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Original Article

EVALUATION OF *MYRISTICA FRAGRANS* AS A PENETRATION ENHANCER IN TRANSDERMAL GEL FORMULATION

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ABSTRACT

Objective: Skin delivery of NSAIDs offers several advantages over oral route associated with potential side effects. The study aims at exploring the potential of *Myristica fragrans* as a penetration enhancer (PE) for transdermal gel of Diclofenac sodium.

Methods: In the present work, methanol extract (ME), chloroform extract (CE) and n-hexane extract (NHE) of *Myristica fragrans*were subjected to preliminary phytochemical screening and TLC. These extracts were evaluated for enhancing *in vitro* & *ex vivo* percutaneous absorption in comparison with synthetic enhancer (SE). The study was performed for Diclofenac sodium as target drug formulated in gel form with Ethanol (95%) as a solvent & carbopol 934p as a gelling polymer using Franz diffusion cell. It was evaluated for different physicochemical parameters.

Results: The different extracts showed the presence of carbohydrates, fats and oils, volatile oil and flavonoids. The gel formulation complied with various physicochemical parameters for evaluation like odour, consistency, grittiness, pH (6.7), stickiness, uniformity, spreadability (6.7 g. Cm/Sec) & viscosity (10160cps). *In vitro* and *Ex vivo* studies showed that both ME and CE showed better % cumulative release (% CR), hence better penetration effect as compared to the SE. Maximum % C. R (27.97) and flux (151.45) was found in a P1H2 batch (ME) while the next highest was P1H4 (CE). The % C. R and flux of gel with synthetic enhancer were low, i. e. 20.8% and 112.62 respectively. Maximum enhancement ratio (ER) (1.913) was observed in P1H2 (ME) batch.

Conclusion: ME and CE may serve as potential penetration enhancers for industrial benefit.

Keywords: Diclofenac sodium, Evaluation, *Myristica fragrans,* Penetration enhancer, Percutaneous absorption.

INTRODUCTION

In the last few decades, there has been exponential growth in the field of herbal medicine. It is getting popularized in developing as well as in developed countries owing to its natural origin and lesser side effects. Herbs are not only utilized as drugs in oral dosage forms but have captured a separate place in the transdermal formulations as well. The transdermal route of administration has been recognized as one of the potential routes for the local and systemic delivery of drugs. The transdermal drug delivery shows fewer side effects as compared to oral [1] and also the dose is reduced. It gives sustained release of drugs to maintain steady state plasma concentration of drugs with short half-life. Other advantages of transdermal drug delivery include better patient compliance and avoidance of the first pass metabolism effect of drugs with poor bioavailability. However a layer of skin called as 'stratum corneum' forms a barrier for the poorly penetrating drugs. Hence penetration enhancers are used to increase the penetration of the drug through the skin. The penetration enhancers act by reducing the viscosity of the mucus, disruption of intercellular lipid, loosening the tight junctions [2]. In this way, increased partitioning of the drug into the tissue can be seen. In the present study Diclofenac sodium was used. Diclofenac sodium is an NSAID type of drug which is widely used for the diseases like rheumatoid arthritis, which is the most common form of arthritis. As per CDC (centers for disease control and prevention) survey, around 22.2% (49.9 million) of adults reported arthritis, with significantly higher age related prevalence in women than in men. By the year 2030, an estimated 67 million adults aged 18 years and older will have doctor-diagnosed arthritis, compared with the 50 million adults in 2007–2009 [3].

When diclofenac sodium is given orally it shows several adverse effects associated with NSAIDs like gastrointestinal effects, bleeding, perforation, ulceration, CNS effects, skin rashes, allergic reactions [4] etc. This problem can be avoided by giving the diclofenac sodium transdermally. Nowadays the gel form of the drug is widely used to avoid the side effects. For the better permeation of the drug various penetration enhancers are used in the formulation. Synthetic penetration enhancers show various side effects and hence they can be replaced by herbal penetration enhancers [5]. There are many evidences indicating the use of herbs as penetration enhancers e. g. cineole [6], menthol [7]. The objective of the study was to explore the use of *Myristica fragrans* (nutmeg) as an herbal penetration enhancer to develop the transdermal gel formulation containing Diclofenac sodium. Thus, it is an effort to avoid the side effects of oral drug delivery as compared to transdermal delivery and also improving patient compliance for the treatment.

For the present work, nutmeg was chosen as the herbal drug for the study. Nutmeg is obtained from seeds of *Myristica fragrans* belonging to family *Myristecaceae.* It mainly contains α-pinene, camphene, β-pinene, sabinene, myrcene, α-phellandrene, α-terpinene, limonene, 1, 8-cineole, linalool, terpinen-4-ol, safrole, methyl eugenol, elimicin and myristicin [8-10].

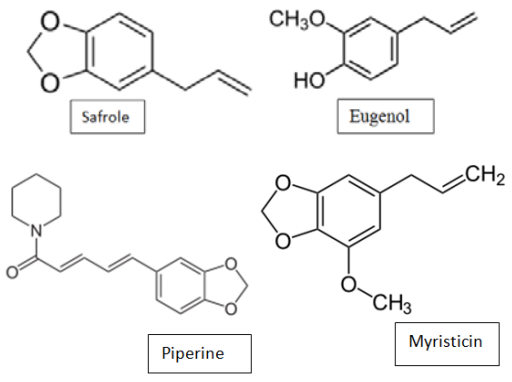

Fig. 1: Structures of various constituents of *Myristica fragrans* and some already proven natural penetration enhancers [11, 12].

The terpenes and essential oils have shown penetration enhancing activity [13]. Also natural penetration enhancers are cost effective [5].

MATERIALS AND METHODS

Materials

Chemicals

Diclofenac sodium (Torrent Pharmaceuticals, Ahmedabad, India), Potassium dihydrogen phosphate, Sodium chloride, N-hexane, Chloroform, Triton X (All from Loba Chemie Pvt. Ltd., Mumbai, India), Disodium hydrogen phosphate, Methanol, Triethanolamine (All from Merck Specialities Pvt. Ltd., Mumbai) Carbopol 934 (Oxford laboratory, Mumbai), HPMC K100M (Colourcon Asia Pvt. Ltd., Goa) carbopol 974p (Lubrizol advanced materials, Inc., Cleveland) (All the chemicals used were of AR).

Equipments

UV Visible Spectrophotometer (ShimadzuUV-1800, Japan), Diffusion cell apparatus (MFDC06, Orchid scientific, Nashik, India), Single Pan Electronic Balance (Shimadzu AY-120, Japan), Digital pH meter (Model EQ-610, Equiptronics, Mumbai, India), Brookfield Viscometer (Model CAP 2000 + L, Brook field Engineering Laboratories, Inc.

Massachusetts, (USA) animals

Whistar rats (150-200g) were maintained in uniform laboratory conditions for acclimatization before performing experiments. Institution animal ethics committee has approved the protocol and the protocol no. is SCOP/IAEC/2012-13/36.

Methods

Collection and authentication of *Myristica fragrans* seeds

The *Myristica fragrans* seeds were purchased from the local market of Pune, Maharashtra and submitted to Agharkar Research Institute (ARI), Pune for authentication.

Preliminary characterization of *Myristica fragrans* seeds

The *Myristica fragrans* seeds and characterized for its properties like colour and weight per seed, and other external characters along with the microscopic features.

Extraction scheme (Maceration)

The extraction was carried out by successive solvent extraction using maceration technique. 100g powder was macerated with n-hexane and extract was obtained after 3 days by vacuum filtration. Then same powder was macerated with fresh n-hexane. Marc obtained after filtration was air dried for 2 days and the same marc was used for extracting with chloroform and then methanol.

Organoleptic evaluation of extract

The organoleptic evaluation refers to the evaluation of colour, odour and special features which include touch and texture. The majority of information on the identity, purity and quality of the material can be drawn from these observations.

% Yield of *Myristica fragrans* seeds extract

The % yield of extracts of seeds was calculated by following Equation

% yield of extracts of *Myristica fragrans* = weight of extract/total weight of seeds *100

Preliminary phytochemical screening of extracts of *Myristica fragrans* seeds

To determine the presence of secondary metabolites from various chemical classes, tests for carbohydrates, proteins, fats and oils, steroids, volatile oils, alkaloids and terpenes etc. were carried out [14].

Chromatographic study of various extracts

Thin layer chromatography (TLC) was carried out using toluene-ethyl acetate (93:7) as a solvent system and vanillin- sulfuric acid as a detection reagent [15]

Preformulation studies of diclofenac sodium

- Melting point
- Determination of solubility: Solubility of diclofenac sodium was determined in water, phosphate buffer saline (PBS) pH 7.4, ethanol, and methanol. 10mg of diclofenac sodium was added to 10 ml of media and solubility was checked at room temperature. [16]
- FTIR: FTIR spectroscopic studies were done to check the interactions of drug and the extracts of nutmeg
- UV-VIS Spectrophotometric method for diclofenac sodium

a. Selection of solvent

Phosphate buffer saline pH 7.4 was selected for analytical purpose; as PBS pH 7.4 is the media for the diffusion study.

b. Study of Beers-Lambert's law

Stock solution of 100µg/ml was prepared. From this solution, solutions of concentrations 4, 8, 12, 16, 20µg/ml were prepared. Absorbances of these solutions were plotted against concentration to obtain calibration curve. UV analysis was carried out at 276 nm.

Formulation of topical gels

Selection and optimization of gelling agents

In the preliminary study, various gelling agents like Carbopol 934p, Carbopol 974p, and HPMC K100M were tried in different concentrations. The concentration of gelling agent was optimized based on the physical characteristics of the formed gel.

Selection of PEs

In order to achieve the increased flux, selection of the best PEs was done. Triton X was used as a synthetic penetration enhancer (SPE). Nutmeg was selected as an herbal proposed penetration enhancer.

Table 1: Formulation of topical gels using different gelling agents

Ingredients %w/w	P1	P2	P3	P4	P5
Carbopol 934p	1	1.5	-	-	-
Carbopol 974p	-	-	1	1.5	-
HPMC K100M	-	-	-	-	1
Diclofenac sodium	1	1	1	1	1
Triethanolamine (50%)	q. s	q. s	q. s	q. s	-
Methyl paraben	0.02	0.02	0.02	0.02	0.02
Propyl paraben	0.1	0.1	0.1	0.1	0.1
Ethanol (95%)	10	10	10	10	10
DW up to (ml)	10	10	10	10	10

Preparation of the gel

Gelling agent was mixed with a small amount of distilled water. Then pH of carbopol gels was adjusted to neutral by the addition of 50% Triethanolamine. The drug was dissolved in ethanol (95%) and then was mixed with the solution of the gel. Then to this mixture preservatives were added and the volume was made up to 10 ml using distilled water. Initially gels were prepared without using PE.

But after optimization of the final batch, penetration enhancers were incorporated in the respective gel formulation.

Evaluation of gel

Appearance

The formulations were observed for their visual appearance, odour, colour, texture, and feel upon application such as grittiness, greasiness, stickiness, smoothness, stiffness and tackiness [17].

pH measurement

The pH of prepared gels was measured using a pH meter. The pH meter was calibrated before each use with standard pH 4 and 7 buffer solutions.

***In vitro* and *ex vivo* permeation study**

The cellulose acetate membrane was used for the *in vitro* permeation study and rat skin was used for *ex vivo* permeation study. 0.5g of gel formulation was applied uniformly onto the membrane. The membrane was then mounted between the donor and receptor compartment of diffusion cell.

Modified Franz diffusion cells [18] were placed on six station magnetic stirring unit. A drug permeation study was carried out for 6 hours at 37 °C. Absorbance of the solutions was measured spectrophotometrically. Drug permeation was calculated by the calibration curve method. *In vitro* permeation study was carried out for the gels with PE as well as gel without PE. But *ex vivo* permeation study was done only for gels with PE.

Flux determination (J)

Feasibility of the drug to pass through the membrane and enter into blood circulation following application of transdermal formulation can be predicted by using the flux calculation.

Flux is the amount of drug (µg) flowing per unit area through the barrier (cm^2) in unit time (hour).

Flux (J) = dM/S. dt [2]

Enhancement ratio (ER)

It is the ratio of the flux value with enhancer to that of flux value without enhancer. [19]

RESULTS AND DISCUSSION

Authentication of *Myristica fragrans* seeds

The *Myristica fragrans* seeds were authenticated at the Agharkar Research Institute (ARI), Pune with specimen number Auth 12-189

Preliminary characterization of *Myristica fragrans* seeds

Myristica fragrans seeds were found to be brown in colour, oval in shape with striations on it. Powder microscopy [14] revealed the presence of lignified tissue and starch grains in abundance.

Organoleptic evaluation and % yield of extract

All the extracts were characterized by various organoleptic properties such as colour, odour, and taste.

Table 2: Organoleptic characteristics of extract with % yield

S. No.	Evaluation	Observation		
		NHE	CE	ME
1	Colour	Yellow	Dark orange	Dark red
2	Odour	Aromatic and characteristic	Aromatic and characteristic	Aromatic and characteristic
3	%yield	9.06	7.65	5.2

Preliminary phytochemical screening

All the three extracts showed the presence of carbohydrates, fats and oils, volatile oil while only CE and NHE showed the presence of flavonoids.

Chromatographic study of extracts of nutmeg

As in table 3.

Preformulation studies of diclofenac sodium

- Melting point: 283 °C
- Determination of solubility: Diclofenac sodium was sparingly soluble in water. Diclofenac sodium was freely soluble in methanol, soluble in95% ethanol.

Therefore, 95% ethanol was used as a solvent for the formulation of gel

- FTIR study

IR interpretation showed that there is no interaction between herbal extract and diclofenac sodium.

Table 3: Chromatographic evaluation of extracts of nutmeg

S. No.	Extract	Retention factor (Rf) (observed)	Probable component
1	CE	0.684	Myristicin
2	ME	0.232	Geraniol or borneol
3	NHE	0.643	Myristicin
		0.986	Safrole

TLC character (table no. 3) and these constituents might be responsible for penetration enhancement activity.

The IR spectrum of diclofenac sodium shows peaks at 1573.26 cm^{-1} due to –C=O stretching of carboxyl ion and at 748.44 cm^{-1} owing to C-Cl stretching. Peak at 1452.97 cm^{-1} is observed due to benzene bonds (-C=C- stretching). These peaks were also found in the IR spectrum of a mixture of diclofenac sodium and chloroform extract. But slight shifting of the peaks is observed in the IR spectrum of a mixture of diclofenac sodium and n- hexane extract. This may be the possible reason of the reduced % C. R of P1H6.

- **UV-VIS Spectrophotometric method for diclofenac sodium**

UV-VIS Spectrophotometric results of diclofenac sodium were found to be linear in the stated concentration range and λ_{max} was found to be 276 nm which was matched with the literature.

Formulation of topical gel

- **Selection and optimization of gelling agents**:

Carbopol 934p [20], carbopol 974p, HPMC K100M were evaluated for gelling ability. The best results of gel formulation were obtained using carbopol 934p as gelling agent. Amongst batches P1 to P5 (table no. 1), P1 was selected based on the properties like viscosity, clarity and transparency of the gel (table No. 4).

Batches of Gel containing penetration enhancers

As in table 5.

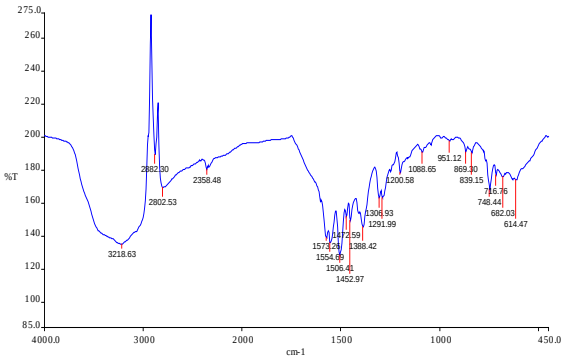

Fig. 2: IR studies of Diclofenac sodium

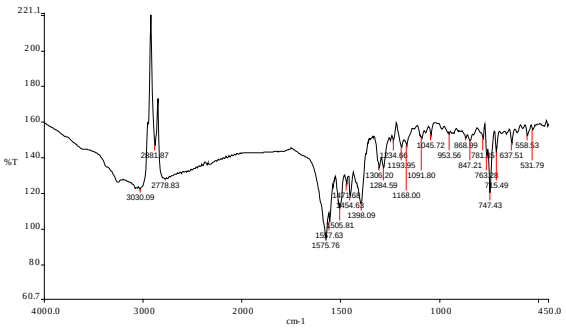

Fig. 3: IR studies of combination of diclofenacsodium and CE

Evaluation and characterization of optimized preliminary gel containing diclofenac sodium

As in table 4.

Evaluation and characterization of gel batches containing PE

As in table 6.

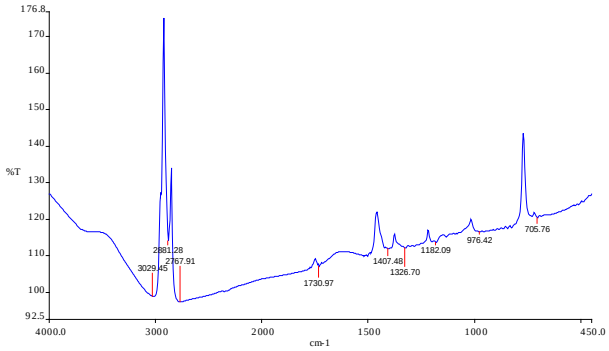

Fig. 4: IR studies of combination of diclofenac sodium and NHE

Table 4: Evaluation and characterization of optimized preliminary gel containing diclofenac sodium

S. No.	Properties	Observations
1	Colour	Transparent
2	Odour	Slightly alcoholic
3	Consistency	Smooth
4	Grittiness	None
5	Uniformity	Good
6	Stickiness	None
7	Viscosity	10160cps
8	Clarity	Clear
9	pH	6.7
10	Spreadability	6.7 (g. Cm/Sec)

Table 5: Batches of Gel containing penetration enhancers

S. No.	Ingredients (%w/w)	P1H1	P1H2	P1H3	P1H4	P1H5	P1H6	P1S1	P1S2
1.	Diclofenac sodium	1	1	1	1	1	1	1	1
2.	Carbopol 934p	1	1	1	1	1	1	1	1
3.	ME	1	2	-	-	-	-	-	-
4.	CE	-	-	1	2	-	-	-	-
5.	NHE	-	-	-	-	1	2	-	-
6.	Triton X 100	-	-	-	-	-	-	1	2
7.	Methyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
8.	Propyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
9.	Ethanol (95%)	10	10	10	10	10	10	10	10
10.	Triethanolamine	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S
11.	DW up to (ml)	10	10	10	10	10	10	10	10

Table 6: Evaluation and characterization of gel batches containing PE

S. No.	Properties	P1H1, P1H2	P1H3, P1H4	P1H5, P1H6	P1S1, P1S2
1	Colour	Pale yellowish	Slightly reddish	Pale yellowish	Transparent
2	Odour	Very slight alcoholic	Very slight alcoholic	Very slight alcoholic	Very slight alcoholic
3	Consistency	Smooth	Smooth	Smooth	Smooth
4	Grittiness	Very less	Very less	Very less	None
5	Uniformity	Good	Good	Good	Good
6	Stickiness	None	None	None	None
7	Clarity	-	-	-	Clear

***In vitro* permeation study**

The formulated gels were subjected to permeation study through a cellulose acetate membrane, to establish % cumulative release (%CR) Initially, batch of gel without PE was studied. Batches of gel containing 1% concentrations of penetration enhancer did not show good results, hence were rejected.

Flux determination: (µg/cm²/h) -(*In vitro* permeation study)

In table 8.

Enhancement ratio (ER)

In table 9.

Table 7: % CR of in vitro permeation study of P1

Time (hours)	% CR
0.5	3.92
1	5.28
2	7.7
3	9.82
4	11.06
5	12.92
6	14.62

% C. R for *in vitro* permeation study of P1S2, P1H2, P1H4, P1H6 was found to be 20.8, 27.97, 23.4, 13.31 respectively at the end of 6 hours.

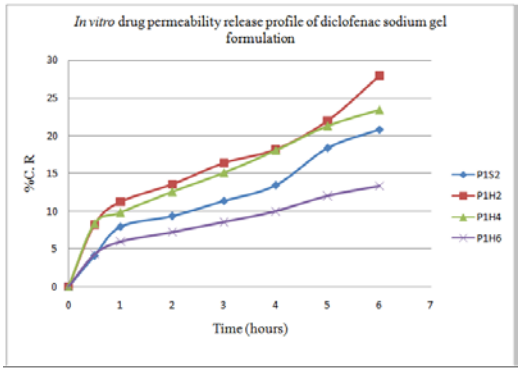

Fig. 5: *In vitro* drug permeability release profile of diclofenac sodium gel formulation

Table 8: Flux (µg/cm²/h) for *in vitro* permeation study

Batch name	Flux
P1	79.16
P1S2	112.62
P1H2	151.45
P1H4	126.7
P1H6	72.07

Table 9: ER for *in vitro* permeation study

ER	Flux
P1S2 (triton X)	1.422
P1H2 (ME)	1.913
P1H4 (CE)	1.6
P1H6 (NHE)	0.9104

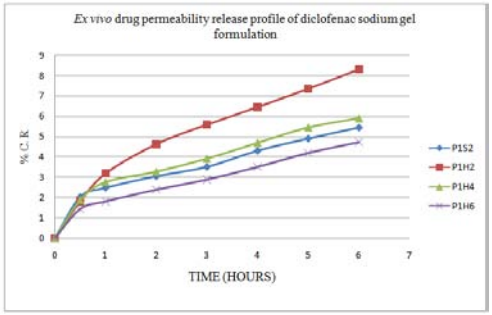

Fig. 6: *Ex vivo* drug permeability release profile of diclofenac sodium gel formulation

***Ex vivo* permeation study**

The formulated gels were subjected to permeation study through rat skin to establish %CR

% C. R for the *ex vivo* permeation study of P1S2, P1H2, P1H4, P1H6 was found to be 4.92, 7.37, 5.44, 4.2 respectively at the end of 6 hours.

Flux determination: (µg/cm²/h)- (*Ex vivo* permeation study)

In table 10.

Table 10: Flux (µg/cm²/h) for *ex vivo* permeation study

Batch name	Flux
P1S2	26.64
P1H2	39.9
P1H4	29.45
P1H6	22.74

This indicates that gels containing 2% penetration enhancer showed better % cumulative release. In *in vitro* studies maximum % C. R (27.97), flux (151.45) and ER (1.913) were found in P1H2 batch. Lowest % C. R (13.31%) was found in P1H6 batch. The % C. R and flux of gel with synthetic enhancer was low, i. e. 20.8% and 112.62 respectively. While *ex vivo* studies showed maximum % C. R (7.37), flux (39.9) in P1H2 batch. Lowest % C. R (4.2%) was found in P1H6 batch. The % C. R and flux of gel with synthetic enhancer were found to be 4.92% and 26.64 respectively. This shows that Methanol extract and chloroform extract showed better penetration enhancing ability than synthetic penetration enhancer (Triton X) and n-Hexane extract. As reported in the literature, many terpenes act as permeation enhancers, the penetration enhancement witnessed here may be attributed to Myristicin, Safrole, geraniol or borneol detected in the extracts of *Myristica fragrans.* Piperine being more widely accepted bioenhancer and the constituents of nutmeg like safrole and myristicine share common 1, 3- benzodioxole nucleus in the chemical structure which may have role in enhancing the permeation of drug through the membrane.

CONCLUSION

The above result shows that %CR of diclofenac sodium was found more in the formulation with natural penetration enhancer than the synthetic penetration enhancer. %CR of P1H2 (ME) and P1H4 (CE) are1. 4 and 1.13times greater than that of P1S2 respectively.

Hence this concludes that *Myristica fragrans*can be used as a potent penetration enhancer to avoid the side effects of synthetic penetration enhancer. This penetration enhancement may be attributed to the presence of terpenes in the *Myristica fragrans*.

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CONFLICT OF INTERESTS

There is no conflict of interest amongst the authors of the present work.

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