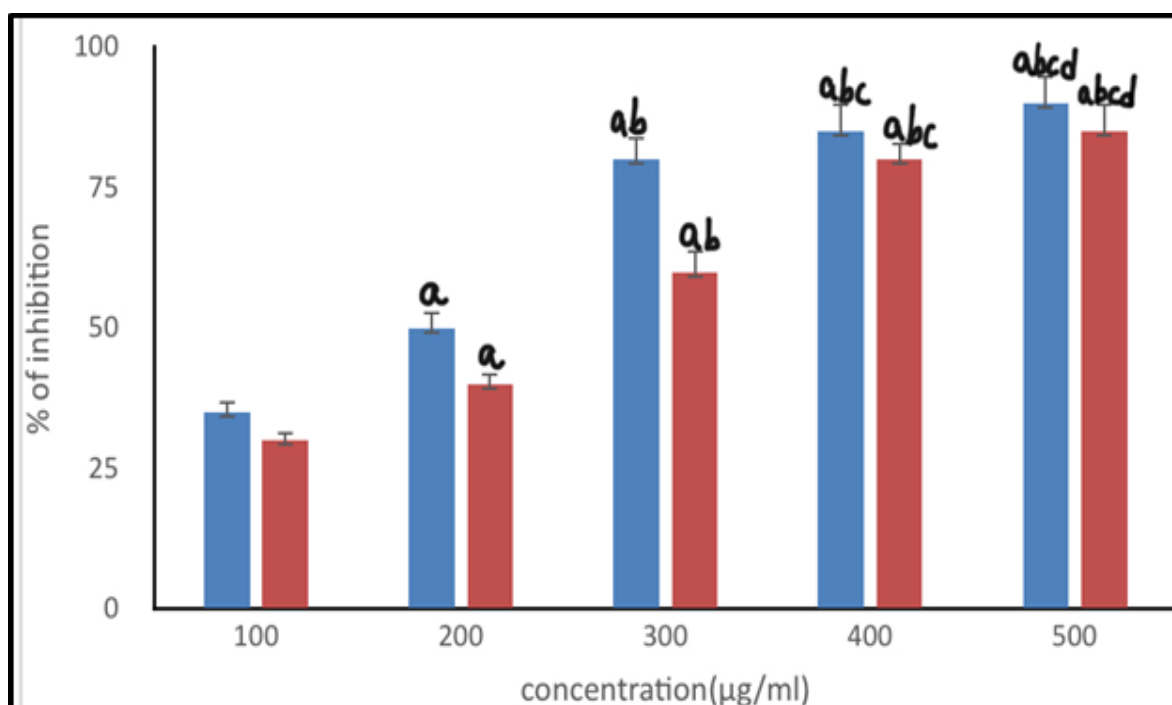


Fig. 1. Each bar represents the mean  $\pm$  SD of 6 observations. Significance is observed at the levels of  $p < 0.05$ . a-compared with 100µg; b-compared with 200µg; c-compared with 300µg and d-compared with 400µg. The X axis represents the concentrations of Chloroform extract of *Kaempferia galanga* rhizome that increases from 100ug/ml to 500ug/ml. The Y axis represents the percentage of protein denaturation inhibition ranging from 0-100%. The blue bar represents the percentage inhibition of protein denaturation by the standard drug-aspirin. The red bar represents the percentage inhibition of protein denaturation by the chloroform extract of *Kaempferia galanga* rhizome

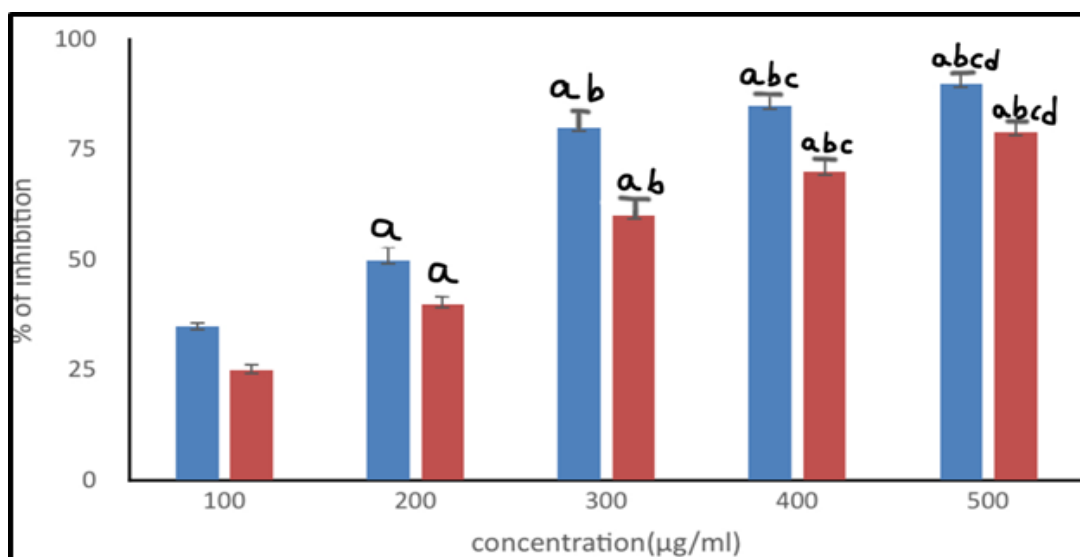


Fig. 2. Each bar represents the mean  $\pm$  SD of 6 observations. Significance is observed at the levels of  $p < 0.05$ . a-compared with 100µg; b-compared with 200µg; c-compared with 300µg and d-compared with 400µg. The X axis represents the concentrations of the Ethanolic extract of *Kaempferia galanga* rhizome that increases from 100ug/ml to 500ug/ml. The Y axis represents the percentage of protein denaturation inhibition ranging from 0-100%. The blue bar represents the percentage inhibition of protein denaturation by the standard drug-aspirin. The red bar represents the percentage inhibition of protein denaturation by the Ethanolic extract of *Kaempferia galanga* rhizome

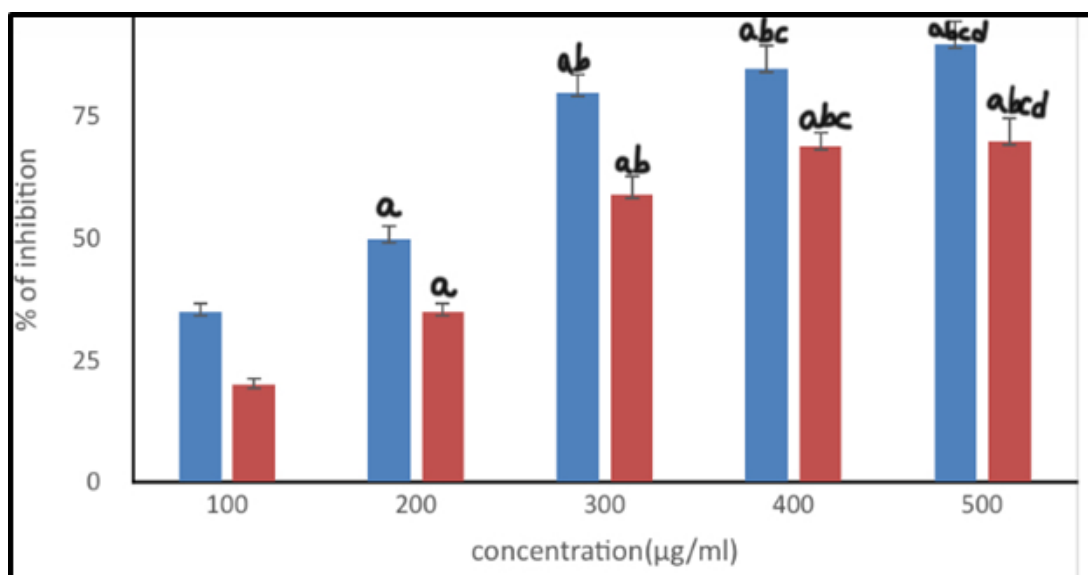


Fig. 3. Each bar represents the mean  $\pm$  SD of 6 observations. Significance is observed at the levels of  $p < 0.05$ . a-compared with 100µg; b-compared with 200µg; c-compared with 300µg and d-compared with 400µg. X axis represents the concentrations of the Aqueous extract of *Kaempferia galanga* rhizome that increases from 100ug/ml to 500ug/ml. Y axis represents the percentage of protein denaturation inhibition ranging from 0-100%. The blue bar represents the percentage inhibition of protein denaturation by the standard drug-aspirin. The red bar represents the percentage inhibition of protein denaturation by the Aqueous extract of *Kaempferia galanga* rhizome

#### 4. DISCUSSION

The results have shown that the protein denaturation inhibition of chloroform, ethanol and aqueous extracts of *Kaempferia galanga* occurred in a dose dependent manner with concentrations ranging from 100-500 µg/ml. It is also observed that the chloroform extract of *Kaempferia galanga* has the maximum anti-inflammatory potential followed by the ethanolic extract and then aqueous extract of *Kaempferia galanga*. In a previous research, it has been observed that on the subcutaneous implantation of cotton pellets in female albino rats, granuloma was induced in them (19). This increased the aggregation of macrophages and the release of histamine, serotonin and bradykinin. Once the chloroform extract was injected cytokine inhibition took place and hence granulomatous inflammation was treated (20) Similarly, in the present study we have found that Chloroform extract of kencur exhibits an excellent anti-inflammatory potential.

Based on a previous research, kencur (*Kaempferia galanga* L.) rhizome ethanol extract can be made as anti-inflammatory plaster (21). A dose of 45 mg/Kg BW rat kencur (*Kaempferia galanga* L.) rhizome ethanol extract provides the most excellent anti-inflammatory effect (22). Carrageenan was injected into male albino rats which induced paw edema in them. This increased the levels of PGE2 in them.(23) When the ethanol extract of kencur rhizome was used in the form of plaster at increasing doses from 18 mg/Kg BW to 45 mg/Kg BW ,after 30 minutes of usage of 45 mg/Kg BW dose a significant decrease in measurement of volume of rat foot was seen from 63ul to 60ul.(24) (25)In another study ,Albino rats were administered with cotton pellets and granuloma was induced (26).On the injection of the ethanol extract of kencur ,granulomatous inflammation showed a marked decrease (27).(28)Similarly in the present study we have found that ethanolic extract of kencur exhibits a very good anti-inflammatory potential.(29,30)

Previous reports reported that colitis was induced in male wistar rats when they were given water with DSS (31). This increased the inflammatory mediators (32).When the aqueous extract of (33) *kaempferia galanga* linn. was injected into these

rats, selective inhibition of COX-2 pathway took place and stepwise colitis healed (34) Similarly in

the present study we have found that aqueous extract of kencur exhibits a good anti-inflammatory potential (35). In a study ((36), where forty male Sprague-Dawley rats were divided into 5 groups, namely: normal group which consumed normal diet, negative control group which consumed high cholesterol diet without treatment, crude oil group, fraction 2 group and EPMC group which consumed high cholesterol diet with sniffing kencur (*Kaempferia Galanga*) crude oil, fraction 2 and EMPC, respectively (37). In the results it was found that the, cholesterol, HDL and triglycerides levels in blood of rats that sniffed oils for 5 weeks were significantly different for every single of the 5 groups( $P < 0.05$ ). Kencur crude essential oil and 3-(4-methoxyphenyl)-ethyl ester (EPMC) decreased the cholesterol and triglyceride level in rats' blood while the fraction2 group which comprised δ-3-carene had a slimming effect (38). Limitations-Since they are all natural products, They might not be found everywhere and may get damaged during culture as well. Future scope-All these three drugs may be recommended for the treatment of inflammation-mediated development of diabetes and associated complications. However, among three extracts analyzed chloroform extract of *kaempferia galanga* can be prioritized as it proved to possess better efficacy.

#### 5. CONCLUSION

Chloroform extract of *Kaempferia galanga* has the best anti-inflammatory potential followed by Ethanolic and then Aqueous extract of *Kaempferia galanga*. After further *in vitro*, *in vivo* studies and clinical trials, chloroform, ethanol and aqueous extracts of *Kaempferia galanga* can be recommended as potential anti-inflammatory drugs in the market.

#### DISCLAIMER

The company name used for this research is commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use this company as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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