JOURNAL OF PHARMACOLOGY AND BIOMEDICINE
www.rbscience.co.in
E-ISSN: 2456-8244

Study of synergistic antiinflammatory action of *Murraya koenigii* and *Ocimum sanctum* leaf extract

Chirayu Bhugra*, Bharat Tyagi

IPS College of Pharmacy, Gwalior, Madhya Pradesh

*Corresponding Author

Email ID – cbhugra123@gmail.com

Abstract

The present work was undertaken with an aim to determine the effect of combined extract of *Murraya koenigii* and *Ocimum sanctum*. The extraction yield in the solvent mixture was found to be 39.6 % for *Murraya koenigii* and 43.7 % for *Ocimum sanctum* 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 were not found to be present in *Ocimum sanctum*. The total phenolic content of the hydroalcoholic extracts of *M. koenigii* and *O. sanctum* were 38.31±1.7 and 54.27±2.1GAE mg/g, respectively. The phenolic content was highest in the combined extract (MSE:OSE, 1:2) of all the three combinations with total phenolics 63.9±3.8 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 52.47% (MKE:OSE, 1:2) at the end of 4h.

Keywords: *Murraya koenigii*, *Ocimum sanctum*, carrageenan, anti-inflammatory, Ibuprofen, combined extracts

Received 27/08/2021; Revised 03/09/2021; Accepted 05/09/2021

Scan QR Code to visit Website

Introduction

Inflammation is normal and necessary protective response to the harmful stimuli such as infectious agents, antigen-antibody reactions, thermal, chemical, physical agents, and ischemia (Goldyne et al., 1984).

All inflammatory diseases have almost a common pathway of generation of disease which involves generation of various inflammatory mediators at various stages due to initial stimulation by one or various etiological factors which may be an infection, an injury or even an allergic stimulus.

Murraya koenigii is semi deciduous, unarmed aromatic small spreading shrub or tree commonly referred as Curry leaf belonging to family Rutaceae. It is a very rich source of organic compounds and known to possess several biological actions (Jain et al., 2017).

Ocimum sanctum L. (Tulsi) is an erect, much branched sub-shrub 30-60 cm tall, with simple opposite green or purple leaves that are strongly scented and hairy stems belonging to family Lamiaceae. The leaf is known source of volatile oils, strerols, flavanols and anthycyans. It is reported to possesses wide array of pharmacological actions (Pattanayak et al., 2010).

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 conditions.

The objective of the present work was to combine the hydroalcoholic extracts of *Murraya koenigii* and *Ocimum sanctum* leaves, and examine the anti-inflammatory potential of the combined extracts using *in vivo* model.

Material and Methods

Collection and preparation of the plant material

The leaves of *Murraya koenigii* and *Ocimum sanctum* were collected from the local surrounding of Bhopal, Madhya Pradesh in the month of January and authenticated. The plant leaves, 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 were stored in closed container till use.

Extraction of leaves

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 ethanol-water (70:30) 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. The evaluation was done for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. Precipitate formation or color intensity was used as analytical response to these tests.

Preparation the combined extracts

The hydroalcoholic extracts obtained from *Murraya koenigii* and *Ocimum sanctum* 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 Content (Ansari et al., 2013)

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 analgesic and anti-inflammatory action

Healthy Wistar rats of either sex, weighing 180-250g were used for the study, fasting 12 hours before the experiment with free access to only water.

Carageenan induced rat paw edema method

The carageenan induced rat paw edema method was used for evaluating the anti-inflammatory activity of the extracts (Kemisetti and Manda, 2018).

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 3 groups (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 – *Ocimum sanctum* 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:

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

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

T= Paw volume (mL) in drug treated group

Results and Discussion

The extraction yield in ethanol-water (70:30) was found to be 39.6 % for *Murraya koenigii* and 43.7 % for *Ocimum sanctum.*

Phytochemical Screening the extracts were tested for the presence of various categories of phytochemicals and the results are presented in Table 1.

The findings suggest the presence of alkaloids, saponin glycosides, phenolics, terpenoids, sterols, and flavonoids in the leaf of the plants.

Table 1 Phytochemical screening of the extracts

Phytochemical tested	Observation	*M. koenigii* extract	*O. sanctum* 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	++	++

Total Phenolic content

The total phenolic content in the extract of *Murraya koenigii* and *Ocimum sanctum* was quantified using Folin-Ciocalteu method using the standard curve of gallic acid in distilled water. The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in table 2. The total phenolic content in extracts, expressed as gallic acid equivalents. The total phenolic content of found in the extract of *Murraya koenigii* and *Ocimum sanctum*

were 38.31±1.7 and 54.27±2.1 GAE mg/g, respectively.

Table 2 Total phenolic content of extracts

Plant	Total phenolic content (GAE mg/g)
Murraya koenigii	38.31±1.7
Ocimum sanctum	54.27±2.1
MKE:OSE (1:1)	47.96±4.6
MKE:OSE (1:2)	63.9±3.8
MKE:OSE (2:1)	58.44±6.1

Data expressed as gallic acid equivalent (GAE) mg per gm of the extract, Values are mean ± SD of triplicate determinations; MKE –*Murraya koenigii* extract, OSE- *Ocimum sanctum* 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 study by Mirje et al (2014) and Gupta et al (2010).

Table 3 shows the effect of extracts and standard drug as compared to the normal saline control at different hours in carageenan-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 52.47% (MKE:OSE, 1:2) at the end of 4h.

Carrageenan-induced acute inflammation is one of the most suitable test procedure to screen anti-inflammatory agents. As shown in the table, the combined extracts of *Murraya koenigii* and *Ocimum sanctum* 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 1).

Table 3 Effect of extracts on rat paw edema

Group	Change in Paw thickness (mm) [% inhibition of edema]			
	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 [44.54%]	0.354 ± 0.025 [46.52%]	0.396 ± 0.024 [52.68%]	0.224 ± 0.027 [69.23%]
MKE	0.442 ± 0.024 [7.14%]	0.534 ± 0.016 [19.33%]	0.568 ± 0.026 [27.36%]	0.546 ± 0.023 [25%]
OSE	0.456 ± 0.011 [4.2%]	0.534 ± 0.016 [20.54%]	0.552 ± 0.013 [29.41%]	0.52 ± 0.012 [28.57%]
MKE:OSE (1:1)	0.448 ± 0.017 [5.88%]	0.522 ± 0.004 [21.29%]	0.546 ± 0.020 [30.17%]	0.518 ± 0.023 [28.84%]
MKE:OSE (1:2)	0.409 ± 0.003 [14.07%]	0.503 ± 0.003 [24.01%]	0.476 ± 0.007 [39.13%]	0.346 ± 0.008 [52.47%]
MKE:OSE (2:1)	0.418 ± 0.013 [12.18%]	0.516 ± 0.023 [22.05%]	0.534 ± 0.018 [31.71%]	0.466 ± 0.046 [35.98%]

Results are mean ± SD (n = 5)

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 synthesis (Seibert et al., 1994). 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.

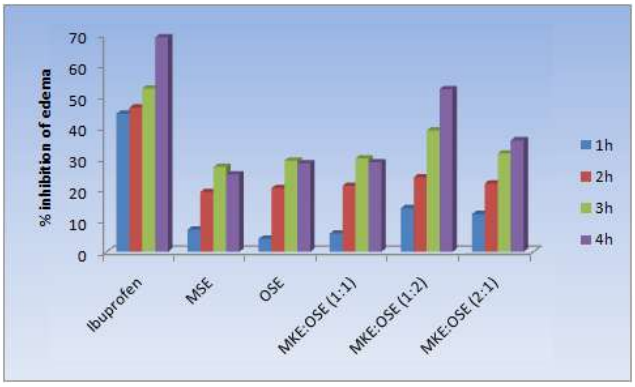

Figure 1 Percent inhibition of edema by ibuprofen, extracts and combined extracts

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 diseases (Bravo, 1998). Flavonoids suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and up-regulate and protect antioxidant defenses (Agati et al., 2012). 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* (George et al., 2015) and for synergistic antimycobaterial action by combining *Combretum heroroense, Citrus lemon* and *Apodytes dimidiate* (Komape et al., 2017).

Conclusion

The objective of the present study was to assess the anti-inflammatory potential of combined extracts of *Murraya koenigii* and *Ocimum sanctum* using the carrageenan induced rat paw edema method. The hydroalcoholic 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.

Acknowledgement

The authors are thankful to the management of IPS College of Pharmacy, Gwalior for providing necessary facilities to carry the research work.

References

Agati G, Azzarello E, Pollastri S, Tattini M. Flavonoids as antioxidants in plants: location and functional significance. Plant Science. 2012; 196: 67–76.

Ansari AQ, Ahmed SA, Waheed MA, Sayyed JA. Extraction and determination of antioxidant activity of Withania somnifera Dunal. Eur J Experimental Biol 2013, 3(5): 502-507.

Bravo L. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutrition Reviews. 1998; 56: 317–333.

George B, Corina B, Gheorghe C. Antioxidant activity of *Humulus lupulus* and *Vaccinium myrtillus*

individual and combined extracts. Romanian Biotechnological Letters. 2015; 20(2): 1027-10286

Goldyne ME, Burrish GF, Poubelle P and Borgeat P (1984). Arachidonic acid metabolism among human mononuclear leukocytes. Lipoxygenaserelated pathways, J Biol Chem., 259: 8815-8819.

Gupta S, George M, Singhal M, Sharma GN, Garg V (2010). Leaves extract of Murraya koenigii for anti-inflammatory and analgesic activity in animal models, J Aadv Pharm Tech Res, 1: 68-77.

Jain M, Gilhotra R, Singh RP, et al. Curry leaf (MurrayaKoenigii): a spice with medicinal property. MOJ Biol Med. 2017;2(3):236–256. DOI: 10.15406/mojbm.2017.02.00050

Kemisetti D, Manda S. Synthesis and comparison of peg-ibuprofen and peg-ketoprofen prodrugs by *in vitro* and *in vivo* evaluation. J Drug Del Ther, 2018; 8(4): 145-154.

Komape NPM, Bagla VP, Kabongo-Kayoka P, Masoko P. Anti-mycobacteria potential and synergistic effects of combined crude extracts of selected medicinal plants used by Bapedi traditional healers to treat tuberculosis related symptoms in Limpopo Province, South Africa. BMC Complementary and Alternative Medicine. 2017; 17: 128. DOI 10.1186/s12906-016-1521-2

Mirje MM, Zaman SU, Ramabhimaiah S (2014). Evaluation of the anti-inflammatory activity of Ocimum sanctum Linn (Tulsi) in albino rats, Int J Curr Microbiol App Sci., 3(1): 198-205.

Pattanayak P, Behera P, Das D, Panda SK (2010). Ocimum sanctum Linn. A reservoir plant for therapeutic applications: An overview. Pharmacognosy Review, 4 (7): 95-105.

Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W, et al. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. Proc Nat Acad Sci, 1994; 91:12013–12017.