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PHARMACEUTICAL FORMULATION & EVALUATION OF HERBAL *MYRISTICA FRAGRANS* CREAM FOR ATOPIC DERMATITIS

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ABSTRACT

Objective: The current study deals with the formulation and evaluation of herbal extracted *Myristica fragrans* cream for atopic dermatitis. **Methods:** The herbal *Myristica fragrans* was processed, phytochemically screened, analytically determined and extracted by ethanol cold maceration technique. The extracted *Myristica* volatile oil was formulated by melting and mixing the oil-aqueous phase containing lanolin as base, liquid paraffin, glyceryl monostearate, propylene glycol, triethanolamine and glycerine at different concentrations. The formulated herbal cream was evaluated for homogeneity, pH, washability, spreadability and carried out the *in-vitro* drug release, *in-vitro* anti-inflammatory study & anti-microbial study. Stability study has been conducted for the optimized formulation at temperatures 40 ± 2°C at 75 ± 5%. relative humidity. **Results:** The formulated herbal cream had showed good clarity, homogeneity, odour, spreadability and washability. It also has shown an increase in the drug release with a slight increase in the *in-vitro* anti-inflammatory activity than compared to marketed drug. The optimum formulation selected was M2 which follows a zero order kinetics with Fickian diffusion phenomenon. The formulation found to be stable with no signs of phase separation and change in color. **Conclusion:** It can be concluded that herbal *myristica fragrans* creams having anti-bacterial and anti-inflammatory property can be used as provision of a barrier to protect the skin from atopic dermatitis.

KEYWORDS: *Myristica fragrans*, cold maceration, *in-vitro* drug release, *in-vitro* anti-inflammatory.

INTRODUCTION

Atopic Dermatitis (AD) is a highly pruritic chronic inflammatory that has the affinity to secrete immunoiglobulin E (IgE).[1] Atopic dermatitis is characterized by pruritic, erythematous and scaly skin lesions that are generally affected in face, extensor surfaces and scalp. The disease can be affected either at the beginning of the birth or during the late onset of disease. This can also occur due to allergens in the air, genetic factors or environmental factors. The skin lesions in acute atopic dermatitis occurs due to the mononuclear cell infiltration resulting in the activation of memory Th2 cells expressing the CLA skin receptor.[2] The increased expression of Thl cytokines accompanied by the infiltration of macrophages and eosinophil's results in the development of chronic acute atopic dermatitis. The severity of the disease increases with the increase in its risk factors. Genetic association with atopic dermatitis results in the loss-of-function mutations in the filaggrin (FLG) gene, which encodes the important barrier protein (pro-) filaggrin. Filaggrin aggregates keratin and interacts with lamellar bodies that leads to changes of skin hydration and skin pH.[3] The activity of serine protease increases the skin pH leading to degradation of corneodesmosomes and intercellular adherence, but attenuates ceramide synthesis leading to lower ceramide content. Finally, these mechanisms result in increased Th2 inflammation and higher penetration of allergens through the skin.[4] Besides loss-of-function mutations in the FLG gene, several other factors, such as DNA methylation state or variations of FLG copy numbers, environmental factors including skin irritation and mechanical damage, low humidity but also the cytokine milieu in the skin with reduction of filaggrin expression by Th2 cytokines, IL-17, IL-22, IL-25 or IL-31 as well as micro-organisms colonizing the skin and topical and systemic treatment are capable to modulate filaggrin expression secondarily. These factors are capable to modulate keratinocyte function and the integrity of the skin barrier.[5] Topical corticosteroids are the most common preferred treatment for atopic dermatitis Topical corticosteroids, topical calcineurin, antibiotics, systemic therapy, certain therapies like Ultraviolet (UV) phototherapy using UVB, narrow-band UVB, UVA, or psoralen plus UVA may be beneficial for the treatment of severe disease. Calcineurin inhibitors should be used as second-line agents and rarely, systemic therapies may be considered in adults. But using these steroids in the long run leads to specific organ damage and adverse effects in skin.

Incomparison with the allopathic drugs herbal medicines can be used in the prevention, diagnosis and treatment of diseases in addition to their nutritional value. *Myristica fragrans*

is the drug obtained from the plant origin, with anti-inflammatory, antibacterial, antiseptic activity. Traditionally, nutmeg is used widely in the treatment of rheumatism, diarrhoea, stomach cramps, flatulence, and 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.

Our interest is to develop a safe and curative herb derived agent for AD using medical knowledge and clinical experience of herbal medicine combining with molecular biology and combinatorial chemistry technologies. In comparison with today's marketed available conventional drugs, herbal drugs are more preferable since it reduces the adverse effect caused by the continual use.

Thus in the present work herbal *Myristica fragrans* cream was formulated to protect the skin against harshness from the environment and any dry conditions of the skin, restoring moisture for the treatment of atopic dermatitis.

MATERIALS AND METHODS

Materials

Lanoline, liquid paraffin, glyceryl monostearate, glycerine were purchased from Omega. Stearic acid from We associations and methanol purchased from Finer.

Methods

Preliminary process, phytochemical screening, characterization of *Myristica fragrans*

Preparation & characterization of *Myristica fragrans* seeds

Myristica fragrans seeds are characterized for its properties like colour, nature. The seeds were washed thoroughly under running tap water and was allowed to dry under shade. The seeds are then powdered using mortar and pestle and stored in a airtight container for further use.[6]

Extraction of *Myristica fragrans*

The powdered *Myristica fragrans* seed was extracted by ethanol cold maceration technique. 150gm of nutmeg powder was weighed and added into a 500mL beaker containing 300 ml of 95% ethanol. The contents were mixed thoroughly and covered tightly inorder to prevent the evaporation of ethanol. Allow the content to keep undisturbed for 7 days. After 7 days, filter the content using a muslin cloth. The filtrate was allowed to evaporate for 5 days. The

volatile oil *Myristica fragrans* was collected and stored in desiccator for further use. The extract was the evaluated for its organoleptic characteristics.[7]

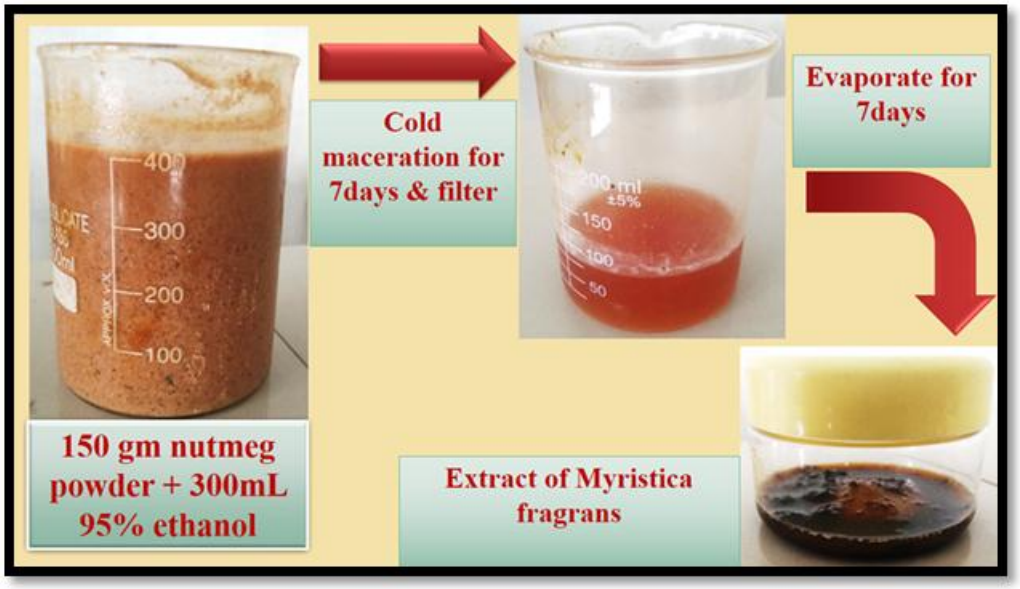


Figure - 1: Extraction of *Myristica fragrans.*

Percentage yield & Phytochemical screening of *Myristica fragrans* volatile oil

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

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

The ethanolic extract of *Myristica fragrans* was tested for the presence of various phytoconstituents like alkaloids, carbohydrates, flavonoids, proteins, glycosides, tannins, steroids, saponins, terpenes etc.

Formulation of Herbal *Myristica fragrans* Cream

The composition of cream formulation representated in Table -1. Initially melt melting lanoline at 50-70°C, 500rpm and to it added volatile oil of *Myristica fragrans*, stearic acid, liquid paraffin, glyceryl monostearate and thoroughly stir to produce an oil phase. Aqueous phase containing propylene glycol, triethanolamine, glycerin and water heated at same temperature as oil phase. Both the phases were mixed slowly with continuous stirring to form the homogenous dispersion. Perfume was added when the temperature downs at 35°C. Preservatives added in the water phase before mixing.[8]

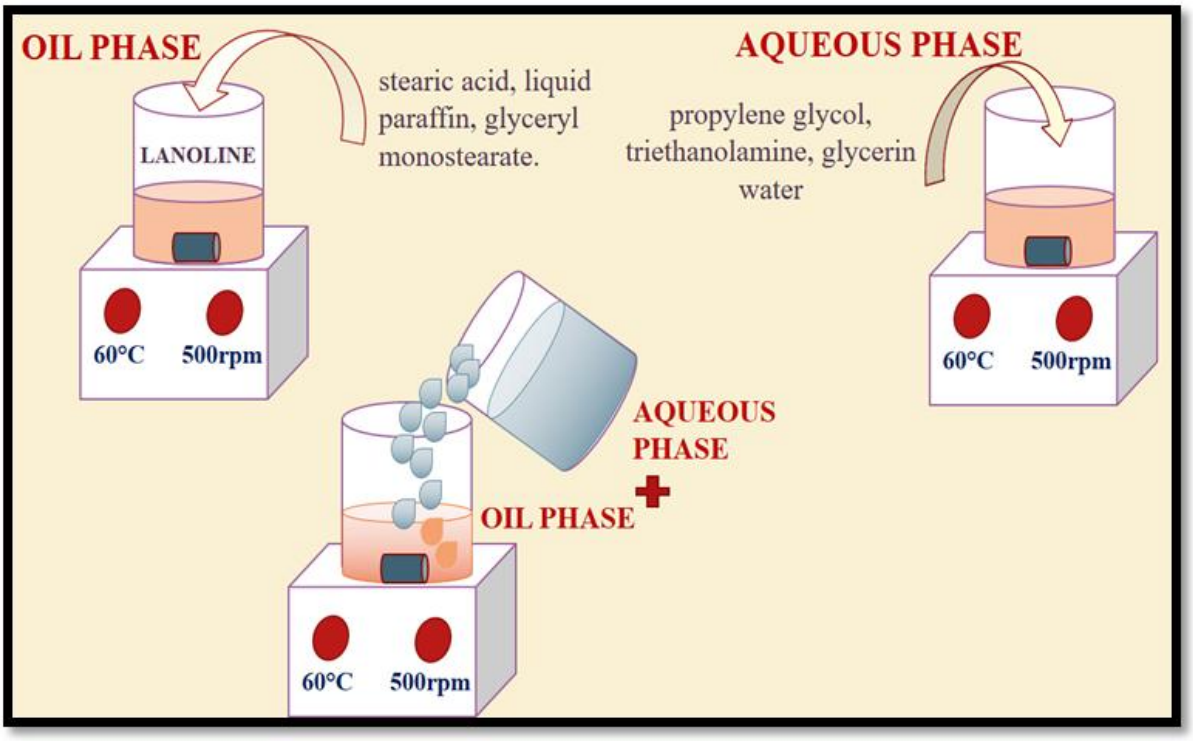


Figure -2: Formulation of herbal *Myristica fragrans* cream.

Table -1: Herbal cream composition.

SI	Ingredients	Quantity for 100gm (%)		
Oil Phase		M1	M2	M3
1.	*Myristica fragrans*	1	2	4
2.	Stearic acid	4.0%	4.0%	4.0%
3.	Liquid paraffin	2.0%	2.0%	2.0%
4.	Lanoline	2.0%	4.0%	8.0%
5.	Glyceryl monostearate	3.0%	3.0%	3.0%
6.	Essential oil	8.0%	8.0%	8.0%
7.	Stearic acid	4.0%	4.0%	4.0%
Aqueous Phase				
8.	Glycerine	4.0%	4.0%	4.0%
9.	Propylene glycol	4.0%	4.0%	4.0%
10.	Triethanolamine	0.2%	0.2%	0.2%
11.	Methyl paraben	0.03%	0.03%	0.03%
12.	Propyl paraben	0.07%	0.07%	0.07%
13.	Perfume	q.s	q.s	q.s
14.	Water	100%	100%	100%

Analytical Method by UV Spectrophotometer

Preparation of standard stock solution

Required volume of essential oil of *Myristica fragrans* was weighed and was made upto 100mL with ethanol to give a solution of 1000 µg/mL.

Preparation of standard calibration curve

From the prepared solution, aliquots of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mL were withdrawn and diluted with ethanol to get the concentration of 1-10 µg/mL. The absorbance was measured from the obtained λ_{max} by using UV spectrophotometer, against ethanol as a blank. Concentration vs absorbance calibration curve was plotted.[9]

Evaluation of formulated herbal cream

The herbal cream products were characterized by pH, spreadability, viscosity, *in-vitro* drug release study, anti-inflammatory activity.

Physicochemical properties

Clarity, colour, appearance and odour were checked for the formulated herbal cream. The herbal cream was tested for homogeneity by visual inspection.

pH measurement

The pH of the 10% w/v cream suspension was determined at 25 °C using a pH meter, standardized using pH 4.0 and 7.0 standard buffers before use and average of triplicates were determined.

Viscosity

Brookfield Synchro -Lectric Viscometer (Model RVT) with helipath stand was used for rheological studies. The sample (50 g) was placed in a beaker and was allowed to equilibrate for 5 min before measuring the dial reading using a T-D spindle at 10 20,30,50,60,100 rpm. According to the speed, the corresponding dial reading on the viscometer was noted. The measurements were carried in triplicate at ambient temperature. Average of three triplicates was computed.

Washability

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

Spreadability

Spreadability refers to the area covered by a fixed amount of cream sample after the uniform spread of sample on the glass slide The spreadability of test samples was determined using the following technique: 0.5 g test formulation was placed within a circle of 1 cm diameter pre-marked on a glass plate over which a second glass plate was placed. Place 500 g weight

on the upper glass plate for 5 min. Spreadability of the formulation was noted with increase in diameter.[10] Average of three determinations was noted. Spreadability was calculated using the following formula:

S = M × 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

Determination of thermal stability

Transferred the cream into a glass bottle using spatula and tapped gently to settle at the bottom. Two-third capacity of bottle was filled up, inserted the plug and tightened the cap. The formulation was kept in the incubator at 45°C for 48 hr.[11]

***In-vitro* drug release studies**

The *in-vitro* diffusion study of the cream was carried out in modified Diffusion cell using cellophane membrane. Soaked the membrane overnight in phosphate buffer pH 5.5 and carefully attached to the diffusion cell (2.3 cm diameter; 4-16 cm^2 area). The herbal formulated cream was spread on the dialysis membrane. 10 ml of phosphate buffer was taken in a beaker, which was used as receptor compartment. The donor compartment was kept in contact with receptor compartment which was placed on a magnetic stirrer and the solution on the receptor side was stirred continuously using a magnetic bead and temperature of the cell was maintained at 37±2 °C. 5ml of the sample solution was withdrawn at regular time intervals and replaced with equal amount of freshly prepared solution.[8]

Mathematical modelling of kinetics

In order to gain vision into the drug release mechanism from herbal *Myristica fragrans* cream, release data of optimized formulations were examined according to the zero order, first order, Higuchi's square root of time mathematical models and Korsmeyer-Peppas model where release exponent n was calculated. An n-value is considered consistent with a diffusion controlled release, where as a value 1.0 indicates a zero order release behavior and intermediate values (0.5 > n >1.0) are defined as anomalous non-fickian transport mechanism.

***In-vitro* Anti-inflammatory activity**

Inhibition of protein denaturation

The *in-vitro* anti-inflammatory activity of formulated herbal cream of *Myristica fragrans* was evaluated by inhibition of protein denaturation method. 10 mg of *Myristica fragrans* cream 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 containing Bovine Serum Albumin (1.5 ml). This mixture was kept at room temperature for 10 minutes, followed by incubated at 27+ 10°C for 15 min. The resulting solution was cooled down and absorbance was recorded at 254 nm. Marketed anti-inflammatory cream 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, Vt = absorbance of test Vc = absorbance of control

***In-vitro* Anti-Microbial Activity**

Disc diffusion method

The efficiency of herbal nutmeg cream was determined by anti-microbial test (disc diffusion method). In this method, the prepared nutrient agar was sterilized and aseptically spread on three sets of Petri plates marked as test, control and standard. Test culture used were *Staphylococcus aureus*. The inoculated plates with test culture was incubated at 37°C for 24h. After incubation, two filter paper discs were placed with herbal cream and a marketed atopic anti-microbial cream were positioned in the marked plate in such a way that the sterile discs can completely absorb the formulation. Sodium Lauryl sulphate Disc was maintained as control which was then incubated for 24h. The efficacy of the product in terms of zone of inhibition of the organism was determined by the test. The test product is more effective if the zone of inhibition, is higher.[12]

Stability testing

Stability by centrifugation

The herbal cream were centrifuged at 3500- 13,500 rpm at interval of 500 rpm for 10 min. The formulation were observed for the phase separation.

Stability studies as per ICH guidelines

For assessing the stability of formulated creams, the following parameters were taken into consideration like color, pH, viscosity, spreadability and thermal stability of formulation. These studies are designed to ensure the stability of product. The study was carried out for thirty days at temperatures 40 ± 2°C and relative humidity at 75 ± 5% using stability chamber.[13-15]

RESULTS AND DISCUSSIONS

Processing, Characterisation, Phytochemical Screening & Extraction of *Myristica fragrans*

Myristica fragrans seeds were characterized for its colour, nature and properties, *Myristica fragrans* seeds were coarsely powdered using mortar and pestle, the powder was extracted by maceration technique. The methanolic extract of *Myristica fragrans* was organoleptically evaluated. *Myristica fragrans* seeds were found to be orange-red and brown in color respectively, and oval in shape with striations on it. The percentage yield of the methanolic extract is 18.89±0.02%. The phytochemical screening of the methanolic extract of *Myristica fragrans* showed positive results for flavonoids, sterols and phenols. The flavonoids have potent anti-inflammatory activity by inhibiting prostaglandin synthesis. Phenolic compounds possess certain biological properties such as anti-carcinogen, anti-inflammation, as well as inhibition of angiogenesis and cell proliferation activities. Phytosterol acts as growth hormones in plants.[16]

Analytical Method by UV Spectrophotometer

- It is reported from the literature review that the absorption maxima (λ_{max}) of myristicin is 254nm.The calibration curve was obtained for a series of concentration in the range of 1-10 µg/ml. It was found to be linear and hence suitable for the estimation of drug.
- Regression analysis of Beer's law plot revealed a good correlation.

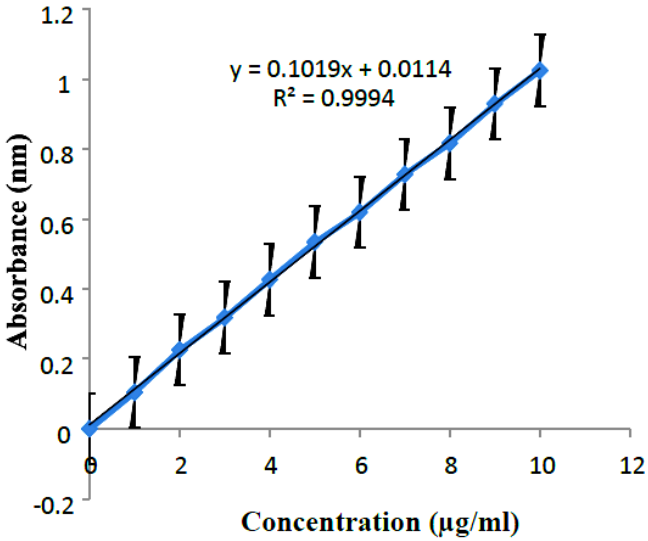


Figure -3: Standard graph of myristicin.

Physicochemical parameters

The herbal cream were characterized by color, consistency, homogeneity, pH, viscosity spreadability, washability and thermal stability study. The methanolic extract of *Myristica fragrans* is yellowish brown in colour, aromatic in nature. The formulated herbal cream was found to be brownish yellow in colour with aromatic odour. The cream had showed excellent clarity and homogeneity. Any change in pH of the product indicates a possible interaction or occurrence of chemical reactions which may provide an idea on the quality of the final product. The pH of human skin normally ranges from 4.5 to 6.0. Due to frequent washing and used of soap, the acidity of the skin is lost. The cream formulation had a pH value of 6.5-7.5 range, which is an acceptable and non-skin irritating pH value. This indicates that the compound can be further modified for formulation and is compatible with the skin spreadability. The formulation M2 have better spreadability when compared with M1 and M3 and hence, easy to apply. The formulated herbal cream were found to be easily washable from the skin. There was no phase separation formed after 48h of incubation indicating its stability.

Table -2: Physicochemical parameters of Herbal *Myristica fragrans* Cream.

Parameters	M1	M2	M3
Color	Brownish-Yellow	Brownish-Yellow	Brownish-Yellow
Consistency	Good	Excellent	Very Good
Homogeneity	Fine	Excellent	Fine
Ph	7.08±0.01	6.52±0.08	7.41±0.12
Viscosity	15470±0.10	19574±0.08	17830±0.07
Spreadability (g	13.56±0.20	18.84±0.08	15.91±0.09

cm/sec)			
Washability	No grittiness	No grittiness	No grittiness
Thermal stability	No phase separation	No phase separation	No phase separation

Evaluation of formulated herbal cream

***In-vitro* Drug release studies**

The major composition for the formulation of herbal cream includes lanoline, stearic acid, liquid paraffin, glycerine and propylene glycol. The herbal *Myristica fragrans* act as a carminative, anti-spot, flavouring agent which also have an excellent anti-bacterial & anti-inflammatory activity. Glycerine and propylene glycol is the most suitable moisturizer and binder which also act as a humectant resulting in hydration of the stratum corneum when applied to the skin. These are consist of a hydroxyl group that allow them to take part in the association process known as hydrogen binding. It is of note that all glycol-type humectants can additionally improve the effect of preservatives (e.g. paraben) since they take away the water from the bacteria (needed for their growth) and improve the solubility of parabens. Lanoline act as a emollients, generally smooth skin by filling spaces between the skin flakes with droplets of oil, and are not usually occlusive unless applied heavily. When combined with an emulsifier, they may help hold oil and water in the stratum corneum. Stearic acid exert their benefits through effects on the skin barrier. Due to the increase in the solubility of extract with the excipients and base, the formulation results in increase in drug release. The percentage in-vitro drug release was found greater in M2 (78.56±0.08%) in comparison with the M1 & M3.

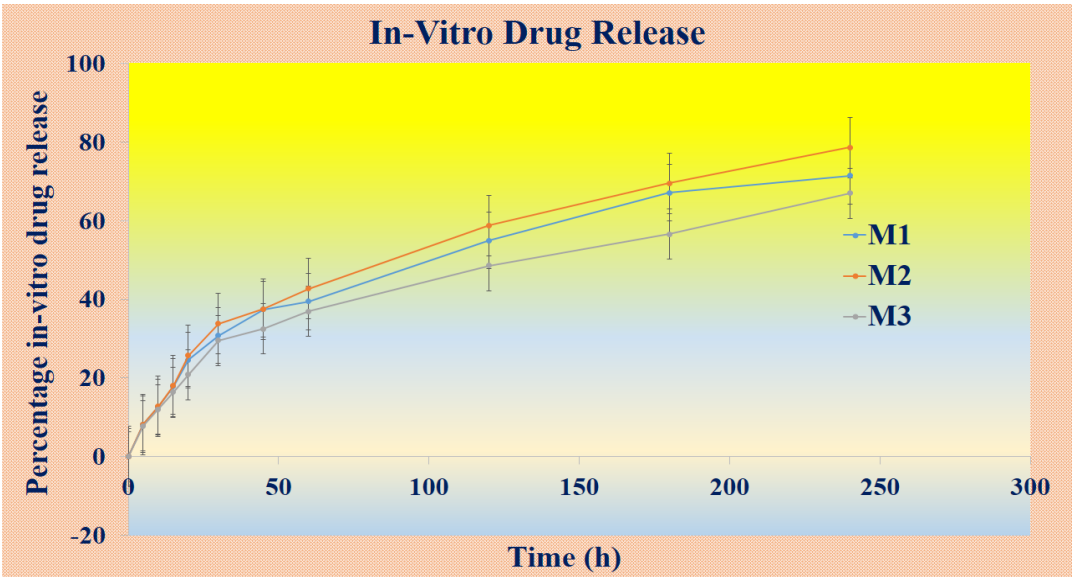


Figure -4: In-vitro drug release study of herbal formulations.

Mathematical Modelling of Kinetics

Based on the *in-vitro* drug release profiles, the drug releases from the herbal cream are governed by various parameters. Solubility of the extract from the carrier (base), diffusion rate into the medium and diffusion of the base within the experimental conditions are important factors that affect the kinetics of drug release. The data obtained from the in-vitro drug release experiments were fitted to different mathematical model i.e zero order, first order, Higuchi and korsmeyer-peppas to predict the kinetics and release mechanisms of the solid dispersions.

The regression coefficient (R^2) values obtained from the mathematically models. the data obtained shows that the formulated herbal nutmeg cream follows zero order kinetics with R^2 value 0.9901 respectively indicating that drug release is independent of drug concentration within the system. The formulated herbal cream tends to exhibit Fickian diffusion as the corresponding values of n is lower than or equal to the standard value of fickian release behaviour. Thus the results point out the diffusion phenomena.

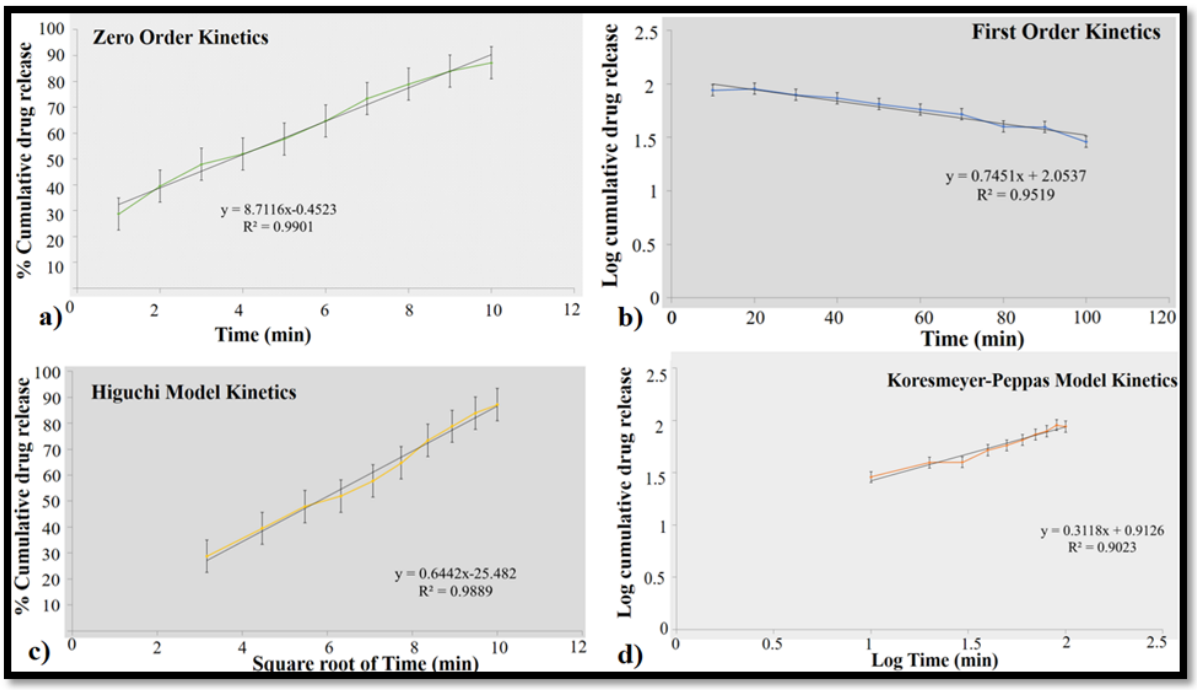


Figure -5: Mathematical model kinetics of optimised herbal formulations.

***In-vitro* anti-inflammatory activity**

In-vitro anti-inflammatory activity of the formulated herbal cream was carried out by Albumin denaturation method. The results indicated that myristicin present in seeds were responsible for anti-inflammatory action. The anti-inflammatory property of Myristicin was

due to inhibition of chemokines, cytokines. Herbal Myristica fragrans cream showed high anti-inflammatory activity by inhibiting the inflammatory cytokines. The percentage inhibition of formulated cream was showed better results compared to marketed drug. Maximum inhibition of 78% was observed at 600 µg/ml for M2 formulation, a standard anti-inflammation drug showed the maximum inhibition 76% at the concentration of 200 µg/ml. Thus the *Myristica fragrans* cream shows slight increase in percentage inhibition compared to marketed drug and hence the formulated herbal *Myristica fragrans* cream have better anti-inflammatory activity compared to the marketed drug.

Table -3: Effect of M2 on protein denaturation.

Samples	Concentration (µg/ml)	% Inhibition of protein denaturation
M2	200	45
M2	400	61
M2	600	78
Marketed drug	200	76

***In-vitro* anti-microbial activity**

Antimicrobial activity was determined by measuring zone of inhibition formed after incubation period. The results showed a good antibacterial effect against *Staphylococcus aureus* for herbal formulated cream. The methanol extract showed characteristic zone of inhibition against *Staphylococcus aureus*. Highest zone of inhibition was observed at 600µg/ml concentration against *Staphylococcus aureus*.[17]

Table -4: Anti-microbial activity on the optimised formulation.

Organisms	Zone of inhibition (mm)		
	Herbal formulations	Commercial formulations	Control
Staphylococcus aureus	25±0.12	18±0.07	05±0.02

Stability study

The stability studies of varies parameters like visual appearance, pH, spreadability, viscosity showed that there were no significant changes after 1 month of study period. During centrifugation studies, it was observed that there was no phase separation in M2 formulation, confirming that formulation was stable at accelerated speed. The optimised formulation had increasing viscosity values after storage at temperatures 40 ± 2°C and relative humidity at 75 ± 5%. M2 formulation were oil-in-water creams; hence, their water content might lose at

fluctuated temperatures. Therefore, the suggested storage condition for this formulation should be at constant temperature. No phase separation and changing in colour as well as odour were observed after stability test. From the results it was observed that the given formulation was relatively stable at accelerated temperature and humidity.

Table -5: Stability Study of M2 Herbal *Myristica fragrans* cream.

Parameters	Observations
Colour	Brownish-Yellow
Consistency	Very good
Phase separation	No phase separation
pH	6.97 ± 0.02
Viscosity	19782 ± 0.08
Spreadability	18.34 ± 0.12

CONCLUSIONS

Myristica fragrans are well known in traditional herbal medicines had been widely accepted in the treatment of various disorders. The markedly available allopathic drugs are either directly derived from herbal resources or have been further enhanced through structural modifications. Natural products have substantially contributed in development of new drugs. Acute and chronic atopic dermatitis are still one of the major health problems of world population. Although there are several steroids and anti-inflammatory drugs available, but their use in the longer run had been discouraged due to its adverse effect. Thus to provide efficient application on the skin, a herbal *Myristica fragrans* cream had been formulated for the treatment of atopic dermatitis. From the results it had been noted that the optimised formulation are homogeneous, emollient, non-greasy and easily removed, with compatible skin pH, with increase in drug release due to the penetrability property into the skin. The stable formulations were safe in respect to skin irritation and allergic sensitization. The herbal cream was O/W type cream, hence can be easily washed with plane water that gives better customer compliance. The formulation has antibacterial activity, anti-inflammatory activity. The extract has emollient properties Hence all these properties are beneficial to normal human keratinocytes, so it is safe & stable thus it may produce synergistic action.

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