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Study of Synergistic Anti-inflammatory action of Ocimum Sanctum and Mangifera Indica leaf extract

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ABSTRACT

The present work focused on preparing hydroalcoholic extracts of *Mangifera indica* and *Ocimum sanctum* leaves and establishing the anti-inflammatory potential of the combined extracts in experimental model. The extraction yield of the hydroalcoholic mixture was found to be 36.2 % for *Mangifera indica* 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 *Mangifera indica* while alkaloids, sterols and glycosides were not present in *Ocimum sanctum.* The total phenolic content of the extracts of *M. indica* and *O. sanctum* were 26.86 ± 0.308 and 37.56 ± 0.665 GAE mg/g, respectively. The phenolic content was highest in the combined extract (CE, 1:2) of all the three combinations with total phenolics 81.53 ± 1.855 GAE mg/g. The extracts were individually and in combination (1:1, 1:2 & 2:1) subjected to determination of anti-inflammatory potential using carrageenan induced rat paw edema model. 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% (MIE:OSE, 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 OSE in the mixture presenting a better inhibition of paw-edema.

Keywords: *Mangifera indica*, *Ocimum sanctum*, paw edema, anti-inflammatory, phenolics

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INTRODUCTION

Plants have played an important role in human health care since ancient times. Medicinal plants continue to be an interesting source of natural products for treating various health conditions [1]. The World Health Organization (WHO) estimates that approximately 65% of the world's population incorporates traditional medicine into medical care. The use of medicinal plant-based natural compounds to treat many illnesses has become a great trend in clinical research. Polyphenolic compounds have drawn significant attention due to their modulation effects on inflammasomes [2]. Inflammation is a defense response of our body to hazardous stimuli such as allergens and/or injury to the tissues [3]. It is a dynamic process that is elicited in response to mechanical injuries, burns, microbial infections, and other noxious stimuli that may threaten the well-being of the host [4]. Arachidonic acid is a key biological intermediate that is converted in to a large number of eicosanoids with potent biological activities [5]. Several medicinal plants have been reported to possess anti-inflammatory action in several animal models. It is important to note that the extraction of plant materials is the first major step to test biological activities, presenting many advantages and some disadvantages compared to the isolation of pure active compounds [6]. Several herbal products available commercially either as food supplements or as ayurvedic medicinal preparations contain multiple ingredients that potentiate the effects of all the ingredients present in them. In consonance with this, it was envisioned that combining the extracts of Mangifera indica and Ocimum sanctum, would be beneficial in potentiating anti-inflammatory effect of both the extracts. The objective of the present investigation was therefore to Hydroalcoholic extraction of Mangifera indica leaf, Hydroalcoholic extraction of Ocimum sanctum whole plant and Evaluation of anti-inflammatory effect of the combined extracts on paw edema in rats.

MATERIALS AND METHOD

Ocimum Sanctum 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 leaves [7]

The plant parts were washed with distilled water and dried under shade. The dried parts were powdered using a low speed blender and were stored in closed container till use. 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 16 h. The extracts were filtered while hot using Whatman filter paper for removal of impurities. The extracts were then concentrated using rotary vacuum evaporator and the solvent was evaporated on water bath. The oleo-resinous/semisolid extracts collected and the excessive moisture was removed by storing the extracts in desiccator until further use.

Preliminary phytochemical screening [8]

The extracts from both the plants were tested qualitatively for phytochemical analysis to confirm the presence or absence of common plant secondary metabolites.

Alkaloids [15]

Test for alkaloids was done using Dragendorff's reagent. Each of the extracts were re-dissolved in 5 ml of 1% HCl and 5 drops of Dragendorff'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 Glycosides [16]

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 deoxy sugar cardenolides. A violet ring might 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.

Tannins [17]

The extracts were dissolved in 5 ml distilled water and boiled gently and cooled. To 1 ml of extract, 2-3 drops of ferric chloride solution was added. The formation of green colored precipitate indicates the presence of tannins.

Flavonoids [18]

To a fraction of the 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.

Saponins [19]

The method of unrelenting 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 continual appearance of foam that lasts for at least 15 min to confirm the presence of saponins.

Steroids [20]

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

Terpenes/terpenoids [21]

The Salkowski test was used to detect the presence of terpenes/terpenoids in the extracts. 5 mL of extract was mixed with 2 ml chloroform; 3 ml concentrated sulphuric acid was then carefully flowed down 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 hydroalcoholic extracts obtained from Mangifera indica and Ocimum sanctum were mixed in three different ratios (1:1, 1:2 & 2:1) respectively.

Total Phenolic Content [9]

The total phenolic content in the hydroalcoholic extracts was determined by Folin-Ciocalteu method. Briefly, 200 µL of each extract (1 mg/ml) was mixed with 3 ml purified water and 0.5 ml of Folin-Ciocalteu reagent. After 2 min, 4 ml of 75 g/L aqueous sodium carbonate solution was added, the mixture was vortexed for 15 sec and allowed to stand for 1 h in dark and the absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Standard solutions of gallic acid (25-125 µg/mL) were prepared in methanol and treated similarly to construct the calibration curve. Results obtained were expressed as milligrams of gallic acid equivalent (GAE) per g of the dry sample, calculated according to the following formula:

$T = C \times V/M;$

Where T is total phenolic content, C- concentration of gallic acid in extract,

V- Volume of extract solution, M is the weight of the extract in g.

The total phenolic content of the various concentrations of the combined extracts was also determined according to the above method.

Evaluation of anti-amnesic potential

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.

Grouping of animal for treatment

The animals were divided in 7 groups with 5 animals in each group. The grouping and treatment per group is presented below.

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

Group II - Standard drug – Ibuprofen

Group III– Ocimum sanctum 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)

Carrageenan induced rat paw edema method [10]

The carrageenan induced rat paw edema method was used for evaluating the anti-inflammatory activity of the extracts.

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.

Paw diameter was measured using vernier caliper directly before the administration of the Carrageenan and thereafter at 1, 2, 3 and 4 h. The results obtained were compared with control group. The percentage inhibition of paw inflammation exhibited by each group was calculated by using following formula:

% inhibition = C-T/ C x 100

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

T= Paw volume (mL) in drug treated group

RESULTS AND DISCUSSION

Extraction Yields

The extraction yield in ethanol-water (70:30) was found to be 36.2 % for Mangifera indica and 43.7 % for Ocimum sanctum.

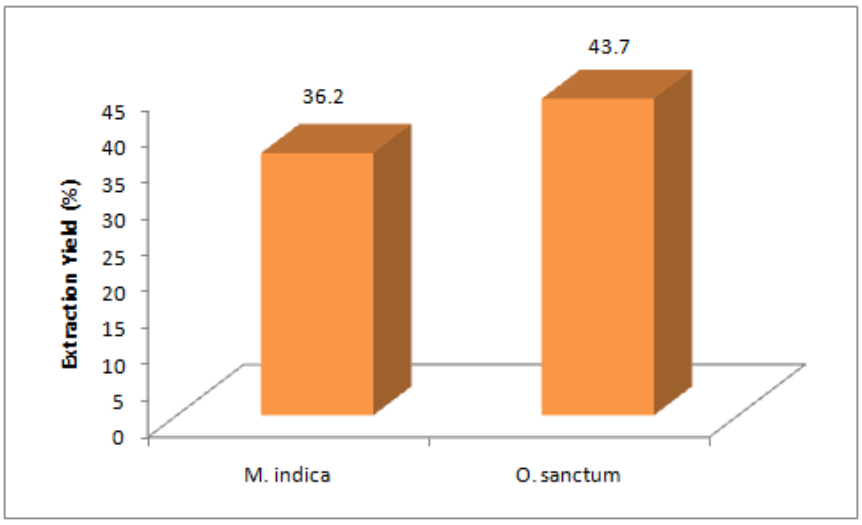

Figure 1: Extraction yields in ethanol-water (70:30)

Phytochemical Screening

A small fraction of the dried extracts were subjected to the phytochemical screening for detecting the presence alkaloids, glycosides, tannins, saponins, flavonoids, steroids and terpenoids. All the extracts were tested for the presence of various categories of phytochemicals and the results are presented in Table.1

Table 1: Phytochemical screening of the extracts

Phytochemical tested	**Observation**	***Mangifera indica* extract**	***O. sanctum* extract**
Alkaloids	Orange colour precipitate/ solution	+	-
Saponins	Continual frothing	+	+
Cardiac glycosides	Brown ring at junction	+	-
Tannins	Green coloured precipitate	+	+
Flavonoids	Yellow coloured precipitate	+	+
Steroids	Formation of Green Colour	+	-
Terpenes/terpenoids	Appearance of Grey colour	+	+

Total Phenolic content

The total phenolic content in the extract of *Mangifera indica* and *Ocimum sanctum* was quantified using Folin-Ciocalteu method. Standard curve of gallic acid was plotted for determining absorption data (Table 2). The linear equation of gallic acid was found to be y = 0.001x + 0.007 (Figure 2) and was used for calculations of phenolic content. The results of the total phenolic content of the extracts are depicted in (Table 3). The total phenolic content of found in the extract of *Mangifera indica* and *Ocimum sanctum* were 26.86 ± 0.308 and 37.56 ± 0.665 GAE mg/g, respectively.

Table 2: Absorbance data of gallic acid (at 765 nm)

Concentration (µg/mL)	Absorbance at 765 nm
0	0
25	0.057
50	0.108
75	0.147
100	0.188
125	0.239

Table 3: Total phenolic content of extracts

Plant	Total phenolic content (GAE mg/g)
Mangifera indica	26.86 ± 0.305
Ocimum sanctum	37.56 ± 0.665
CE, 1:1	54.86 ± 3.510
CE, 1:2	81.53 ± 1.855
CE, 2:1	74.06 ± 0.945

Data expressed as gallic acid equivalent (GAE) mg per g of the extract, Values are mean ± SD of triplicate determinations; CE – Combined extract of *Mangifera indica* and *Ocimum sanctum* extract.

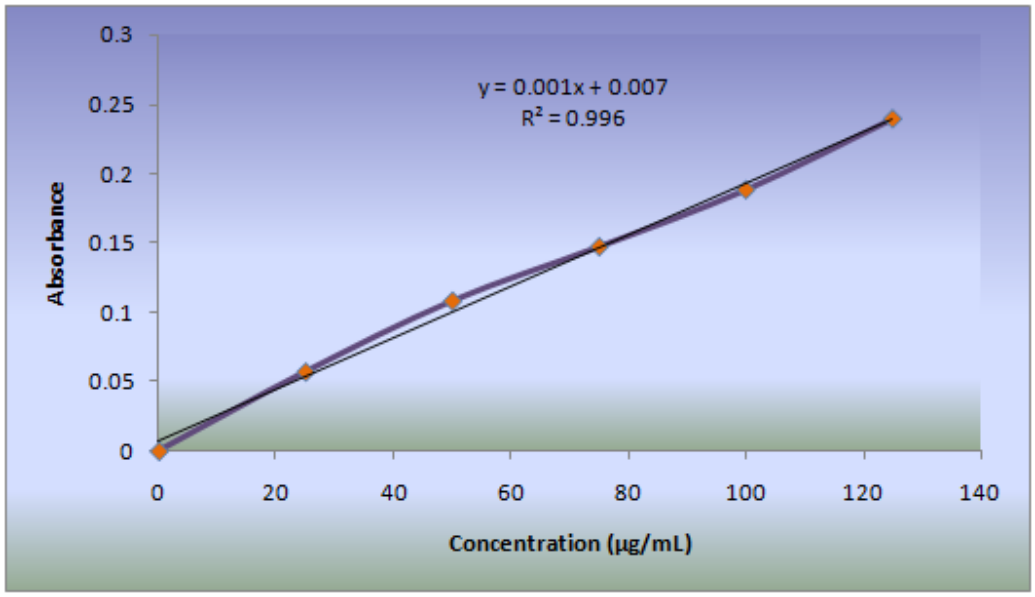

Figure 2: Calibration curve of gallic acid

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. 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 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% (MIE:OSE, 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 OSE 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
MIE	0.442 ± 0.024	0.534 ± 0.016	0.568 ± 0.026	0.546 ± 0.023
OSE	0.456 ± 0.011	0.534 ± 0.016	0.552 ± 0.013	0.52 ± 0.012
MIE:OSE (1:1)	0.448 ± 0.017	0.522 ± 0.004	0.546 ± 0.020	0.518 ± 0.023
MIE:OSE (1:2)	0.409 ± 0.003	0.503 ± 0.003	0.476 ± 0.007	0.346 ± 0.008
MIE:OSE (2:1)	0.418 ± 0.013	0.516 ± 0.023	0.534 ± 0.018	0.466 ± 0.046

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
MIE	7.14	19.33	27.36	25
OSE	4.2	20.54	29.41	28.57
MIE:OSE (1:1)	5.88	21.29	30.17	28.84
MIE:OSE (1:2)	14.07	24.01	39.13	52.47
MIE:OSE (2:1)	12.18	22.05	31.71	35.98

As shown in the table, the combined extracts of *Ocimum sanctum* 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.

Percent inhibition of edema by ibuprofen, extracts and combined extracts

The presence of phenolics and flavonoids in the extracts are expected to be accountable for the anti-inflammatory potential exhibited by these plant extracts. Flavonoids are highly effective predators of several oxidizing molecules, like singlet oxygen, and several other free radicals implicated in several diseases [11]. Flavonoids inhibit reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and up-regulate and protect antioxidant defenses [12]. Additionally phenolics are known to confer oxidative stress tolerance on plants.

The combination of the two separate species to form a combined an extract accentuates 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 [13] and for synergistic antimycobaterial action by combining *Combretum heroroense, Citrus lemon* and *Apodytes dimidiate* [14].

CONCLUSION

The objective of the present study was to assess the anti-inflammatory potential of combined extracts of Mangifera indica and Ocimum sanctum using the carageenan induced rat paw edema. 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 management of inflammatory conditions.

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