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Phytochemical and Pharmacological screening of some Selected Indian Medicinal Plants as Anti-inflammatory agent

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Phytochemical, Pharmaological, Anti-inflammatory, In-vivo.

ABSTRACT:

The objective of the current study is to formulate and check out the in vivo potential of formulations containing Mentha longifolia [F1], Curcuma caesia [F2], Adhatoda vasica [F3], Carica papaya [F] and Piper longum [F4]. These plants are present in many nations and continents, including Asia, Mauritius, South Africa, Mexico, China, the West Indies, East Africa, and Brazil. It is also used in traditional medicine for a number of indications. The hydro alcoholic extracts of plants were used for the main phytochemical study and in vivo anti-inflammatory evaluation. On behalf of physical property of gels, F1 formulation was selected for in vivo potential. The plant contains phenolic chemicals, fatty acids, flavonoids, tannins, and glycosides, according to phytochemical analysis. According to the findings, F1 formulation has possessed good anti-inflammatory activity.

INTRODUCTION

Inflammation is a defence reaction that triggers several physiological reactions that reduce tissue damage and eliminate pathogenic insults. This kind of mechanism involves a complicated chain of processes, including the enlargement of capillaries, venules, and arterioles with an increase in vascular permeability, fluid exudation including plasma proteins, and leukocyte migration into the inflammatory area. Monocytes and lymphocytes are the first cells to infiltrate a given lesion during inflammation, followed by PM. [1-3] Inflammation's goal is to obliterate and get rid of the harmful substance. However, inflammation will isolate and contain the injury if it doesn't happen or if it takes a long time. The goal in each area is to promote the recovery of damaged tissue while causing the least amount of physiologic harm to the host. Inflammation is a major component of stress responses and results from them. [4-7] On behalf of published scientific data, there is a lack of anti-inflammatory activity of *Mentha longifolia* (fruits), *Curcuma caesia* (rhizomes), *Adhatoda vasica* (leaves), *Carica papaya* (leaves)and *Piper longum* (fruits). The sleeted plants have traditionally mentioned to cure inflammation.

MATERIALS AND METHOD

Collection and authentication of plant material

Selected parts of *Mentha longifolia* (fruits), *Curcuma caesia* (rhizomes), *Adhatoda vasica* (leaves), *Carica papaya* (leaves) and *Piper longum* (fruits) were collected from National Botanical Research Institute, Lucknow, India in month of February 2021and authenticated by Department of Pharmacognosy & Phytochemistry, Intregral University, Lucknow, India (Ref No. IU/PHAR/HRB/21/06, IU/PHAR/HRB/21/05, IU/PHAR/HRB/21/04, IU/PHAR/HRB/21/03, IU/PHAR/HRB/21/02).

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Drugs and chemicals

Methanol, Toluene, Formic acid, Ethyl acetate, DPPH purchased from SDFCL, Mumbai. Ethanol was purchased from Changshu Yangyuan chemical, China. Ascorbic acid was purchased from Thomas Chemical Laboratory, Mumbai. Ursolic acid and lupeol was supplied by Sigma Aldrich, Germany. All the reagents used in the experiment were of analytical grade.

Animal's approval, source and their housing

Wister rats180-200gm (male/ female) were used for the study after approval of Institutional Animal Ethics Committee (IAEC) under the Registration Number CPCSEA/2022/41 by Ref No: RKDF/CPCSEA/22-41. Animal's room conditions were maintained with natural light and dark cycle at controlled room temperature of 20-25 º C and they were left for 7 days for acclimatization to animal room on standard pellet diet and water *ad libitum.*

Extraction of plant material

Extraction was carried out using methanol-water (90:10) using Soxhlet apparatus till clear solution in siphon was obtained. The plant parts were air-dried at room temperature for 10 days and pulverized by grinder. Five hundred grams of the powered plant material was defatted with petroleum ether then extracted with methanol and water for 24 to 36 h by Soxhlet extraction method. Then, methanol was separated under reduced pressure to obtain solid mass after that the powder material again treated with ethanol and water using Soxhlet extraction method then extracts were dried and stored in air tight amber-coloured bottle in refrigerator until further use [8].

Identification of Primary and Secondary metabolites by phytochemical screening

Different leaf extracts were treated with different reagent for the presence and absence of various primary and secondary metabolites [9, 10].

Preparation of nanogel formulation [11]

The F1, F2, F3, F4 and F5 contain *Mentha longifolia*, *Curcuma caesia*, *Adhatoda vasica*, *Carica papaya and Piper longum* extracts and other pharmaceutical agents shows in Table 1.

Table 1 Formulation

Formulation code	E1 (mg)	E2 (mg)	E3 (mg)	E4 (mg)	E5 (mg)	Eudragit L 100 (mg)	Ethanol (mL)	Carbopol (mg)	Propylene (mL)	Glycerine	Triethanolamine (mL)
F1	100	100	100	100	100	120	20	50	1	1	Qs
F2	100	100	100	100	100	120	20	100	1	1	Qs
F3	100	100	100	100	100	120	20	150	1	1	Qs
F4	100	100	100	100	100	120	20	200	1	1	Qs
F5	100	100	100	100	100	120	20	250	1	1	Qs

Preparation of nanosuspension

Sonication method was used, weighed quantity of extracts and eudragit were dissolved in ethanol and stirred well on magnetic stirrer to mix. The solution was sonicated using probe sonicator utilizing probe number 6 at pulse cycle of 1 sec with 4 sec off time and pulse power of 40%. The total sonication time was 10 min.

Preparation of nanogel

Nanosuspension was added carbopol followed by addition of glycerine and polypropylene glycol. The mixture was stirred and allowed to swell. Triethanolamine was added drop wise if needed for gelling the solution.

Evaluation of gel [12]

Spreadability – 1g gel was place between two slides 20cm x 10 cm and pressed with 100 g weight. The weight was removed and a fixed weight of 250 g was

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added to the pan attached to one end of the top slide. The Distance moved by the top slide in 10 seconds was recorded and spreadabilty was calculated by formula: S = m*l/t where m is weight on pan, l is distance moved by slide, t is time.

Viscosity – Brookfield viscometer was used. The gel was placed in beaker and spindle no. 64 was dipped in the gel. The spindle was allowed to rotate at 100 rpm and the viscosity reading was directly read on the display

pH – pH meter was used. 1 g of the gel was dissolved in 100 ml water and the pH was measured by dipping the electrode of pH meter in the gel solution.

Homogenity – The gel was visualized for consistency and homogenous character.

Grittiness – The gel was rubbed between fingers and sensed for particles.

Anti-inflammatory screening [13]

Carrageenan induced rat paw edema

On behalf of physical properties, F1 gel were selected for *in vivo* screening through carrageenan induced rat paw edema. Male wistar rat 180-200 g were used**.** Animal were divided in 3 groups Group I (control)-administered with gel base**,** Group II (standard)-administered with diclofenac gel**,** Group III (test) – administered with polyherbal gel.0.1mL carrageenan (1% w/v solution in normal saline) was injected into sub platnar tissue of right hind paw of each animal. The paw diameter was measured using screw gauge at various time points: before injection (-1), immediately after injection (0h), 1h, 2h, 3h & 4h.1g of gel was rubbed on paw with index finger 50 times paw diameter was measured at each time point as in induction

% inhibition of edema was calculated by formula: 100*(1-((y-x)/(b-a)))

Where x – initial paw thickness of test group

Y – paw thickness of test group at time point

A – initial paw thickness of control group

B- paw thickness of control group at time point

Papaya latex induced edema method [14]

Male wistar rat 180-200 g were used, Animal were divided in 3 groups namely Group I (control)-administered with gel base, Group II (standard)-administered with diclofenac gel, Group III (test) – administered with polyherbal gel.0.1mL papaya latex (0.25% w/v solution in acetate buffer) was injected into sub plantar tissue of right hind paw of each animal.The paw diameter was measured using screw gauge at various time points: before injection (-1), immediately after injection (0h), 1h, 2h, 3h & 4h.1g of gel was rubbed on paw with index finger 50 times. Paw diameter was measured at each time point as in induction.

% inhibition of edema was calculated by formula: 100*(1-((y-x)/(b-a)))

Where x – initial paw thickness of test group

Y – paw thickness of test group at time point

A – initial paw thickness of control group

B- paw thickness of control group at time point

RESULTS AND DISCUSSION

Phytochemical screening

Different extracts contain a range of primary and secondary metabolites, including alkaloids, glycosides, saponins, tannins, flavonoids, carbohydrate, and protein. For humans, the discovery of various metabolites such as alkaloids, glycosides, saponins, tannins, flavonoids, carbohydrate, protein, etc. is particularly advantageous because they can be used to treat a variety of ailments (Table 2).

Table 2 Phytochemical analysis of different extracts activity

Phytoconstituents	**HAML**	**HACC**	**HAAV**	**HACP**	**HAPL**
Alkaloids	+	++	+++	+++	+++

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Glycosides	+	+	++	+++	+++
Tannins	++	+++	+++	++	+++
Flavonoids	+++	+++	+++	+++	++
Fats and oil	+	+	+	+++	+
Carbohydrates	++	++	++	+++	+++
Reducing sugar	+	+	+	++	++
Proteins	+++	++	+++	+++	+++
Saponin	-	-	+	+++	+
Terpenoids	++	++	+++	+++	+++

HAML- Hydroalcoholic extract of *Mentha longifolia*, HACC- Hydroalcoholic extract of *Curcuma caesia*, HAAV- Hydroalcoholic extract of *Adhatoda vasica*, HACP- Hydroalcoholic extract of *Carica papaya*, HAPL- Hydroalcoholic extract of *Piper longum*

Evaluation of herbal gels

Different evaluation parametersof gel like viscosity, spreadability, homogeneity, grittiness and are shown in Table 3. From these data, the viscosity of gels was found to be between 3997 to 6954. Spreadability of gels were found to be between 17.5 to 27.5 etc.

Table 3 Evaluation parameters of different gels

Formulation Code	**Viscosity (cps)**	**Spreadability (g.cm/sec)**	**Homogeneity**	**Grittiness**	**pH**
F1	3997	25	Homogenous	Non gritty	6.49
F2	4712	20	Homogenous	Non gritty	6.57
F3	4953	27.5	Homogenous	Non gritty	6.69
F4	5562	17.5	Homogenous	Non gritty	6.59
F5	6954	17.5	Non-Homogenous	Non-Homogenous and gritty	6.59

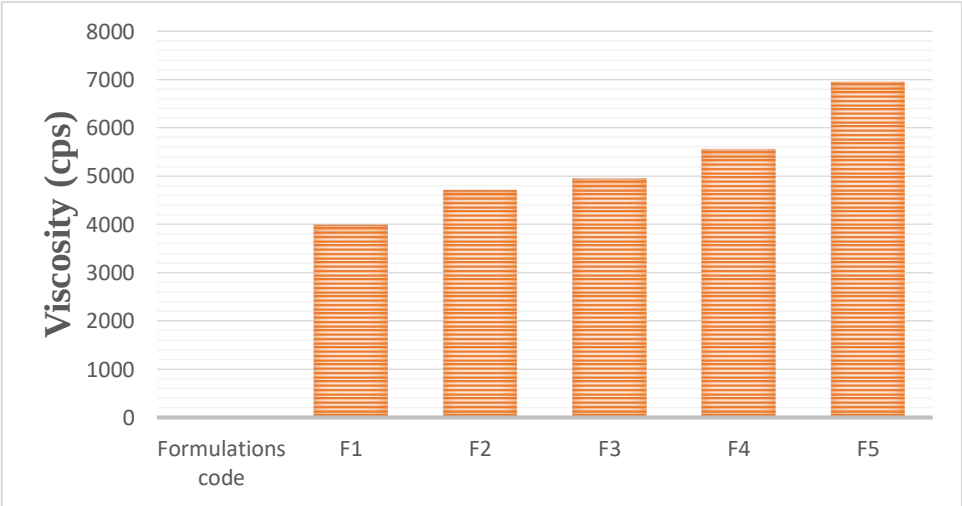

Figure 1. Viscosity analysis

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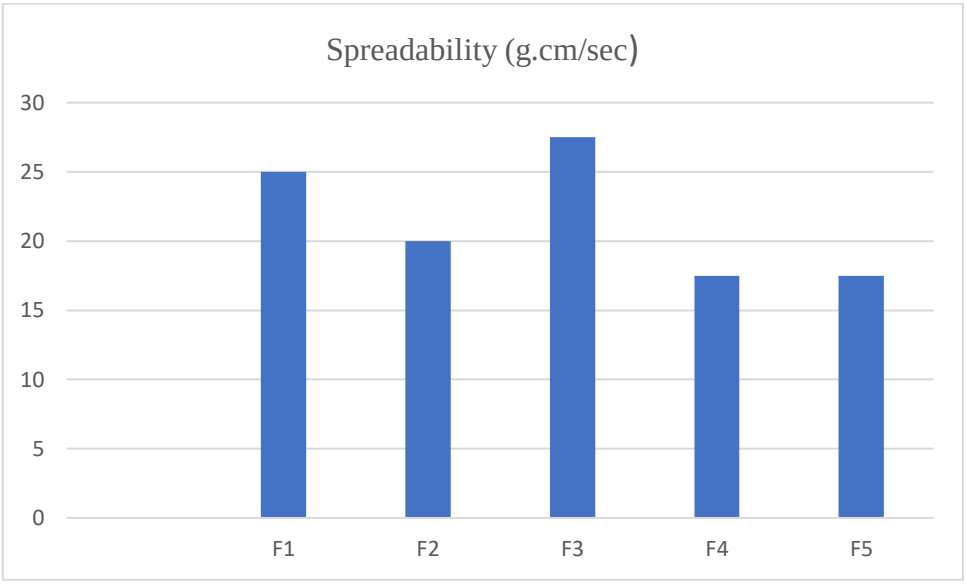

Figure 2. Spreadibility analysis

Pharmacological screening of F1 formulation

Different pathogens (viruses, bacteria), poisons, toxic chemicals, and tissue injury can all cause inflammation. [15]These damaging stimuli set off a chain of chemical reactions that activate leukocytes, which then secrete inflammatory cytokines, [16] such as interleukin-1β (IL-1β), interleukin-6, tumor necrosis factor-α (TNF-α). These cytokines interact with and activate receptors (IL-6R, TNFR-1, TNFR-2, TLR4, GM-CSFR etc.) [17]. Receptor activation results in the phosphorylation of a number of signalling molecules, including Janus kinase (Jak), nuclear factor kappa-B (NF-kB), and mitogen-activated protein kinase (MAPK), which in turn activates a number of transcription factors. The level of inflammatory mediators in resident tissue cells is controlled by this coordinated activation of signalling molecules, which also attracts inflammatory cells from the circulation. [18,19]As a result, acute inflammation serves as a protective mechanism, removing harmful stimuli and starting a repair process that returns the organism to its normal state of homeostasis [20].

From the Table 3.3, topical administration of different formulations shows anti-inflammatory action but F3 showed promising anti-inflammatory activity by reducing the carrageenan induced mice paw edema volume. In the present study carrageenan induced 3.96, 3.99, 5.43, 5.72, 5.77 and 5.51mm edema formation at -1, 0, 1, 2, 3 and 4 h respectively. It was observed that formulation F3 showed significant decrease in edema volume (11.5%) after 1 h. Moreover, at the same dose of formulation F3 considerable reduction in the edema volume (23.8 %) was observed, however,78.7 % reduction in edema volume was observed in respective sample after 4 h. The results were compared with standard anti-inflammatory drug such as diclofenac gel which showed effective inhibition (98.7 %) at 4 h [20].

Table 4 Paw edema condition

Group	**Paw diameter (mm)**					
	-1	**0**	**1**	**2**	**3**	**4**
I (Control)	3.96	3.99	5.43	5.72	5.77	5.51
II (Standard gel)	3.75	3.82	4.79	4.52	4.11	3.77
III (Test gel)	3.91	3.95	5.21	5.25	4.58	4.24

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The paw diameter was measured using screw gauge at various time points: before injection (-1), immediately after injection (0h), 1h, 2h, 3h & 4h.

Table 5 Percentage inhibition of edema

Group	% inhibition of edema in hours					
	-1	**0**	**1**	**2**	**3**	**4**
I (Control)	3.96	3.99	5.43	5.72	5.77	5.51
II (Standard gel)	3.75	3.82	29.2517	56.25	80.1105	98.70968
III (Test gel)	3.91	3.95	11.56463	23.86364	62.98343	78.70968

The paw diameter was measured using screw gauge at various time points: before injection (-1), immediately after injection (0h), 1h, 2h, 3h & 4h.

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REFERENCES

1. Punchard N.A., Whelan C.J., Adcock I., 2004. The Journal of Inflammation. J Inflamm (Lond). 27; 1(1):1.
2. Medzhitov R., Inflammation 2010: new adventures of an old flame. *Cell.*;140:771–776.
3. Ferrero-Miliani L., Nielsen O., Andersen P., Girardin S., 2007. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. *Clin Exp Immunol.* ;147:227–235.
4. Nathan C., Ding A. 2010. Nonresolving inflammation. *Cell.* ; 140:871–882.
5. Zhou Y., Hong Y., Huang H., 2016. Triptolide Attenuates Inflammatory Response in Membranous Glomerulo-Nephritis Rat via Downregulation of NF-κB Signaling Pathway. *Kidney and Blood Pressure Res.* 41:901–910.
6. Pahwa R., Goyal A., Jialal I., 2022. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL). Chronic Inflammation.
7. Germolec D.R., Shipkowski K.A., Frawley R.P., Evans E., 2018. Markers of Inflammation. Methods Mol Biol. 1803:57-79.
8. Sharma A.K., Chaudhary R.K., Singh S., Ved A., Shukla K.S., Singh A., Singh M.P., Kulshreshtha M., 2023. HPTLC Studies, in silico Docking Studies, and Pharmacological Evaluation of *Elaeocarpus ganitrus* as a Gastroprotective Agent. Cent Nerv Syst Agents Med Chem. 23(1):13-31
9. Kulshreshtha M., Dwivedi H., SinghM.P., 2018. Antimicrobial effects of leaves of Indian herbal plants with reference to peptic ulcer. Env. Dis. 3(1):18-26.
10. Kulshreshtha M., Chaudhary K.R, Roy S., Shukla S.K., Singh A., Singh P.M., 2023. Pharmacological Investigation on Unraveling Mechanism of Action of *Qusqualis indica* Leaves for Predicted Treatment of Peptic Ulcer Disease, Current Functional Foods. 1(2) : e200323214728 .
11. Ravali P., Swetha M., Battu S., 2021. Formulation And Evaluation of Nanogels Prepared With Herbal Extracts For Antifungal Activity. International Journal of All Research Education and Scientific Methods. 9(7): 1110-1120
12. Vaishya M.K, Sharma V., Rai J.P., 2021. Tazarotene loaded niosomal gel for effective acne treatment. Journal of Pharmacology and Biomedicine. 5(1): 235-241

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13. Gupta R., Gupta G.D., 2017. Formulation Development and Evaluation of Anti-inflammatory Potential of Cordia obliqua Topical Gel on Animal Model. Pharmacognosy Journal. 9(6) Suppl: S93-S98
14. Gupta O.P, Sharma N., Chand D. A., 1992. Sensitive and Relevant Model for Evaluating Anti-Inflammatory Activity-Papaya Latex-Induced Rat Paw Inflammation. Journal of Pharmacological and Toxicological Methods. 28(1): 15-19
15. Medzhitov R., 2010. Inflammation 2010: New Adventures of an Old Flame. *Cell* 140:771–6. doi:10.1016/j.cell.2010.03.006
16. Jabbour, H.N., Sales, K.J., Catalano, R.D., Norman, J.E., 2009. Inflammatory Pathways in Female Reproductive Health and Disease. *Reprod* 138:903–19. doi:10.1530/rep-09-0247
17. Kaminska, B., 2005. MAPK Signalling Pathways as Molecular Targets for Anti-inflammatory Therapy-From Molecular Mechanisms to Therapeutic Benefits. *Biochim Biophys Acta (Bba) - Proteins Proteomics* 1754:253–62. doi:10.1016/j.bbapap.2005.08.017
18. Lawrence, T., 2009. The Nuclear Factor NF-kB Pathway in Inflammation. *CSH Perspect Biol* 1:a001651. doi:10.1101/cshperspect.a001651
19. Libby P., 2007. Inflammatory Mechanisms: the Molecular Basis of Inflammation and Disease. *Nutr Rev* 65:S140–6. doi:10.1111/j.1753-4887.2007.tb00352.x
20. Ferrero-Miliani L., Nielsen O.H., Andersen P.S. Girardin, E., 2007. Chronic Inflammation: Importance of NOD2 and NALP3 in Interleukin-1beta Generation. *Clin Exp Immunol* 147:227–35. doi:10.1111/j.1365-2249.2006.03261.