Nat. Volatiles & Essent. Oils, 2021; 8(5): 6387 - 6397

A Comparative Study On The Anti-Inflammatory Activities Of Ethanolic Leaf Extracts On Mentha Piperita (Peppermint) And Murraya Koenigii (Curry Leaves)

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ABSTRACT

Background:
Peppermint (*Mentha piperita*) is a plant genus which is placed in the Lamiaceae family. As a strewing herb, it is scattered around the house as a deodorizer. Curry leaves (*Murraya koenigii*) are very popular leaf spices which are used very often in very small quantities for their distinct aroma because of the presence of volatile oil & Its ability to improve digestion. Inflammation refers to the body's process of fighting against things that harm, such as infections, injuries, and toxins, in an attempt to heal itself. The aim of this study is to analyse the antioxidant and anti-inflammatory activities of these leaf extracts.
Methods:
Ethanolic leaf extracts of *Mentha piperita* and *Murraya koenigii* were analysed for its antioxidant and anti-inflammatory potential. The plants were freshly collected from the garden and the essence was extracted by using mortar and pestle. The data were analyzed statistically by a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to see the statistical significance among the groups. The results with $p<0.05$ level were considered to be statistically significant.
Results:
The Phytochemical screening of the leaf extract of *Mentha piperita* has shown a presence of flavonoids, alkaloids, terpenoids, saponins, and steroids and Phytochemical screening of the leaf extract of *Murraya koenigii* has shown a strong presence of proteins, flavonoids, alkaloids, terpenoids (++), saponins, and steroids. The DPPH radical scavenging activity showed that standard drug (vitamin C) had more inhibition at all the levels of concentration than the two plant extracts, for example at 500 ug/ml the inhibition percent for standard drug (Vitamin C) is 78% for *Mentha piperita* it is 59% and for *Murraya koenigii* it is 67% can be seen.
Conclusion:
From the study it was evident that both the leaf extracts exhibited anti-inflammatory potential. The Comparative anti-inflammatory potential of *Murraya koenigii* extract was significantly more than *Mentha piperita* extract.
Key words: *Mentha piperita, Murraya koenigii*, anti-inflammatory activity, ethanolic leaf extract, antioxidant, innovative technology, novel method, therapeutic efficacy.

INTRODUCTION:

Inflammation is the process of a body fighting against the things that harms it, such as infections, toxins and injuries, in an attempt to heal by itself. When toxins damage cells, the body releases chemicals that trigger a response from the immune system (1). The anti-inflammatory drugs make up about half of analgesics, the remedying pain by the inflammatory reduction as opposed to opioids. (2) Diclofenac is the standard drug used against inflammation and it is also reported to have some side effects (3). Peppermint [*Mentha piperita*] is a plant genus which is placed in the Lamiaceae family. In the home, mint has long been used as a food constituent (4). As a strewing herb, it is scattered around the house as a deodorizer. Today, the commonly used things by it are the sachets and potpourris. Small amounts of dried mint oil is added to soaps, while peppermint oil is sometimes used in aromatherapy to improve alertness (5).

Curry leaves [*Murraya koenigii*] are the popular leaf Spices which are used very often in small quantities for their distinct aroma because of the content which shows the presence of the volatile oil & Its ability to improve digestion. They have a slightly pungent smell, bitter or feebly acidic taste (6). They also contain proteins, carbohydrates, fibre, minerals, carotene, nicotinic acid, calcium and oxalic acid (7). Both the plants are used as a stimulant, stomachic, febrifuge, analgesic and for the treatment of diarrhoea, dysentery; insect bites and also used to allay the heat of the body (7,8). The antioxidant potential in turn depends upon the total polyphenolic compounds, essential oils and other compounds (9). In curry leaves, the carbazole alkaloids that are recently isolated are of the mahanimbine and koenigii, which showed higher antioxidant activities. The acrylic hydroxyl group of alkaloids in curry leaves showed higher antioxidant potential (10).

Herbs are considered as a very good source of natural antioxidants, but very limited work has been reported for the utilisation of herbs as a possible antioxidant (11,12). Most of the research works have been done on these leaves except on their anti-inflammatory activities (11–13). Therefore this work is intended to extract the essence of the medicinal values of *Mentha piperita* and *Murraya koenigii*, using the ethanol as a solvent for a portion. (14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24),(25),(26),(27),(28),(29),(30),(31),(32),(33). The aim of this study is to analyse the antioxidant and anti-inflammatory activities of these leaf extracts (11–13,34).

MATERIALS AND METHOD:

The plants were freshly collected from the garden and the essence was extracted by using mortar and pestle.

Phytochemical screening test

Test for phlobatannin

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red colour precipitate indicates the presence of phlobatannin (35).

Test for carbohydrates

3-5 drops of Molisch reagent was added to 1 mL of the extract, after 1 minute 1 mL of concentrated sulphuric acid was added carefully along the sides of the test tube. The mixture was allowed to stand for two minutes and then it was diluted with 5 mL of distilled water. The development of a dull violet or red ring at the junction of the liquids marked the presence of carbohydrates(36).

Test for flavonoids

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids(37).

Test for alkaloids

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and a further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids(38).

Test for terpenoids

2 ml of sample along with 2ml of chloroform and 3ml of con. H_2SO_4 was added. Red color ppt obtained indicates the presence of terpenoids(38,39).

Test for proteins

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The presence of proteins resulted in the formation of purple colour(40).

Detection of saponins

Foam test: 1 ml sample of the extract was vigorously shaken with water and persistent foam was observed.

Test for steroids

One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicated the presence of steroids (41).

DPPH free radical scavenging activity of ethanolic leaf extracts of *Mentha piperita* and *Murraya koenigii*

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical was assessed by the method of Hatano et al, (1989). Briefly, DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0. 5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

$$\text{DPPH radical scavenged (\%)} = \frac{\text{Control OD-Sample OD X100}}{\text{Control OD}}$$

***In vitro* anti-inflammatory activity of ethanolic leaf extracts of *Mentha piperita and Murraya koenigii* by albumin denaturation inhibition assay**

The anti-inflammatory activity was studied by using inhibition of albumin denaturation analysis which was studied according to the method of Leelaprakash and Mohan Dass (2010). Briefly, the reaction mixture containing test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using a small amount of 1N HCl. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51°C for 20 min, after cooling the samples the turbidity was measured at 660nm (UV 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) X 100/ Abs control

STATISTICAL ANALYSIS

The data were subjected to statistical analysis using one – way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the groups. In Duncan's test, significance was considered at the level of $p<0.05$.

RESULTS:

Table 1: Phytochemical screening of *Mentha piperita* and *Murraya koenigii*

Phytochemical	***Mentha piperita***	***Murraya koenigii***
proteins	-	+
amino acids	-	-
flavonoids	+	+
alkaloids	+	+
terpenoids	+	++
saponins	+	+
steroids	+	+

The Phytochemical screening of the leaf extract of *Mentha piperita* has shown a presence of flavonoids, alkaloids, terpenoids, saponins, and steroids and Phytochemical screening of the leaf extract of *Murraya koenigii* has shown a strong presence of proteins, flavonoids, alkaloids, terpenoids (++), saponins, and steroids [shown in the above table chart]. The DPPH radical scavenging activity showed that standard drug (vitamin C) had more inhibition at all the levels of concentration than the two plant extracts, for example at 500 ug/ml the inhibition percent for standard drug (Vitamin C) is 78% for *Mentha piperita* it is 59% and for *Murraya koenigii* it is 67% can be seen [Figure 1]. In albumin denaturation test the same results are observed as seen in the DPPH radical scavenging activity test standard drug (Diclofinac) has shown more percentage of inhibition than both the leaf extracts, For example at 200 ug/ml concentration the inhibition shown by standard drug (Diclofinac) is 43%, for *Mentha piperita* it is 16% and for *Murraya koenigii* it is 30% [Figure 2].

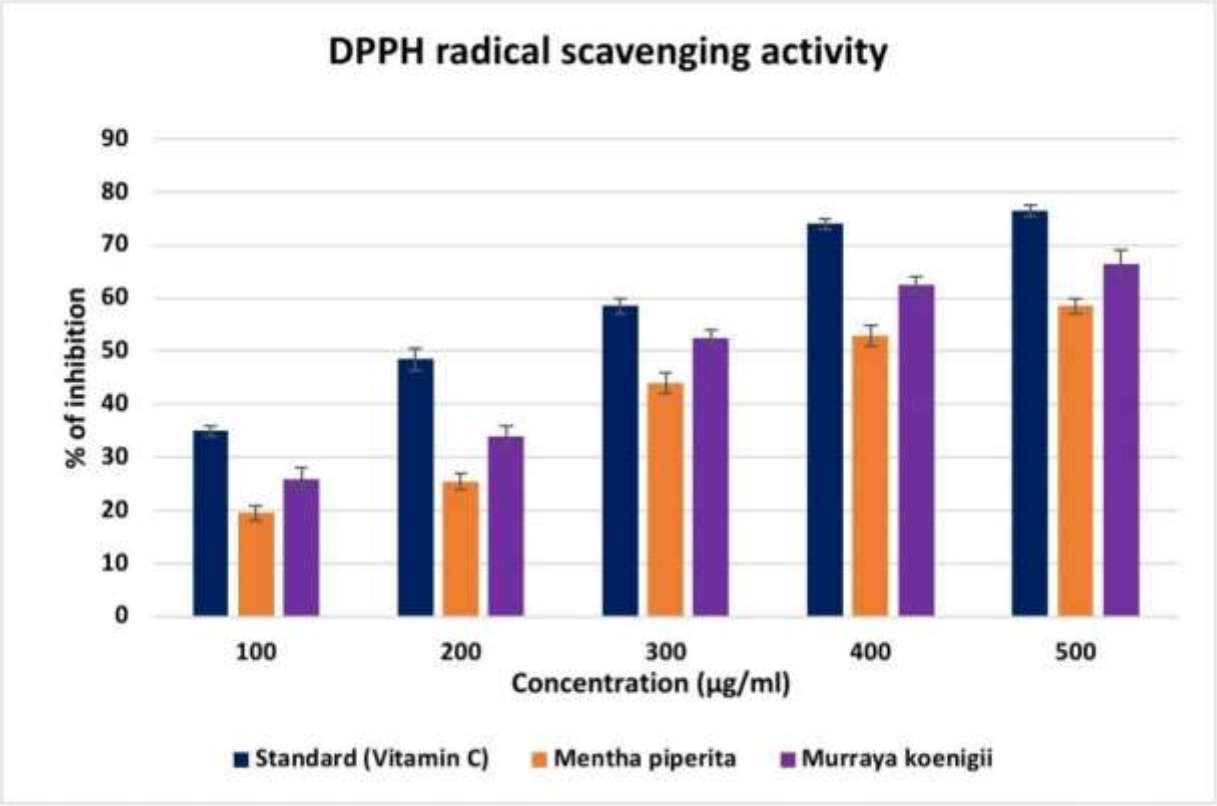

Figure 1: DPPH radical scavenging activity of *Mentha piperita and Murraya koenigii*. Each bar represents Mean ± SEM of 3 independent observations. Significance was considered at the levels of $p < 0.05$. X-axis depicts concentration of plant extract (0.1 to 0.5mg/ml). The Y-axis depicts % of inhibition. Vitamin C was used as a standard drug.

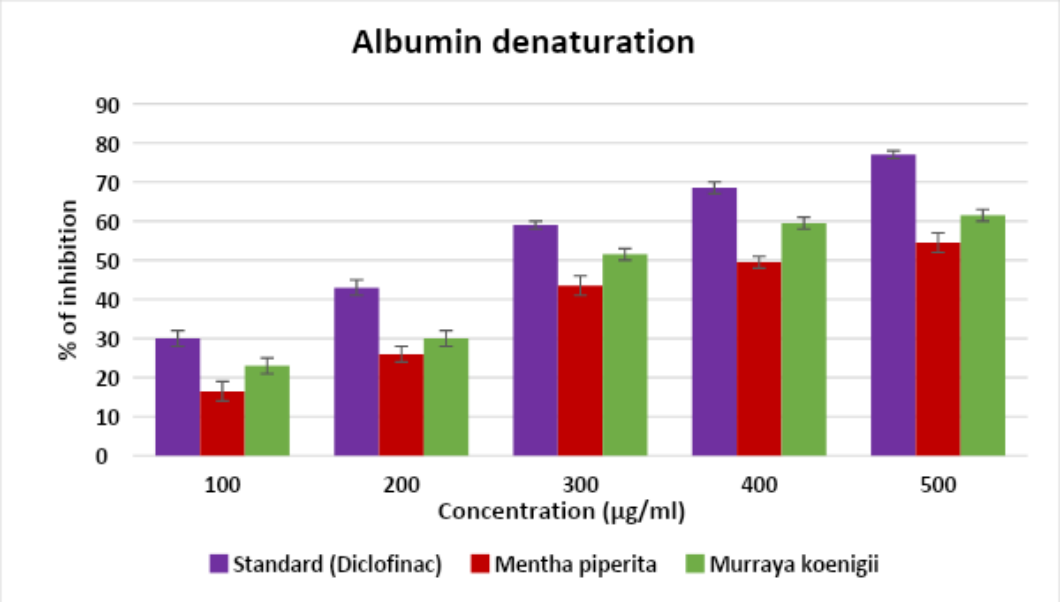

Figure 2: Anti-inflammatory activity of *Mentha piperita and Murraya koenigii*. Each bar represents Mean ± SEM of 3 independent observations. Significance was considered at the levels of $p < 0.05$. X-axis depicts concentration of plant extract (0.1 to 0.5mg/ml). The Y-axis depicts % of inhibition. Diclofinac was used as a standard drug.

DISCUSSION:

Phytochemical screening of both the leaf extracts (*Mentha piperita* and *Murraya koenigii*) showed a strong presence of flavonoids, alkaloids, terpenoids, saponins, and steroids (Table 1). From the previous studies it is proved that the secondary metabolites and the other chemical constituents of the medicinal plants accounts for their medicinal values (14). For instance, saponins are the glycosides of both triterpenes and steroids which exhibit hypotensive and cardiac depressant properties. Hence, the results indicate that the presence of these phytochemicals in the extracts, thus these extracts might contribute to various beneficial activities. The antioxidant activity of the plant extracts was determined by different in vitro methods such as the DPPH free radical scavenging assay and reducing power methods. The results are in accordance with the other study which described methanol extracts of leaves (400 mg/kg) of *Murraya koenigii* as potent anti- inflammatory agents in carrageenan induced inflammation in albino rats which is similar to this present study. Further studies are needed to isolate and identify some active compounds which might be responsible for anti-inflammatory activity (15).

The antibacterial activity of the leaf extract of *M. koenigii* as recorded in previous study may therefore be attributed to the presence of above phytochemicals i.e. Alkaloids, Carbohydrates, Cardiac glycosides, Phenol, Phylobatannins, Tannins, Terpenoids, in Ethanol extracts and Alkaloids, Carbohydrate, Cardiac glycosides, in Methanol extracts and Alkaloids, Carbohydrate, Phenols, Terpinoids, Tannins, Quinons in Aqueous extracts (16). The previous study on aqueous extracts have shown the higher solubility for more

phytoconstituents, and hence consequently showed highest antibacterial activity (17). It indicates that leaves of *M. koenigii* may possess compounds with antimicrobial properties. These results of previous articles have also shown the antioxidant and inflammatory activity of ethanol extracts which are similar to the present study with *Mentha piperita* and *Murraya koenigii* (18,19). The highest zone of inhibitions were recorded in the case of Curry leaves (*M. koenigii*) with solvent methanol and Distilled water against *Staphylococcus aureus* 10 mm and 8 mm also with solvent DMSO against *Lactobacillus* sp. 07 mm. The Curry leaves have shown the maximum zone of inhibition with solvent methanol and Distilled water against *Staphylococcus aureus* 10 mm and 08 mm. The minimum concentration of the extract observed as MIC. This result of the previous study supports the previous claim of (42). In case of *S. typhi* showed no sensitivity for all tested with plant extracts (20,21,).

Ethanolic extracts of *Mentha piperita* and *Murraya koenigii* have shown a strong *in vitro* antioxidant activity and increased in a concentration dependent manner. Vitamin-c is used as the standard drug for assessing antioxidant activity. *Murraya koenigii* extracts exhibited significantly more antioxidant activity (IC_{50}= 280µg/ml) (Graph 1) than *Mentha piperita* extract in all the concentrations. *Murraya koenigii* extract possessed significantly more anti-inflammatory activity (IC_{50}= 300µg/ml) (Graph 2) than *Mentha piperita* extract and increased in a dose dependent manner as compared to the standard drug "diclofenac". Synthetic drugs used in the treatment of inflammation exhibit more side effects such as headache, dizziness, stomach ache, wind or loss of appetite, feeling sick (nausea), vomiting, diarrhoea and mild rash (43). Hence, there is always a need for a replacement of these synthetic drugs with herbal extracts which are natural in origin and have less side effects. Limitations of this study were low case study on anti-inflammatory activities on ethanolic leaf extracts, low budget, lesser time limit. Future studies with large sample sizes should be conducted for more reliable results and to make the context evident and to produce an alternative standard drug with purely natural products.

CONCLUSION:

From the study it was evident that both the ethanolic extracts possessed antioxidant activity and anti-inflammatory activity. The anti-inflammatory activity of *Murraya koenigii* extract was significantly more than *Mentha piperita* extract, which was analysed by studying the inhibition of albumin denaturation. . Further studies are needed to validate these herbal extracts for their medicinal properties.

ACKNOWLEDGEMENT

The authors would like to thank Saveetha Dental College and Hospitals, Saveetha Institute of medical and technical Sciences,Saveetha University for providing research laboratory facilities to carry out the study.

SOURCE OF FUNDING

The present study was supported by the following agencies.

- Saveetha Institute of Medical and Technical Sciences (SIMATS)
- Saveetha Dental College
- Saveetha University
- Keshav Enterprises, Kanchipuram.

STATEMENT OF CONFLICT OF INTEREST

The author declares that there is no conflict of interest in the present study.

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