



Study of Synergistic Anti-inflammatory Action of *Murraya Koenigii* and *Mangifera Indica* Leaf Extracts

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ABSTRACT

The objective of the present study was to assess the anti-inflammatory potential of combined extracts of *Murraya koenigii* and *Mangifera indica* causing the carrageenan induced rat paw edema method. The extraction yield in methanol was found to be 26.8 % for *Murraya koenigii* and 31.6 % for *Mangifera indica*. The findings of preliminary phytochemical analysis suggest the presence of alkaloids, saponin glycosides, phenolics, terpenoids, and flavonoids in the leaf of the *Murraya koenigii* while alkaloids and glycosides were not found to be present in *Mangifera indica*. The total phenolic content of the hydroalcoholic extracts of *M. koenigii* and *M. indica* were 34.27 ± 1.7 and 43.11 ± 2.1 GAE mg/g, respectively. The phenolic content was highest in the combined extract (MSE:MIE, 1:2) of all the three combinations with total phenolics 61.22 ± 4.1 GAE mg/g. The extracts were individually and in combination (1:1, 1:2 & 2:1) subjected to determination of anti-inflammatory potential by carrageenan induced rat paw edema method using ibuprofen as the standard drug. Ibuprofen at dose of 10 mg/Kg inhibited 69.23% edema after 4h of administration whereas the maximum edema inhibition exhibited by the combined extracts was 55.22% (MKE:MIE, 1:2) at the end of 4h.

Keywords: *Murraya koenigii*, *Mangifera indica*, carrageenan, Anti-inflammatory, paw edema, Extract

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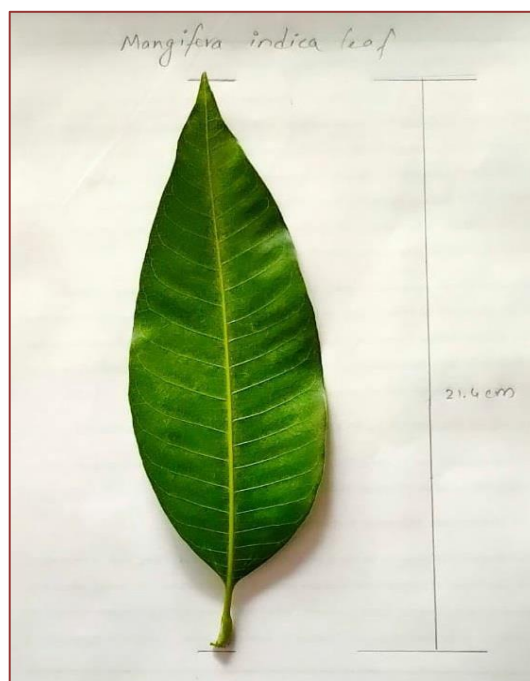
Received 01 May 2022, Accepted 22 June 2022

INTRODUCTION

Inflammation is normal and necessary protective response to harmful stimuli such as infectious agents, antigen-antibody reactions, thermal, chemical, physical agents, and ischemia . It is caused by a variety of stimuli, including physical damage, UV irradiation, microbial attack, and immune reactions. The Ayurvedic system of medicine relies on the use of an amalgamation of several herbs to treat an ailment. In accordance with the principle of Ayurveda, it was therefore envisioned that combining the extracts of two different plants in various concentrations would be beneficial in exerting additive or synergistic action in any particular ailing condition. The objective of the present work was therefore to Methanolic extraction of *Murraya koenigii* and *Mangifera indica* leaves (in vitro) and Study of the synergistic anti-inflammatory potential of the combined extracts using in (vivo model).



Leaves of *Murraya koenigii*



***Mangifera indica* leaf**

MATERIALS AND METHOD

Murraya Koenigii and Mangifera Indica was collected from local surrounding, Bhopal. Glacial acetic acid was purchased from Rankem, Mumbai and HCL, Methanol, ferric chloride solution, sulphuric acid, ammonia solution, acetic anhydride, chloroform and Dragendroff Reagent and other Reagents & chemicals were purchased from oxford fine chemicals, Mumbai, Thermo fisher scientific, Mumbai.

Extraction of leaves²

The plant leaves, were authenticate from Saifia Science College, Bhopal, M.P after authentication, were washed with distilled water and dried under shade. The dried leaves were powdered using a blender at low speed. The powdered leaves of both the plants were used for the extraction process. 100 g of powder was evenly packed in soxhlet apparatus and extracted with 300 ml of methanol by hot continuous extraction process for about 14 h. The extracts were filtered while hot using Whatman filter paper for removal of impurities. The extracts were then concentrated by distillation in order to reduce the volume to one-tenth. The concentrated extracts were transferred to 100 ml beakers and the solvents were evaporated on water bath. The oleo-resinous/semisolid extracts collected and the excessive moisture was removed by placing the extracts in desiccators. The dried extracts were stored in desiccators for further procedures of analysis.

Preliminary phytochemical screening

The extracts from both the plants were subjected to qualitative phytochemical analysis for testing the presence or absence of common plant secondary metabolites.

Alkaloids³

Test for alkaloids was done using Drangend off's reagent. Each of the extracts were re-dissolved in 5 ml of 1% HCl and 5 drops of Drangend off 's reagent was added to each extract solution. A colour change (orange to orange red precipitate) was observed to infer the presence or absence of alkaloids.

Cardiac Glycosides⁴

The Keller-Killani test was performed for detecting the glycosides in the extracts. The plant extracts were dispersed in methanol (5 ml) and were treated with 2 ml of glacial acetic acid, containing one drop of ferric chloride solution. To this was added 1 ml of concentrated sulphuric acid. Brown ring formed at the interface may indicate the presence of deoxysugar cardenoloides. A violet ring may appear just below the brown ring, while in the acetic acid layer, a greenish ring may also form gradually throughout the layer, if the cardiac glycosides are present.

Tannins⁵

The extracts were dissolved in 5 ml distilled water and boiled gently and cooled. To 1 ml of each extract, 3 drops of ferric chloride solution was added. The formation of green coloured precipitate indicates the presence of tannins.

Flavonoids⁶

To a portion of the aqueous filtrate of each plant extract, diluted ammonia solution (5 ml) was added, followed by addition of concentrated sulphuric acid. The formation of a yellow precipitate indicated the presence of flavonoids.

Saponins⁷

The method of persistent frothing was used to detect the presence of saponins in the extracts. 1 g of each extract was boiled with 5 ml distilled water and filtered. To the filtrate was added 3 ml distilled water, shaken vigorously and heated. The samples were observed for the persistence appearance of foam lasting for at least 15 min confirmed the presence of saponins.

Steroids⁸

To 0.5 g of each extract, 2 ml acetic anhydride was added followed by the addition of 2 ml sulphuric acid. Change in color from violet to blue or green indicates the presence of steroids.

Terpenes/terpenoids⁹

The Salkowski test was used to detect the presence of terpenes/terpenoids in the different extracts. Five milliliters of extract was mixed in 2 ml chloroform and 3 ml concentrated sulphuric acid was then carefully added along the walls of the test tube to form a layer. The formation of greyish colour indicates the presence of terpenes/terpenoids.

Preparation the combined extracts

The methanolic extracts obtained from *Murraya koenigii* and *Mangifera indica* were mixed in three different ratios (1:1, 1:2 & 2:1) respectively and used for determination of the total phenolic content and anti-inflammatory action using the reported methods reported in the succeeding sections. The anti-inflammatory activity of the combined extracts was compared to that of the individual extracts and studied statistically for significance.

Total Phenolic Content¹⁰

The total phenolic content in the methanolic extracts of both the plants was determined by Folin-Ciocalteu method. For total phenolic content determination, 200 µL of each extract (1 mg/ml) was mixed with 3 ml purified water and 0.5 ml of Folin-Ciocalteu reagent. After 3 min, 2 ml of 20% w/v sodium carbonate aqueous solution was added and the mixture was allowed to stand for 1 h in dark and the absorbance was measured at 750 nm using a UV-Vis spectrophotometer.

Standard solutions of gallic acid (10-100 ppm) were similarly prepared to obtain a calibration curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample. The total phenolic content of the various concentrations of the combined extracts was also determined according to the above method.

Evaluation of anti-inflammatory action

Animals

Healthy wistar rats of either sex, weighing 180-250g were used for the study. The animals were housed in cages in the animal house of SS Institution of pharmacy, Bhopal during the course of experimental period and maintained at 12 day and night schedule with a temperature [17-26°C] maintained at standard experimental condition. The animals were fed with standard rodent pellet feed and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water. The experiment was performed in accordance with the approval of protocol from the animal ethical committee of the institute.

Carrageenan induced rat paw edema method

The carrageenan induced rat paw edema method was used for evaluating the anti-inflammatory activity of the extracts¹¹. Paw oedema was induced by subcutaneous injection of 0.1mL (1% solution) of Carrageenan into the plantar surface of the right hind paw of the rat. Extracts were administered in dose of 100 mg/kg in different groups of animals, 30 min prior to carrageenan injection. Ibuprofen (10 mg/kg i.p.) was used as a standard anti-inflammatory drug which was administered 30 min prior to carrageenan injection. Animals were divided into 7 groups and 6 animals in each group (n = 6) as follows

Group I - Control - treated with vehicle (normal saline)

Group II - Standard drug – Ibuprofen

Group III– *Murraya koenigii* extract (100 mg/kg)

Group IV – *Mangifera indica* extract (100 mg/kg)

Group V – Combined extract 1:1 (100 mg/kg)

Group VI – Combined extract 1:2 (100 mg/kg)

Group VII – Combined extract 2:1 (100 mg/kg)

Paw diameters were measured immediately before the administration of the Carrageenan and thereafter at 1, 2, 4 and 6 h using vernier caliper. The results obtained were compared with control group. The percentage inhibition of paw inflammation exhibited by each group was

calculated by using following formula:

$$\% \text{ inhibition} = \frac{C-T}{C} \times 100$$

C= Paw volume (mL) in vehicle treated group (control)

T= Paw volume (mL) in drug treated group

Results and Discussions

Extraction Yields

The extraction yield in methanol was found to be 26.8 % for *Murraya koenigii* and 31.6% for *Mangifera indica* (Figure 1).

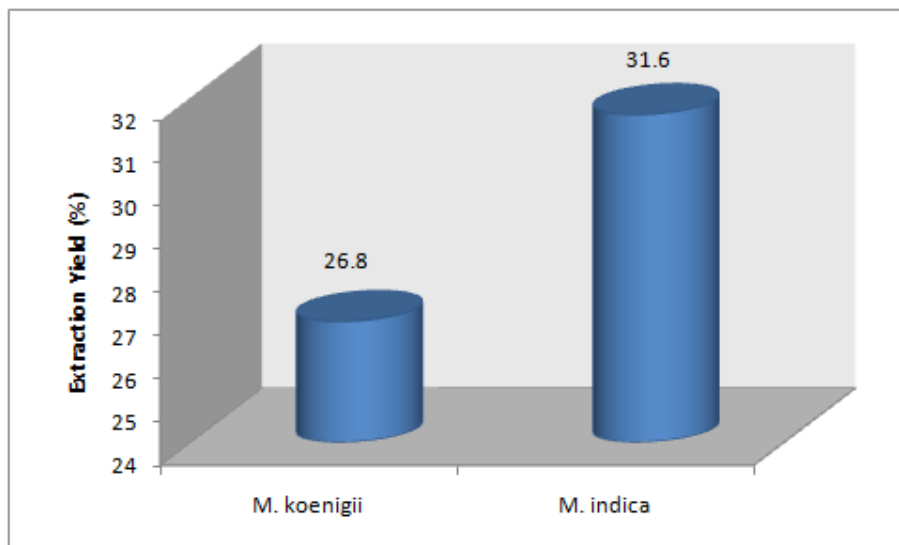


Figure 1: Extraction yields in methanol

Phytochemical Screening

A small fraction of all the dried extracts were subjected to the phytochemical screening for detecting the presence alkaloids, glycosides, tannins, saponins, flavonoids, steroids and terpenoids. Small amount of each extract is suitably resuspended into the sterile distilled water/ethanol for the screening purpose. All the extracts were tested for the presence of various categories of phytochemicals and the results are presented in Table 1

Phytochemical screening of the extracts

Table 1: Phytochemical Screening Of Extract Result

Phytochemical tested	Observation	<i>M. koenigii</i> extract	<i>M. indica</i> extract
Alkaloids	Orange color precipitate/solution	+	-
Saponins	Continual frothing	+	+
Cardiac glycosides	Brown ring at junction	-	-
Tannins	Green colored precipitate	+	+
Flavonoids	Yellow colored precipitate	+	+
Steroids	Formation of Green Color	-	+
Terpenes/terpenoids	Appearance of Grey color	+	+

The presence absence of alkaloids and glycosides in the methanolic leaf extracts of *M. indica* has also been reported¹².

Total Phenolic content

The total phenolic content in the extract of *Murraya koenigii* and *Mangifera indica* was quantified using Folin-Ciocalteu method. Standard curve of gallic acid was calculated and plotted in distilled water for determining absorption data (Table 2). The linear equation of gallic acid was found to be $y = 0.004x + 0.003$. The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in table 3. The total phenolic content in extracts, expressed as gallic acid equivalents. The total phenolic content of found in the extract of *Murraya koenigii* and *Mangifera indica* were 34.27 ± 1.7 and 43.11 ± 2.1 GAE mg/g, respectively.

Table 2: Absorbance data of gallic acid (at 750 nm)

Concentration ppm	Absorbance at 750 nm
0	0
10	0.056
20	0.11
30	0.148
40	0.187
50	0.255
60	0.292
70	0.344
80	0.391
90	0.446
100	0.497

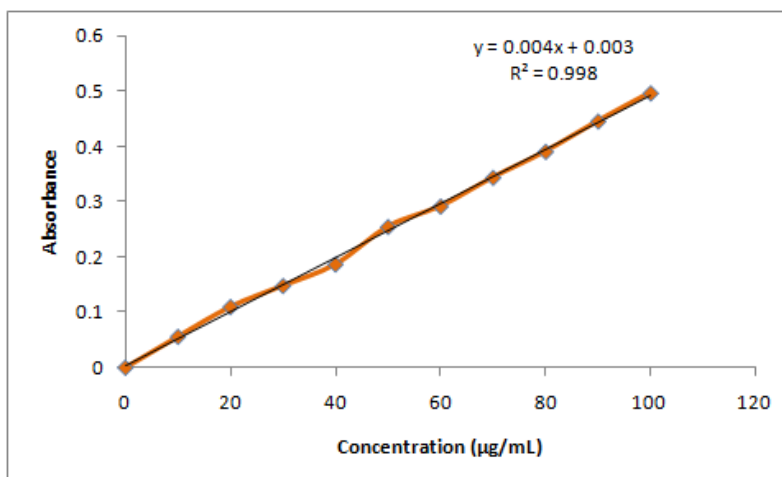


Figure 2: Calibration curve of gallic acid

Table 3: Total phenolic content of extracts

Plant	Total phenolic content (GAE mg/g)
<i>Murraya koenigii</i>	34.27±1.7
<i>Mangifera indica</i>	43.11±2.1
MKE:MIE (1:1)	49.77±6.4
MKE:MIE (1:2)	61.22±4.1
MKE:MIE (2:1)	54.87±7.3

Data expressed as gallic acid equivalent (GAE) mg per gm of the extract, Values are mean ± SD of triplicate determinations; MKE –*Murraya koenigii* extract, MIE- *Mangifera indica* extract.

Determination of Anti-inflammatory Potential

The extracts were individually and in combination (1:1, 1:2 & 2:1) subjected to *in vivo* determination of anti-inflammatory potential using carrageenan-induced rat paw edema method. The dose of extracts was selected on the basis of a previous study¹³.

Table 4 and 5 shows the paw thickness measured with respective treatments and the effect of extracts and standard drug as compared to the normal saline control at different hours in carrageenan-induced rat paw edema model using vernier caliper. Ibuprofen at dose of 10 mg/Kg inhibited 69.23% edema after 4h of administration whereas the maximum edema inhibition exhibited by the combined extracts was 55.22% (MKE:MIE, 1:2) at the end of 4h. The anti-inflammatory action was consistent with the phenolic content of the extracts; with a higher amount of MIE in the mixture presenting a better inhibition of paw-edema.

Table 4: Effect of extracts on rat paw-edema

Group	Paw thickness (mm)			
	1h	2h	3h	4h
Normal Saline	0.476 ± 0.025	0.662 ± 0.024	0.782 ± 0.033	0.728 ± 0.030
Ibuprofen	0.264 ± 0.038	0.354 ± 0.025	0.396 ± 0.024	0.224 ± 0.027
MKE	0.442 ± 0.024	0.534 ± 0.016	0.568 ± 0.026	0.546 ± 0.023
MIE	0.412 ± 0.0234	0.508 ± 0.0260	0.52 ± 0.027	0.436 ± 0.023
MKE:MIE (1:1)	0.406 ± 0.011	0.456 ± 0.024	0.514 ± 0.053	0.398 ± 0.048
MKE:MIE (1:2)	0.38 ± 0.032	0.406 ± 0.032	0.462 ± 0.031	0.326 ± 0.015
MKE:MIE (2:1)	0.396 ± 0.013	0.444 ± 0.021	0.494 ± 0.021	0.376 ± 0.030

Results are mean ± SD (n = 5)

Table 5: Percent inhibition of paw edema presented by treatment

Group	Change in Paw thickness (mm) [% inhibition of edema]			
	1h	2h	3h	4h
Ibuprofen	44.54	46.52	52.68	69.23
MKE	7.14	19.33	27.36	25
MIE	13.44	23.26	33.5	40.11
MKE:MIE (1:1)	14.71	31.11	34.27	45.33
MKE:MIE (1:2)	20.17	38.67	40.92	55.22
MKE:MIE (2:1)	16.81	32.93	36.83	48.35

Carrageenan-induced acute inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. As shown in the table, the combined extracts of *Murraya koenigii* and *Mangifera indica* were able to inhibit much more edema formation as compared to that inhibited by each of the extracts alone suggesting additive effect in the anti-inflammatory potential on combining the extracts (figure 3).

Carrageenan-induced paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis¹⁴. Therefore, it can be inferred that the inhibitory effect of extracts and their combinations on carrageenan-induced inflammation may be due to inhibition of the enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis.

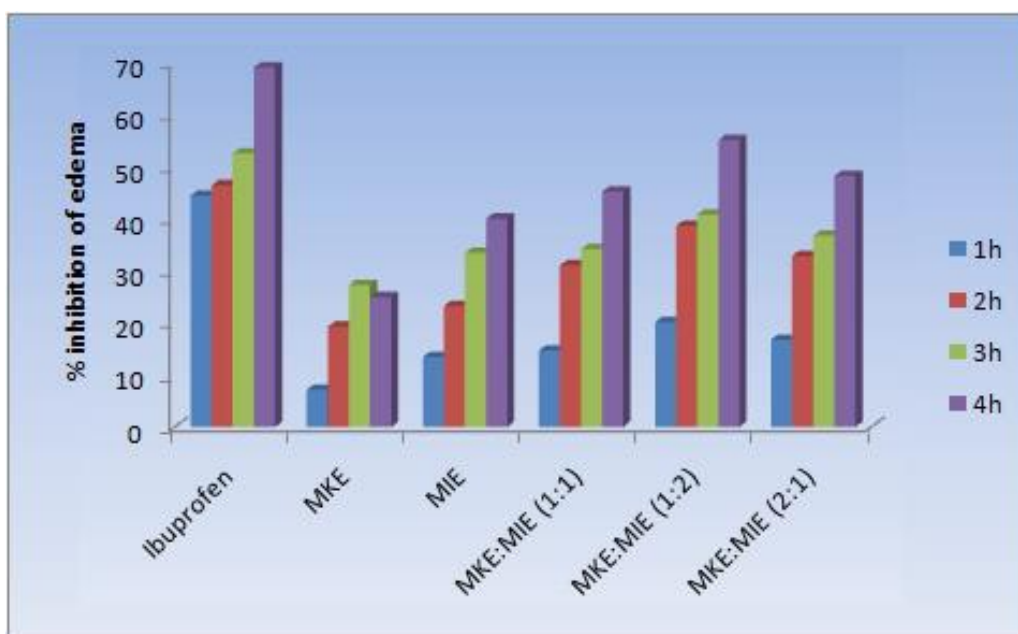


Figure 3: Percent inhibition of edema by ibuprofen, extracts and combined extracts

The presence of phenolics and flavonoids in the extracts might be responsible for the anti-inflammatory potential exhibited by these plant extracts. Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases¹⁵. Flavonoids suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and up-regulate and protect antioxidant defenses¹⁶. On the other hand phenolics are known to confer oxidative stress tolerance on plants.

The association of the two different species to obtain an extract potentiates the antioxidant action of the extracts in comparison to the individual species. Similar results were earlier

reported for improved antioxidant activity on combining *Humulus lupulus* and *Vaccinium myrtillus*¹⁷ and for synergistic antimycobacterial action by combining *Combretum hereroense*, *Citrus lemon* and *Apodytes dimidiata*¹⁸.

CONCLUSION

The objective of the present study was to assess the anti-inflammatory potential of combined extracts of *Murraya koenigii* and *Mangifera indica* using the carrageenan induced rat paw edema method. The methanolic extract of both the plants were found possess anti-inflammatory action. The results obtained led to the conclusion that mixing extracts of different species of plants can lead to synergistic or additive bioactivity thereby paving newer therapies for treatment of inflammation.

ACKNOWLEDGMENTS

The authors would like to thank the management of SS Institute of pharmacy for providing the necessary facilities to conduct the animal experimentation.

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