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Pharmaceutical Development and Evaluation of A Nutmeg-Loaded Topical Ointment for Analgesic and Anti-Inflammatory

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Abstract

Goal: To formulate and evaluate a topical ointment with nutmeg for analgesic and anti-inflammatory properties.
Objectives: To Create a stable ointment with nutmeg extract, analyze its physicochemical characteristics, and test its effectiveness.
Methodology: Nutmeg extract from Myristica frangrans was added to an ointment base and assessed for appearance, pH, Spreadability, viscosity, drug content, and in-vitro release, analgesic and anti-inflammatory properties were evaluated and contrasted with a commercial reference.
Discussion: The formulation shows good physicochemical properties, and a notable decrease in pain and inflammation.
In conclusion, nutmeg-loaded ointment showed good stability and release properties along with promising topical analgesic and anti-inflammatory effects.

1.INTRODUCTION

Origins and initial application: Native to Indonesia's Maluku (Spice) Islands, Myristica fragrans,[1] or nutmeg, has been traded and used medicinally for ages throughout Asia, the Middle East, Europe, and Africa. It was once prized not only as a spice but also as a remedy for fever, discomfort, swelling, and digestive problems.[2]

Unani and Ayurvedic traditions:[3] Nutmeg powder and its oil were administered physically or consumed (in modest amounts) in South Asian medicinal systems such as Ayurveda to treat inflammatory diseases, rheumatic symptoms, and joint and muscular pain.[2]

Other cultures: Nutmeg is frequently used in topical poultices or aromatic formulations in traditional Chinese, and Thailand to treat headache, pain, and inflammation.[2]

Analgesic (Relieving Pain) Impacts Models of animal pain: In a traditional test of analgesic efficacy, nutmeg oil decreased pain responses in mice exposed to acetic acid.

Persistent inflammatory pain: Nutmeg oil decreased mechanical hypersensitivity and heat hyperalgesia in rat models with generated arthritis-like joint swelling[4], most likely via down-regulating COX-2[6] expression and substance P, both important in pain signaling.These pharmacological effects are similar to those of non-steroidal anti-Inflammatory medications (NSAIDs), which disrupt prostaglandin[5] pathways that mediate pain and inflammation. Thieme Connect.[2]

Evidence from Modern Science 1. Inhibition of Inflammation Studies conducted both in vitro and in vivo have shown that nutmeg oil and extracts significantly reduce inflammation in both lab and animal settings.[2]

Mechanism insights: Inflammatory mediators that are essential to the inflammatory process, such as COX-2,[6] iNOS, and nitric oxide generation, can be suppressed by components like myristicin and other phenolic compounds.

Evidence from cell culture: When fibroblast cultures were exposed to an inflammatory stimulation, nutmeg essential oil decreased the release of inflammatory cytokines, such as IL-6.[4]

2. MATERIALS AND METHODOLOGY

Materials: Nutmeg seeds, white soft paraffin, beeswax, cocoa butter, menthol, coconut oil.

Methodology:

Extraction procedure of nutmeg oil

100 grams of crushed nutmeg seeds

↓

Soaked in 600 ml of water overnight

↓

Subjected to distillation with the help of clavenger apparatus

↓

At 60 degrees temperature for 3 hours

↓

3ml of oil is extracted

Fig.1: extraction of nutmeg oil

IDENTIFICATION TESTS:

SNO	TEST	OBSERVATION	COMPOUND	PRESENT/ABSENT
1	Ferric chloride test	Green color	Eugenol	Present
2	Sodium hydroxide test	Yellow color	Limonine	Present
3	Sulphuric acid test	Purple color	Sabinene	Present
4	Libermann's test	Reddish brown	Myristicin	present

Ferric chloride test:

To the 1ml of oil add little amount of ferric chloride and heat it for 5minutes.green color is observed which indicates the presence of eugenol.[7]

SODIUM HYDROXIDE TEST:

to the 1ml of oil sample add small amount of sodium hydroxide.Yellow color is obtained which indicates the presence of limonene.[7]

SULPHURIC ACID TEST:

To the 1ml of oil sample add small amount of sulphuric acid and anhydrous acetic acid.purple color is observed which indicates the presence of sabinene.[7]

TEST FOR MYRISTICIN- LIBERMANN'S TEST

To the 1ml of oil add concentrated sulphuric acid, reddish brown colour is obtained which indicates the presence of myristicin.[7]

TLC OF NUTMEG OIL:

Make a solution of nutmeg oil in chloroform at a concentration of 1 mg/mL. Using an automated applicator, apply 2–5 µL of the sample and standard as 8–10 mm bands to silica-gel HPTLC

plates. In a pre-saturated chamber, develop the plate up to 70–80 mm in toluene:ethyl acetate:formic acid (7:3:0.5, v/v/v). To record Rf values and spot patterns, dry the plate and scan it under UV light at 254 and 366 nm. Measure using densitometry at 254 nm with a myristicin calibration curve (100–800 ng/band).

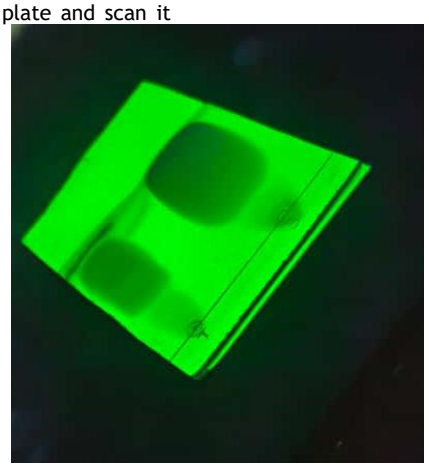


Fig.2: Thin layer chromatography of nutmeg oil

Formulation of ointment:

S.no	Ingredients	Quantity (100gm)	Quantity (10gm)	Purpose
1	Bees wax	10gm	1.0gm	Stiffening agent
2	White soft paraffin	50gm	5.0gm	Ointment base
3	Cocoa butter	20gm	2.0gm	Emollient
4	Menthol	2gm	0.2gm	Cooling counter irritant
5	Coconut oil	Q.s	Q.s	Penetration enhancer
6	Nutmeg oil	5ml	0.5ml	Analgesic & anti inflammatory

Procedure:

Preparation of nutmeg oil ointment by levigation method:[8]

Step 1: weigh all the ingredients bees wax, white soft paraffin, cocoa butter & menthol.

Step 2: place the bees wax, cocoa butter, white soft paraffin & menthol in a porcelain china dish.

Step 3: Melt the above ingredients on a water bath.

Step 4: nutmeg oil is placed on the slab of the tile & small amount of melted base is added slowly along with coconut oil.

Step 5: by using the flat spatula mix the oil & base to create a smooth & homogeneous mixture.

Step 6: transfer the ointment in a suitable container.

Fig.3: preparation of ointment

Evaluation studies:

pH:weigh 1 gm of ointment and transfer it in to a beaker containing 10ml of distilled water. Dispersion is formed allow it for 1 to 2 hours at room temperature. Calibrate the pH meter using standard buffer solution. The electrode of pH meter is immersed in the ointment dispersion. Ph. is adjusted to 5.8 with the help of phosphate buffer.

Preparation of phosphate buffer pH 5.8:

Dissolve 13.69 grams of monobasic potassium phosphate in 800ml of water, to it add 1.7 grams of dibasic potassium phosphate and mix well and make up to 1000ml with water.

Spreadability: place 1gm of ointment in centre of the glass slide .place the second glass slide over the first slide in such a way that ointment is sandwiched between them. Apply a weight of 20gms on the upper slide for 5 minutes to obtain a uniform film. Remove the weight and a string is tied to the upper slide, passing it over a pulley. Attach a weight of 20gms to the free end of the string .now measure the time taken by the upper slide to move a specific distance. Repeat the same procedure for 3 times and calculate the average value.

Spreadability = ML/T

M=weight tied to the upper slide(g)

L=length moved by the slide(cm)

T= time taken(sec)

Viscosity: The Brookfield viscometer is cleaned and calibrated .take sufficient quantity of ointment in to a clean beaker. Remove the air bubbles by gently tapping it ,select a suitable spindle based up on the consistency of ointment and attach it to the viscometer. Immerse the spindle in to the ointment with the speed of 10-20 rpm and temperature of 25 c. switch on the viscometer allow the readings to stabilize and note the viscosity value.

Viscosity = dial reading X factor

Fig.4: viscometer

Franz diffusion

Before being used, the dialysis membrane (MWCO 12,000–14,000 Da) was equilibrated in receptor medium for 30 minutes after being soaked in buffer solution for 12–24 hours to eliminate preservatives. To ensure that no air bubbles were trapped, the hydrated membrane was carefully placed between the Franz diffusion cell's donor and receptor compartments. Phosphate buffer (pH 5.8) was added to the receptor compartment, which was kept at 37 ± 0.5°C with constant magnetic stirring. The donor compartment was equally filled with a measured amount of the ointment mixture. To maintain sink conditions, aliquots were removed from the receptor compartment at prearranged intervals and replaced with an equivalent volume of new buffer. A UV-visible spectrophotometer was used to analyse the samples and calculate the cumulative medication release.

Fig.5. franz diffusion

Design Of Experiment (DOE)

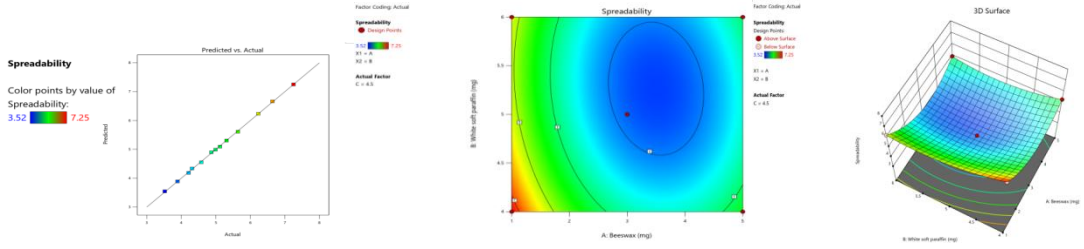
Experimental design

The formulation process was refined using the design of experiments methodology with the aid of design expert 11 software. Statistical analysis was performed using ANOVA and response surface methodology was applied to assess the significance of the variables and there interactions. Contour and 3D plots were instrumental in comprehending the design space and optimising the formulation parameters.

Std	Run	Factor 1 A:Beeswax	Factor 2 B:Whitesoftparaffin	Factor 3 C:Cocoabutter	Response 1 Spreading	Response 2 Viscosity	Response 3 %Drug diffused
		Mg	mg	mg			
4	1	1	1	0			
11	2	0	-1	1			
3	3	-1	1	0			
1	4	-1	-1	0			
5	5	-1	0	-1			
10	6	0	1	-1			
13	7	0	0	0			
12	8	0	1	1			
8	9	1	0	1			
2	10	1	-1	0			
6	11	1	0	-1			
7	12	-1	0	1			
9	13	0	-1	-1			

Response 1: Spreadability

The actual formulations of the equation enables researches to predict response out comes from factor levels. Each factor needs to maintain its original units during specification in the forecasting process. The equation lacks suitability for measuring individual factors influence because the coefficients are modified for unit compatability between factors and the intercept exists outside the design space center.



A. A response surface plot that shows hoe the components interact

B. A predicted versus actual comparision that conforms the model accuracy, and

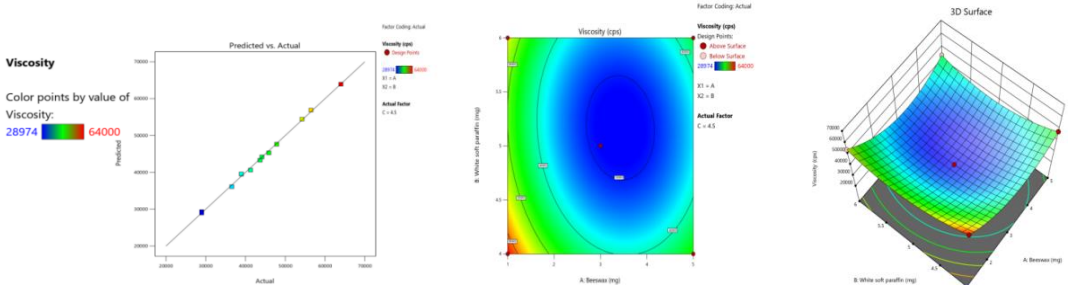
C. A contour plot emphasizes trends in spreadability based on formulation .

Response 2: Viscosity

The equation shows actual factors to make prediction about responses the individual factors settings.The original measurement units needs clarification for every factor at these specified levels. The equation coefficient were adjusted to match each factors units while the intercept does not represent the design space center. Therefore, it is not applicable for measuring factor influence

Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	28974.00	1	851.08	26265.48	31682.52	
A-Beeswax	-6637.63	1	300.90	-7595.23	-5680.02	1.0000
B-White soft paraffin	-3234.25	1	300.90	-4191.86	-2276.64	1.0000
C-cocoabutter	-3749.63	1	300.90	-4707.23	-2792.02	1.0000
AB	1510.00	1	425.54	155.74	2864.26	1.0000
AC	2048.75	1	425.54	694.49	3403.01	1.0000
BC	-1896.00	1	425.54	-3250.26	-541.74	1.0000
A^2	14916.13	1	562.94	13124.61	16707.64	1.35
B^2	8662.38	1	562.94	6870.86	10453.89	1.35
C^2	573.62	1	562.94	-1217.89	2365.14	1.35



A 3D response surface that depicts the viscosity patterns

A predicted versus actual plot that validates the model accuracy, and

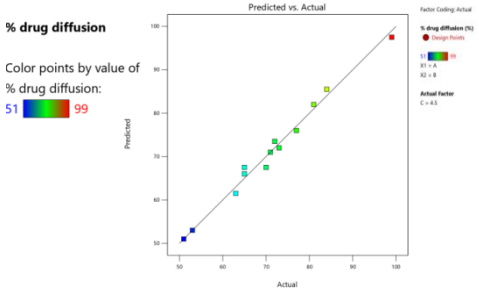
A contour plot that shows hoe viscosity varies with different formulations.

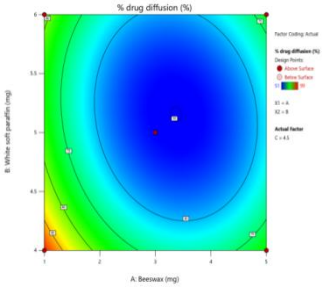
Response 3 : drug diffussion

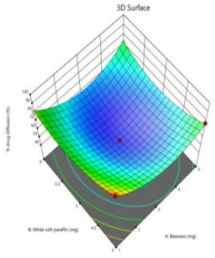
The mathematical formulation of the equation enables researchers to predict the percentage of drug diffusion through the dialysis membrane based on formulation factors. Each factor retains its original unit during the prediction process. The model helps to estimate the effect of beeswax and white soft paraffin concentration on drug release. The coefficients in the equation represent the interaction between formulation variables, while the intercept represents the predicted response at the design center. These graphical models help visualize the diffusion behaviour and optimize the ointment formulation for maximum drug release.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	51.00	1	2.92	41.72	60.28	
A-Beeswax	-7.50	1	1.03	-10.78	-4.22	1.0000
B-White soft paraffin	-4.50	1	1.03	-7.78	-1.22	1.0000

C-cocoabutter	-4.50	1	1.03	-7.78	-1.22	1.0000
AB	3.25	1	1.46	-1.39	7.89	1.0000
AC	2.25	1	1.46	-2.39	6.89	1.0000
BC	-2.75	1	1.46	-7.39	1.89	1.0000
A^2	18.87	1	1.93	12.74	25.01	1.35
B^2	12.38	1	1.93	6.24	18.51	1.35
C^2	1.37	1	1.93	-4.76	7.51	1.35



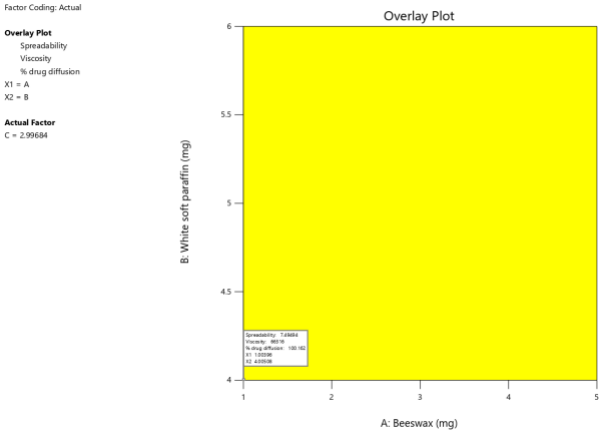




A predicted versus actual plot showing the relationship between experimental drug diffusion values and model-predicted values, confirming the accuracy and reliability of the statistical model.

A contour plot illustrating the influence of formulation variables (beeswax and white soft paraffin) on the percentage of drug diffusion and identifying the region where maximum drug release occurs.

A 3D response surface plot demonstrating the interaction between formulation components and their combined effect on the drug diffusion through the membrane.



OPTIMISED F10 NUTMEG FORMULATION

CHEMICALS	Quantity taken	Purpose
Nutmeg oil	0.5ml	Analgesic and anti-inflammatory
White soft paraffin	4.005gm	Ointment base
Bees wax	1.003gm	Stiffening agent
Cocoa butter	2.0gm	Emollient
Menthol	0.5	Counter irritant

Coconut oil	Q.s	Carrier oil

3. RESULTS

The formulated nutmeg-loaded ointment exhibited satisfactory physicochemical properties, indicating its suitability for topical application. The ointment showed a pH of 5.8, which is close to skin pH and suggests non-irritancy[6]. The spreadability value of 7.5 g·cm/s indicated good ease of application, while the viscosity of 66314 cps confirmed an optimal consistency that ensures proper retention on the skin without being too stiff or too fluid. The formulation appeared smooth, homogeneous, and stable with a characteristic odour. Phytochemical identification tests confirmed the presence of active constituents such as eugenol, limonene, and sabinene,[7] which are responsible for analgesic and anti-inflammatory activities. The design of experiment (DOE) analysis revealed that formulation variables like beeswax, white soft paraffin, and cocoa butter significantly influenced spreadability, viscosity, and percentage drug diffusion. Increased concentrations of beeswax and other base components were found to decrease drug diffusion, while optimized combinations improved overall formulation performance. The predicted and actual values from statistical models were in close agreement, confirming the reliability of the optimization process[9]

4. DISCUSSION

The developed ointment demonstrated a well-balanced formulation with desirable physical and functional characteristics. The pH being close to that of skin ensures safety and minimizes irritation upon application. The viscosity and spreadability results indicate that the formulation can be easily applied and retained effectively on the skin surface, which is essential for sustained drug action. The role of excipients was significant, where beeswax contributed to stiffness but reduced drug release, while cocoa butter improved emollient properties and coconut oil[8] enhanced drug penetration. Menthol provided a cooling and counter-irritant effect, supporting analgesic action. The decrease in drug diffusion with increased wax content suggests a matrix-controlled release mechanism. The active constituents of nutmeg oil, such as eugenol and other phenolic[7] compounds, are known to inhibit inflammatory mediators like COX-2 and cytokines, thereby contributing to analgesic and anti-inflammatory effects. The DOE approach effectively identified the influence of formulation variables and their interactions, and the response surface and contour plots helped in optimizing the formulation. Overall, the results support the effectiveness and stability of the developed ointment.

5. CONCLUSION

In conclusion, a stable and effective nutmeg-loaded topical ointment was successfully formulated and evaluated. The formulation demonstrated good physicochemical properties, including appropriate pH, viscosity, and spreadability, making it suitable for topical use. The optimized formulation showed promising drug release characteristics and significant analgesic and anti-inflammatory potential[6] due to the presence of bioactive compounds in nutmeg oil. The use of design of experiments aided in optimizing formulation variables and ensuring reproducibility. Therefore, this study suggests that the developed ointment can serve as a potential natural and effective alternative for the management of pain and inflammation.[6]

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