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Research Article

FORMULATION OF *MYRISTICA FRAGRANS* (NUTMEG) TOPICAL GEL AND ITS *IN VITRO* EVALUATION FOR ANTINFLAMMATORY ACTIVITY

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Abstract

The purpose of this research was to formulate and evaluate an anti-inflammatory gel using ethanolic extract obtained from seeds and arillus of *Myristica fragrans*. In the present work, ethanolic extract of *Myristica fragrans* was prepared by maceration technique. The gels were formulated using different concentrations of carbopol 934, ethanolic extract of *Myristica fragrans* and piperine as permeability enhancer. The formulated gel was characterized using parameters like clarity, homogeneity, stability, extrudability, washability and *in-vitro* anti-inflammatory activity. The *in-vitro* anti-inflammatory property of the gel was determined using protein denaturation technique and was compared to marketed diclofenac gel. The results obtained indicate a concentration dependent increase in percentage inhibition of albumin denaturation by both the formulated gels (66 ± 0.3 %) and marketed gels (63.7 ± 0.2 %).From the present findings, it was concluded that the topical anti-inflammatory gel prepared from the extract of seeds and mace of *Myristica fragrans* have greater *in-vitro* anti-inflammatory property compared to the marketed gel formulation.

Keywords: *Myristica fragrans,* Maceration, *in-vitro* anti-inflammatory, Protein denaturation.

Introduction

Inflammation is the host response to tissue injury and plays a central role in the pathogenesis of various diseases. The classical signs of acute inflammation include pain, heat, redness, swelling and loss of function. Neutrophils act as the first line of defence followed by the macrophages, T cells and B cells. Once they are activated, they produce inflammatory mediators such as anaphylotoxins of the complement cascade, kinins of the coagulation system, leukotrienes, prostaglandins and neuropeptides. Cytokines responsible for early responses are IL- 1α, IL-1β, IL-6 and

TNF-α. Other pro-inflammatory mediators include leukemia inhibiting factor (LIF), IFN-γ, oncostatin M (OCM), ciliary neurotrophic factor (CNTF), transforming growth factor beta (TGF-β), IL-11, IL-12, IL-17, IL-18, IL-8 and a variety of other chemokines that attract inflammatory cells .

The indigenous system of medicine like Siddha, Ayurveda and Unani is said to be the milestones of Indian system of medicine. India is the birth place of this system of medicine in which herbs are used as the ailments to treat human diseases. These herbs were replaced by the synthetic drugs for a short while, but for the last few years they have come back to their position due to their effectiveness without side effects which are prominent in the allopathic system of medicine. It is used in the prevention, diagnosis and treatment of diseases in addition to their nutritional values.

Myristica fragrans is the drug obtained from the plant origin, which is used as a spice and flavouring agent in the food industry. It is commonly known as nutmeg. It consists of dried kernels and mace of *Myristica fragrans* belonging to the family Myristicaceae**.** The *Myristica fragrans* contains mainly four parts which include skin, fruit, mace and seed.

Fruit is the pericarp which is fleshy, mace is the arillus which covers the hard endocarp. It is brilliant scarlet when fresh and turns horny, brittle, brownish yellow on drying. It contains large amounts of oil. The seed is firm, fleshy and whitish in appearance. Nutmeg is traditionally used widely in the treatment of rheumatism, diarrhoea, stomach cramps, flatulence, anxiety, anti-inflammatory, aphrodisiac. Myristicin is the main psychoactive constituent of nutmeg. It is also the major component of the aromatic ether fraction of the essential oil of mace which provides anti-inflammatory activity.

Fig. 1: Seed and arillus of Nutmeg.

Gels are a dispersion of molecules of a liquid within a solid in which solid is the continuous phase and liquid is the dispersed phase. Gels consist of a three-dimensional network that spans the volume of a liquid medium. The internal

network structure may result from physical bonds or chemical bonds, as well as crystalline or other junctions that remain intact within the extending fluid.

The types of gel include hydrogel, organogels, xerogel, nanocomposite hydrogels. The present study was performed to compare the *invitro* anti-inflammatory effect of gel prepared out of *Myristica fragrans* seed extract with marketed gel using protein denaturation technique.

Materials and Methods

Materials

In India, nutmeg is mainly cultivated in Thrissur, Ernakulam and Kottayam districts of Kerala and parts of Kanyakumari and Tirunelveli districts in Tamilnadu. Seeds and arillus were collected from Piravam (Ernakulam district) in Kerala. It was identified and authenticated by Dr. L. Jose, M.Sc. Ph.D, Department of Botany St. Albert's College, Ernakulam, Kerala, India with specimen number Auth 09 -21. Marketed Diclofenac gel was used as the standard, which was bought from AIMS hospital pharmacy Kochi, Kerala, India.

Methods

Preliminary characterization of *Myristica fragrans* arillus and seeds

The *Myristica fragrans* arillus and seeds are characterized for its properties like colour and its characters.

Preparation of *Myristica fragrans* powder

After collection of plant materials, seeds and arillus were washed thoroughly under running tap water. They were dried under shade and coarse powdered using mortar and pestle and was stored in an airtight container.

Fig. 2: *Myristica fragrans* powder.

Extraction of *Myristica fragrans* powder (Maceration)

Extraction of *Myristica fragrans* powder was performed by maceration technique. 150 gm of nutmeg powder was weighed and transferred to a 500 ml beaker. 300 ml of ethanol (95%) was added to the beaker, the contents were

mixed thoroughly and where kept undisturbed for 7 days. After 7 days the content was filtered with muslin cloth. The filtrate was collected and allowed to evaporate for another 7 days. The ethanolic extract was collected and stored in desiccator for further use.

Organoleptic evaluation of *Myristica fragrans* extract

The organoleptic evaluation of *Myristica fragrans* extract refers to the evaluation of colour, odour etc.

Percentage yield of *Myristica fragrans* extract

The percentage yield of *Myristica fragrans* extract was calculated by following equation

Percentage yield = weight of extract / total weight of seeds *100

Phytochemical screening of *Myristica fragrans* extract

The ethanolic extract of *Myristica fragrans* was tested for the presence of various phytoconstituents like carbohydrates, proteins, glycosides, amino acids, flavonoids, alkaloids etc.

Identification of Myristicin compound in the *Myristica fragrans* extract

Myristicin is the active compound which produces the anti-inflammatory effect of nutmeg. The presence of Myristicin in the ethanolic extract of nutmeg was identified using TLC (Toluene: Ethyl acetate (93:7) and the obtained R_f value was compared with the standard Rf value of Myristicin

R_f value = Distance travelled by the sample / Distance travelled by the solvent front

Formulation of topical gel containing *Myristica fragrans* extract

Table-1: Composition of topical *Myristica fragrans* gels formulation.

SL. NO	INGREDIENTS	MF_1	MF_2	MF_3
1	*Myristica fragrans* extract	1 ml	1 ml	1 ml
2	Carbopol 934	250 mg	200 mg	150 mg
3	Propylene glycol	2 ml	2 ml	2 ml
4	Ethanol (95%)	4 ml	4 ml	4 ml
5	Methyl paraben	0.12 gm	0.12 gm	0.12g
6	Triethanolamine	0.01 ml	0.01 ml	0.01 ml
7	Piperine	0.05 mg	0.05 mg	0.05 mg
8	Purified water	Q.S to 20 gm	Q.S to 20 gm	Q.S to 20gm

Preparation of the *Myristica fragrans* gel

20 gm *Myristica fragrans* gel was prepared by simple dispersion method. Different concentrations of Carbopol 934 were used to prepare the formulation. Required amount of Carbopol 934 and adequate amount of purified water was taken in a beaker. It was mixed to get a uniform mixture using magnetic stirrer and kept overnight for swelling. 1 ml of the ethanolic *Myristica fragrans* extract and Piperine was dissolved in ethanol and added to the polymer solution along with propylene glycol and methyl paraben with gentle stirring. Few drops of triethanolamine was added and mixed well which result in the formation of *Myristica fragrans* gel.

Evaluation of formulated Gels

Physical evaluation

Physical parameters of the formulated gels such as clarity, colour, appearance, odour were checked

Homogeneity

The gel was tested for homogeneity by visual inspection after the gels have been set in the container, for the presence of any aggregate.

Measurement of pH

The gel was placed in digital pH meter, measured the pH in triplicate and average values were calculated

Spreadability

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2 g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A 1 kg weighted was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 g. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability.

Spreadability was calculated using the following formula:

$$S = M \times L / T$$

Where,

S = spreadability

M = is the weight in the pan tied to the upper slide

T = time take to separate the slide completely from each other

Extrudability: Extrudability is the force required to exude material out of the tube; determining the consistency of preparation. It was measured using closed collapsible tube filled with formulated gel.

The weight in gram required to extrude 0.5cm ribbon of the gel in 10 sec was noted. Extrudability was calculated using the formula,

Extrudability =Applied weight to extrude gel from tube (in gm) / Area (in cm^2)

Washability

Formulated *Myristica fragrans* Gels was applied to the skin and washability was determined.

***Invitro* Anti-inflammatory activity**

Inhibition of protein denaturation

The *in-vitro* anti-inflammatory activity of formulated gel of *Myristica fragrans* was evaluated by inhibition of protein denaturation method. 10 mg of *Myristica Fragrans* gel were transferred to a 100 ml flask previously washed with distilled water and DMF.

The volume was made up with phosphate buffer (0.2 M, pH 7.4). The different concentrations were pipetted out into a 10ml standard flask and volume was made up with phosphate buffer. 1.5 ml of solution were pipetted into a clean test tube into which 1.5 ml of Bovine Serum Albumin (1.329 gm in 10 ml phosphate buffer). This mixture was kept at room temperature for 10 minutes, followed by incubated at 27 ± 1^0C for 15 min.

The resulting solution was cooled down and absorbance was recorded at 660 nm. Marketed Diclofenac gel was taken as a positive control. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using:

% Inhibition = 100× [Vt/Vc-1]

Where, V_t **=** absorbance of test

V_c = absorbance of control

Results

Myristica fragrans arillus and seeds were found to be orange - red and brown in color respectively, and oval in shape with striations on it. The ethanolic extract of *Myristica fragrans* is orange red in colour, aromatic in nature (Fig 3). The percentage yield of the ethanolic extract is 9.16%.

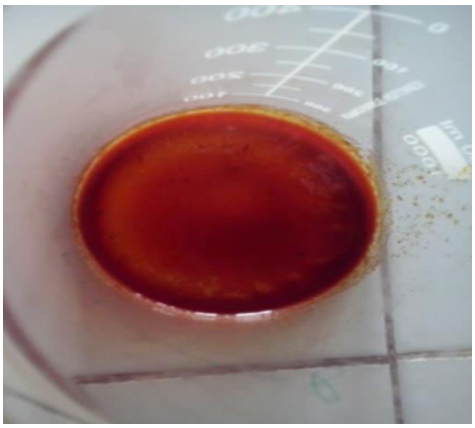


Fig. 3: Extract from seeds of *Myristica fragrans*.

Phytochemical screening of *Myristica fragrans* extract (Table 2).

Table-2: Phytochemical screening of *Myristica fragrans* extract.

Chemical test	Report
Carbohydrates	
Molish's Test	-
Barfodd's Test	-
Benedict's Test	-
Fehling's Test	-
Glycosides	
Legal's Test	-
Borntrager's Test	-
Proteins and aminoacids	
Ninhydrin Test	-
Flavonoids	
Shinoda's Test	+
Lead acetate Test	+
Alkaloids	
Dragendroff's Test	-
Mayer's Test	-
Wagner's Test	-
Hager's Test	-
Tannins	
Ferric chloride Test	+

Identification of Myristicin in the *Myristica fragrans* extract

Four spots were obtained by TLC and R_f values were calculated to obtain **0.7,** 0.53, 0.38, 0.25 from which 0.7 determines the reference R_f value of Myristicin (Fig. 4)

Fig.4: TLC of *Myristica fragrans* extract in Toluene: Ethyl acetate (93:7).

Formulated topical *Myristca fragrans g*el

The *Myristca fragrans g*el was prepared (Fig. 5), using three different concentrations of Carbopol 934 and were further subjected to physicochemical evaluation of various parameters (Table 2).

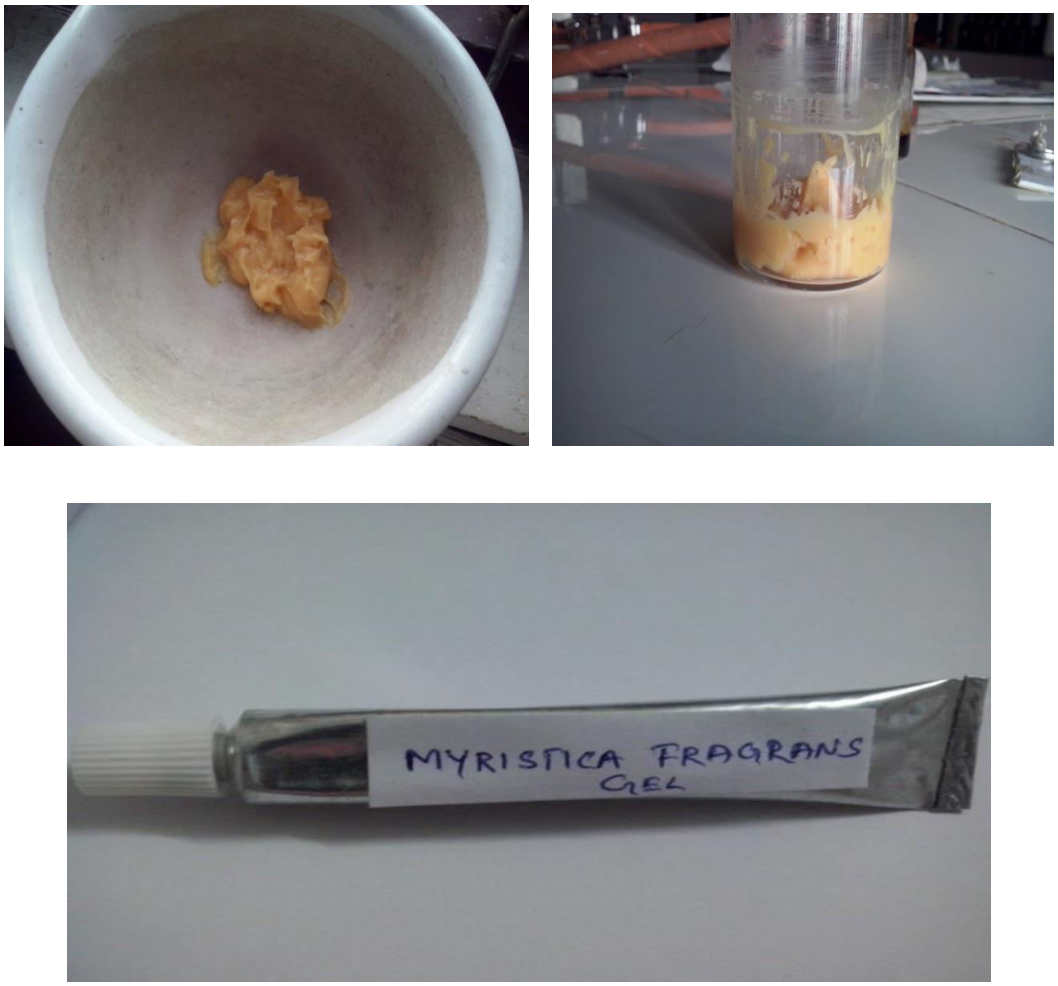

Fig. 5: Formulated *Myristica fragrans* gel.

Table-2: Physicochemical evaluation parameters of formulated gel.

Parameters	MF_1	MF_2	MF_3
Colour	brownish yellow	brownish yellow	brownish yellow
Odour	aromatic	aromatic	aromatic
Clarity	+	++	++
Homogeneity	+	++	+
Spreadability (gcm/sec)	1.1 ± 0.01	1.6 ± 0.08	0.8 ± 0.03
Extrudability (g/sec)	0.9 ± 0.07	0.79 ± 0.13	0.1 ± 0.10
pH	6.4 ± 0.04	6.4 ± 0.08	6.4 ± 0.02

(+ Satisfactory, ++ good)

Optimization of Batch

***Invitro* Anti-inflammatory activity**

Invitro anti-inflammatory activity of the optimized gel (MF2) was done by Albumin denaturation method. (Fig. 6).

Table-8: Percentage Inhibition of gel (MF2).

SL NO	CONCENTRATION(mcg/ml)	% INHIBITION
1.	10	29.6 ± 0.12 %
2.	20	38.7 ± 0.10 %
3.	30	66 ± 0.01 %

Table-9: Percentage Inhibition of marketed gel formulation.

SL NO	CONCENTRATION(mcg/ml)	% INHIBITION
1.	10	9.1 ± 0.11 %
2.	20	43.32 ± 0.03 %
3.	30	63.7 ± 0.05 %

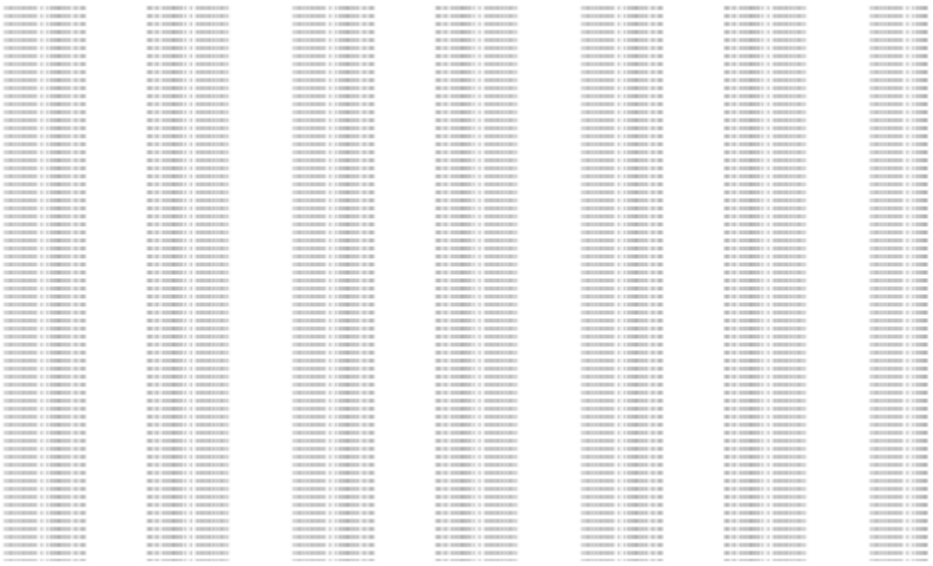

Fig. 6: Percentage inhibition of gels

Discussion

Myristica fragrans arillus and seeds were characterized for its colour, nature and properties, *Myristica fragrans* arillus and seeds were coarsely powdered using mortar and pestle, the powder was extracted by maceration technique. The ethanolic extract of *Myristica fragrans* was organoleptically evaluated. The phytochemical screening of the ethanolic extract of *Myristica fragrans* showed positive results for flavonoids and tannins. The flavonoids have potent anti-inflammatory activity by inhibiting prostaglandin synthesis. Identification of Myristicin was identified by TLC spot and it was obtained in R_f value 0.7. The *Myristca fragrans g*el was formulated using three different concentrations of Carbopol 934 and were further subjected to physicochemical evaluation of various parameters. The formulated gel was found to be brownish yellow in colour with aromatic odour. MF2 and MF3 were showed more clarity and MF1 was more homogenized than MF1 and MF2. The spreadability of the gel MF2 was found to be 1.6 ± 0.08 gcm/sec which shows that the gel have a good spreadability and hence, easy to apply. The extrudability of the gel MF2 was found to be 0.79 ± 0.13g/sec which shows that the gel can easily come out through the tube without application of much pressure. The gel MF2 shows a pH of 6.4 ± 0.08 which is similar to the skin pH and compatible for topical preparation. All formulated gels were found to be easily washable from the skin. After analysis of all batches of formulations for their evaluation parameters like pH, Viscosity, Spreadability, and Extrudability, the formulation MF2 gel was showed good results. Hence, MF2 batch was used for further evaluation of *in-vitro* anti-inflammatory study. *Invitro* anti-inflammatory activity of the optimized gel (MF2) was done by albumin denaturation method. The results indicated that myristicin present in arillus and seeds were responsible for anti-inflammatory

action. The anti-inflammatory property of Myristicin was due to inhibition of chemokines, cytokines. Ethanolic extract of nutmeg seed showed high anti-inflammatory activity by inhibiting the inflammatory cytokines. The percentage inhibition of formulated gel (MF2) was showed better results compared to marketed Diclofenac gel. The IC_{50} value of marketed gel and the formulated gel was found to be 24.15mcg/ml, 22.87 mcg/ml respectively. The *Myristica fragrans* gel shows slight increase in percentage inhibition compared to marketed gel and hence the formulated gel of *Myristica fragrans* have better anti-inflammatory activity compared to the marketed gel.. The clinical applications of topical *Myristica fragrans* gel were anti-inflammatory activity, antioxidant activity, antimicrobial activity, antibacterial activity, and pesticidal activity. Other medicinal plants having similar anti-inflammatory potential were ethanolic extracts of the roots of *Mangifera indica, Ricinus communis,* leaves of *Adhatoda vasica. Myristica fragrans* gel is possibly safe use for topical applications.

Conclusion

In recent years there has been a tremendous increase in the inflammatory disorders in the society. The use of NSAIDs and DMARDs were common in population and their demand weree being increased. In this study we have formulated and prepared an anti-inflammatory topical gel of *Myristica fragrans* which is a common drug used in Ayurvedic system of medicine and a spice used in daily life. Various physicochemical evaluation parameters along with *invitro* anti-inflammatory activity of the gel were performed. The formulated gel showed good clarity, homogeneity, and odour. spreadability, washability and extrudability. It also has excellent *invitro* anti-inflammatory activity than the marketed Diclofenac gel. Thus it surely will improve the patient compliance and safety profile.

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