

## Anti-inflammatory activity of *Murraya koenigii* – An *in vitro* study

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### ABSTRACT

**Background:** In the ancient time, many naturally occurring plants played an important role in discovery in many therapeutic agents. It has been shown to have beneficial role such as therapeutic uses include beneficial effects on the cardiovascular system, antibiotic, anticancer, anti-inflammatory, and hypoglycemic effects. **Objective:** The study was designed to evaluate the anti-inflammatory effect of *Murraya koenigii* leaf ethanolic extract by *in vitro* analysis. **Materials and Methods:** The plant leaf powder was loaded into Soxhlet extractor and subjected to extraction with ethanol. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator. **Results:** Protein denaturation is a process, in which proteins lose their tertiary structure and secondary structure by the application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat-induced albumin denaturation. Maximum inhibition of 88% was observed at 500 µg/ml. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition of 99% at the concentration of 100 µg/ml compared with control. **Conclusions:** Results indicate that the ethanol extracts of *M. koenigii* possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols. The extract fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat-induced albumin denaturation.

**KEY WORDS:** Anti-inflammatory activity, *Ethanol extract*, *In vitro analysis*, *Murraya koenigii*

### INTRODUCTION

*Murraya koenigii* is known as “Curry Patta” in Hindi and widely used as a spice and condiment in the Indian subcontinent and other tropical countries.<sup>[1]</sup> It is a highly valued plant for its characteristic aroma and medicinal value. It belongs to the family Rutaceae.<sup>[2]</sup> Conventionally, the plant is used as a stimulant, stomachic, febrifuge, analgesic, and for the treatment of diarrhea, dysentery, and insect bites and also used to allay heat of body.<sup>[3]</sup> Plants have been the major source of drugs in Indian system of medicine and other ancient systems in the world. Most of the drugs used at present for anti-inflammatory and analgesic effects are synthetic in nature and prolonged use of which cause

severe side effects and exhibit toxic effects.<sup>[4]</sup> In this regard, a novel possibility of evaluating herbs in pain therapy arises. Inflammation is a physiologic series of responses generated by the host in response to infection or other insults. Inflammation can have rapid onset and last a short period of time (acute inflammation) or it can persist due to a continuous stimulus or injury (chronic inflammation).<sup>[5,6]</sup> The signs of inflammation are the result of vasodilatation and increased vascular permeability, leading to exudation of fluid and plasma proteins and recruitment of leukocytes to the site of injury. In this study, we evaluated the anti-inflammatory and analgesic activity of the aqueous extract of the leaves of *M. koenigii*.

### MATERIALS AND METHODS

#### Plant Material

The whole plants of *M. koenigii* collected in fresh condition from Chennai region, Tamil Nadu. The

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voucher specimens of the plants were authenticated by the Department of Plant Biology and Plant Biotechnology, Presidency College, Chennai, Tamil Nadu, India. The plant was dried under shade then ground into a uniform powder using a blender and stored in polythene bags at room temperature.

### Preparation of Extracts

The plant leaf powder was loaded into Soxhlet extractor and subjected to extraction with ethanol. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator.

### Assessment of *in vitro* Anti-inflammatory Activity

#### Inhibition of albumin denaturation

The anti-inflammatory activity of *M. koenigii* was studied using inhibition of albumin denaturation technique which was studied according to the method of Leelaprakash and Dass (2010). The reaction mixture was consisted of test extracts and 1% aqueous

solution of bovine albumin fraction; pH of the reaction mixture was adjusted using small amount of 1 N HCl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min, after cooling the samples, the turbidity was measured at 660 nm (Ultraviolet-visible Spectrophotometer Model 371, Elico India Ltd.). The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as follows: Percentage inhibition = (Abs Control–Abs Sample)×100/Abs control.

### Statistical Analysis

Results are expressed as mean±SD. The difference between experimental groups was compared by one-way analysis of variance followed by Dunnett's multiple comparison test (control vs. test) using the software GraphPad InStat.

## RESULTS

Effect of *M. koenigii* leaf ethanolic extract on heat-induced protein denaturation. Protein denaturation is a process, in which proteins lose their tertiary structure and secondary structure by the application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat-induced albumin denaturation. Maximum inhibition of 88% was observed at 500 µg/ml. Aspirin, a standard anti-inflammation drug showed the maximum inhibition of 99% at the concentration of 100 µg/ml compared with control [Table 1, Figures 1 and 2].

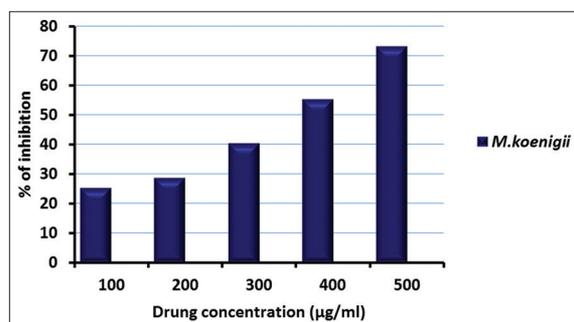


Figure 1: Inhibition of protein denaturation

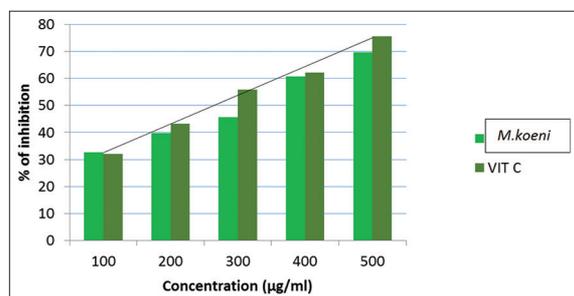


Figure 2: Nitric oxide radical scavenging activity of *Murraya koenigii* seed ethanolic extract

Table 1: Efficacy of anti-inflammatory activity of *M. koenigii*

Treatment (s) concentration	Concentration (µg/ml)	Absorbance at 660 nm	% inhibition of protein denaturation
Control	-	0.923	
<i>M. koenigii</i> extract	100	0.326	34.39
<i>M. koenigii</i> extract	200	0.357	37.75
<i>M. koenigii</i> extract	300	0.413	43.82
<i>M. koenigii</i> extract	400	0.645	68.95
<i>M. koenigii</i> extract	500	0.824	88.35
Aspirin (standard drug)	100	0.927	99.51

*M. koenigii*: *Murraya koenigii*

an unwritten science, is well established in some countries and traditions and has become a way of life in almost 80% of population in rural areas. Chronic anti-inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population.<sup>[10,11]</sup> At present, although synthetic drugs are dominating the market, element of toxicity that these drugs entail cannot be ruled out. Their prolonged use may cause severe adverse effects on chronic administration,<sup>[12]</sup> the most common being gastrointestinal bleeding and peptic ulcers.<sup>[13]</sup> Consequently, there is a need to develop a new anti-inflammatory agent with minimum side effects. Search for safe and effective anti-inflammatory agents has been given priority in scientific research in herbal system of medicine. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat-induced albumin denaturation. Maximum inhibition of 88% was observed at 500 µg/ml. Aspirin, a standard anti-inflammation drug showed the maximum inhibition of 99% at the concentration of 100 µg/ml compared with control. It is concluded that the methanol extracts of *M. koenigii* possess anti-inflammatory properties.

## CONCLUSIONS

In the present study, results indicate that the methanol extracts of *M. koenigii* possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols. The extract fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat-induced albumin denaturation.

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