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"Design and Development of Nanoemulgel using herbal oil extract's of *thymus vulgaris, syzygium aromaticum, melaleuca alternifolia* for Acne treatment"

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ABSTRACT:

Topical drug delivery has several advantages such as avoids systemic toxicity, prolonged action, controlled drug release, higher loading capacity. The objective of this work to formulate nanoemulgel of thyme, clove and tea tree oil to treat acne. Developed Formulation was characterized for physical evaluation spreadability, extrudability, drug content and in-vitro drug diffusion study. The nanoemulgel was clear and transparent indicating the dissolution of drug in base. The maximum % drug content was found to be 99.04% which is within acceptable range. The nanoemulgel prepared by this method meets the quality requirement of nanoemulgel and may be developed as new formulation of combined drugs.

Keywords: *Thymus Vulgaris, Syzygium Aromaticum, Melaleuca Alternifolia,* Acne cream, Emulgel.

INTRODUCTION

In a Greek word of acne which means "Prime of life".Acne is a common inflammatory disorder which is common among the adolescent age groups.Even though it is not a life-threatening diseasebut it affects the patient's self-esteem.It is a chronic inflammatory disease.Acne is also called as acne vulgaris.It occurs on the parts like: face, neck, upper chest, upper back, etc.[1]

THYME

Thyme is *thymus Vulgaris* which killed more bacterial than the highest concentration of benzoyl peroxide. Thyme exact actually boasts acne-fighting abilities given its antibacterial effect on Propionibacterium the bacterium known to cause breakouts. [2]

1. Thyme is the herb of some members of the genus thymus of aromatic perennial ever evergherbs in the mint family Lamiaceae.
2. Thymes have culinary, medicinal and ornamental uses to the species most commonly cultivated and used for culinary purposes.

Figure no.1: Thyme Leaves

3. Thyme is known for the aroma and flavor of its dried leaves & flowering tops.

CLOVE

Clove oil contains compound called eugenol *Syzygium Aromaticum* that helps in treating acne byreducing the inflammation and redness. The antibacterial and antiseptic properties of cloves kill acne- causing bacteria and keep breakouts at bay. [3]

Clove are the aromatic flower buds of a tree in the family *Myrtaceae*.

1. They are native to the Maluku Islands in Indonesia and are commonly used as spice, flavouring or fragracne in consumer products, such as toothpaste, soaps or cosmetics. [4]
2. Nowadays, many reports confirm the antibacterial, antifungal, antiviral and anticarcinogenic properties of clove.
3. Cloves may be used to give aromatic and flavor qualities to hot beverages, often combined with other ingredients such as lemon and sugar.

Figure no.2: Clove plant

TEA TREE

Tea tree is *Melaleuca alternifolia* oil found to have antimicrobial effects against Cutibacterium Acnes, a type of bacteria that is found in healthy normal skin, but one that's also known to be involved in the formation of acne. [5]

1. Tea tree oil, also known as melaleuca oil, is an essential oil with a fresh camphoraceous odor and a color that ranges from pale yellow to nearly colorless and clear.
2. Tea tree oil is claimed as useful for treating dandruff, acne, lice, herpes, insect bites, scabies and skin fungal or bacterial infections.
3. Melaleuca alternifolia is a small tree that can grow to about 7 m (20 ft) with a bushy crown and whitish, papery bark.

Figure no.3: Tea Leaves

NANOEMULGEL

Nano-emulgel is an emerging drug delivery system intended to enhance the therapeutic profile of lipophilic drugs. [6] Nanoemulgel which known as the formation of nanoemulsion based on hydrogel is the addition of the nanoemulsion based on hydrogel is the addition of the nanoemulsion system intergraded into hydrogel matrix which influences better skin penetration. [7]

Flow chart of Nanoemulgel formulation

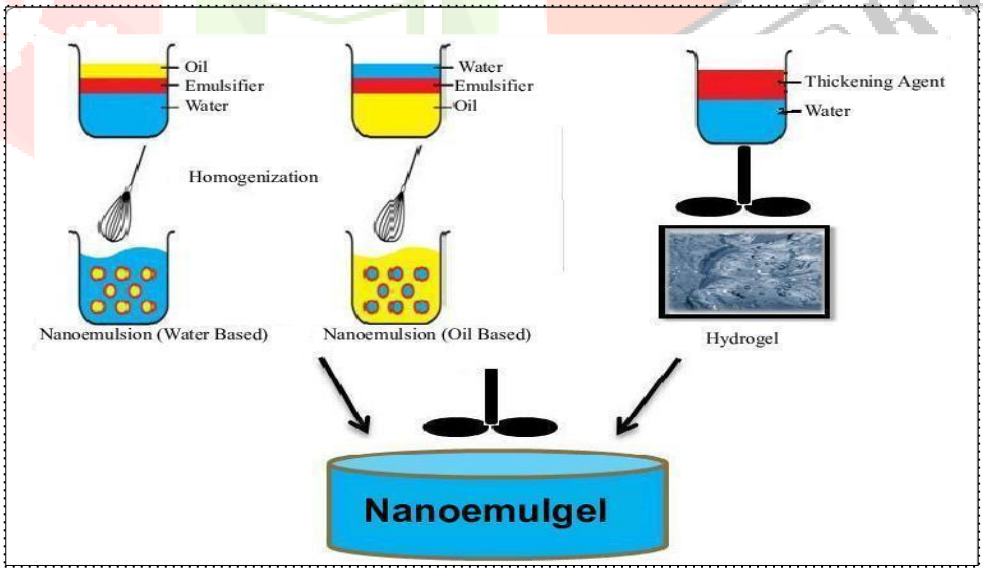

Figure no.4: Nanoemulgel

Components

Oil:

The selection of the oil phase is the most crucial parameter to obtain a stabilized Nanoemulsion so that the maximum amount of drug could solubilize it.Usually, the oil which has maximum solubilizing potential for a selected drug candidate is selected as an oily phase for the formulation of nanoemulsions.This helps to achieve maximum drug loading in the nanoemulsions.

Ex. Oils used in Nanoemulsion is Thyme Oil, Clove Oil, Tea Tree Oil.

Surfactant:

Surfactants are vital components used for stabilizing the nanoemulsion system.The anionic, cationic, and non-ionic types of surfactants were used in this system. Due to their different chemical nature, proper selection of surfactants becomes a crucial factor in obtaining a stabilized delivery system. [8]

Ex. Surfactant used in Nanoemulsion is Tween 80.

Co-surfactant:

Cosurfactant plays an essential role in reducing the polarity of surfactant to obtain a stabilized nanoemulsion.

Ex. Cosurfactants used in Nanoemulsion is Polyethylene Glycol 400.

Gelling Agents (hydrogels):

The unique physical properties of hydrogel have reflected a particular interest in drug delivery applications. These are the semisolid system with three dimensional, cross-linked network of organic and inorganic molecules and inhibition by liquid due to high porosity. [9]

Ex. Gelling Agents is Carbopol 940.

METHODS OF PREPARATION OF NANOEMULSION

Table no.1: % Composition of Optimized Topical Nanoemulsion

Sr.no.	Ingredients	% Composition w/w
01	Thyme Oil	0.62
02	Clove Oil	0.61
03	Tea Tree Oil	0.65
04	Isopropyl Myristate	0.50
05	Tween 80	9
06	Polyethylene Glycol 400	4.00
07	Distilled Water	Q.S

METHOD OF PREPARATION OF NANOEMULGEL

Nanoemulgel containing thymol,eugenol,tea tree oil was prepared using Carbapol 940 as gelling agent in different concentration (0.5-1.0%)for topical applications.Optimized Nanoemulgel was prepared by dissolving 0.3% of Carbapol 940 in purified water and left for complete swelling. After that 1.5% of glycerin is added into the system. [10] Addition of Triethanolamine in sufficient quantity resulted into formation of gel matrix homogenously mix to obtain clear, transparent hydrogel.Finally 6.0% optimized. [11] Topical Nanoemulsion was homogenously dispersed into hydrogel system to obtain nanoemulgel preparation containing 0.75% of thymol, 0.75% of eugenol, 0.75% of tea tree oil.

Table no.2: % Composition of Optimized Topical Nanoemulgel

Sr No.	Ingredients	% Composition (w/w)
01	Carbapol 940	0.3
02	Glycerin	1.5
03	Nanoemulsion	6.0
04	Triethanolamine	0.45
05	Distilled Water	q.s to 30

Figure no.5: Prepared Nanoemulgel in Lab

EVALUATION PARAMETERS:

Physical Stability:

Physical stability of nanoemulgel identified visually by its color, homogenecity consistency, spreadability.

Spreadability Test:

Spreadability of optimized Nanoemulgel formulation was calculated by compressing the known amount (0.5g) of sample under glass plates of known weight. Various plates were subsequently placed over the sample at intervals of 1 min. [12]

Extrudability:

A sealed collapsible tube consist of Nanoemulgel was enforced firmly at the folded end to determine extrudability characteristics of the optimized formulation force needed to extrude small ribbon length of gel in fixed time was observed to optimize the extrudability characteristics of prepared Nanoemulgel formulation. [13]

pH:

Accurately weigh amount (2.5g) of nanoemulgel was diluted with known amount (25ml) ofdistilled water. The pH of nanoemulgel formulation was determined using digital pH meter and standardized using standard buffers at pH 4.0, 7.0 before use.

Viscosity:

Rheology of gel formulation containing nanoemulgel of thyme, clove and tea tree was studied by Brookfield digital viscometer.

Drug Content Analysis:

Nanoemulgel was determine by diluting 100mg of Nanoemulgel in 100ml of phosphate buffer 7.4and filter out the solution and take 1ml from filtrate make volume up to 10ml and determine using U.V. Spectrophotometer with λ_{max} at 278 nm. [14]

ANTIBACTERIAL ACTIVITY:

The prepared nano-emulgel shows antibacterial activity when tested microbiologically by cup-plate method using marketed tetracycline solution as a standard, pure thyme oil, pure clove oil, pure tea tree oil. Prepared nanoemulgel and Saline water as a blank. The plates were incubated at 27° C for 72 hours. The zone of inhibition is measured around each cup was compared with that of standard & pure thyme oil, pure clove oil, pure tea tree oil. [15]

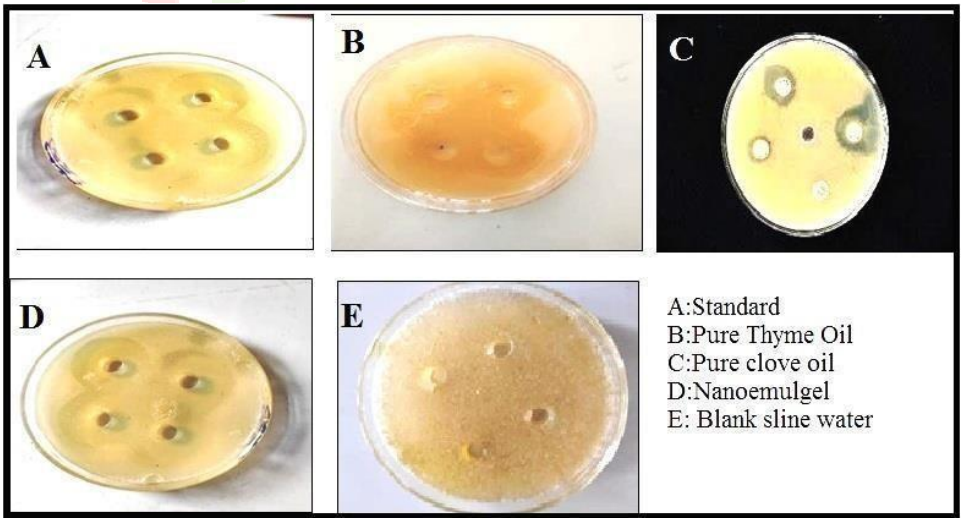

Figure no.6: Antibacterial Activity

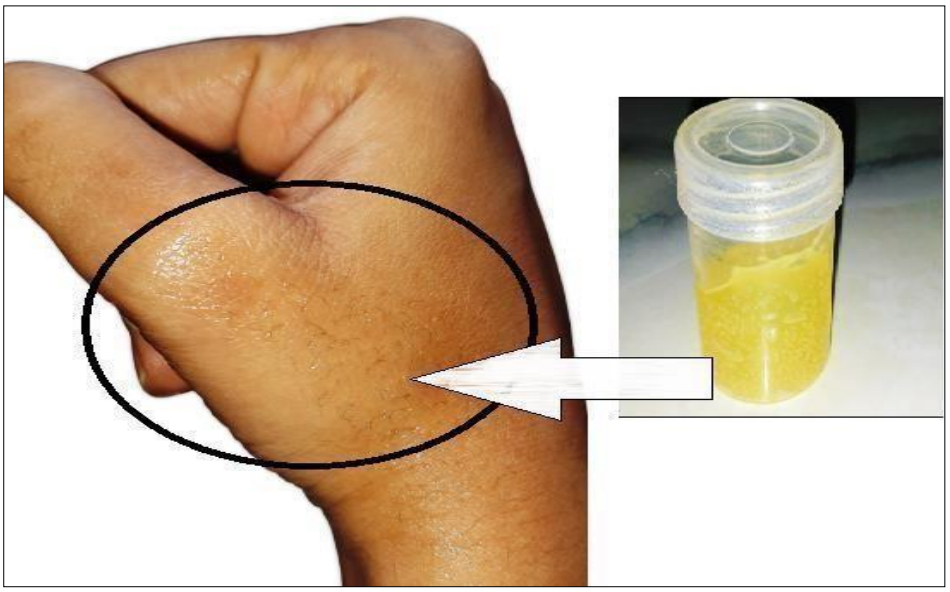

Figure no.7: (a) Antibacterial Activity

PREPARATION OF STANDARD CURVE OFTHYME

To determine standard curve of thyme we have to find out the absorbacne of thyme. Take standard solution which can be prepared by adding a drop of thyme oil into 2 ml of ethyl alcohol as a co-solvent, then add into buffer solution of pH 7.4 & make volume up to 10ml. [16] Now, take 7 test tubes where first test tube contains 0.2ml of standard solution & make volume up to 5ml by standard buffer solution of pH 7.4 than in next test tube add 0.4ml of standard solution & volume make up by buffer solution of pH 7.4 up to 5ml, follow above procedure for preceding next test tube of varying concentration (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4ml). [17]Then each 7 test tube test under U.V Spectrophotometer & on the basis of absorption. The following Standard curve shows lambda maximum at 293nm. [18]

Table no.3: Standard curve of Thyme

Sr no.	Sample Concentration(µg/ml)	Lambda max value(λ) nm	Absorbance(nm)
1	0.2	293	0.05
2	0.4	293	0.08
3	0.6	293	0.189
4	0.8	293	0.241
5	1.0	293	0.328
6	1.2	293	0.241
7	1.4	293	0.328

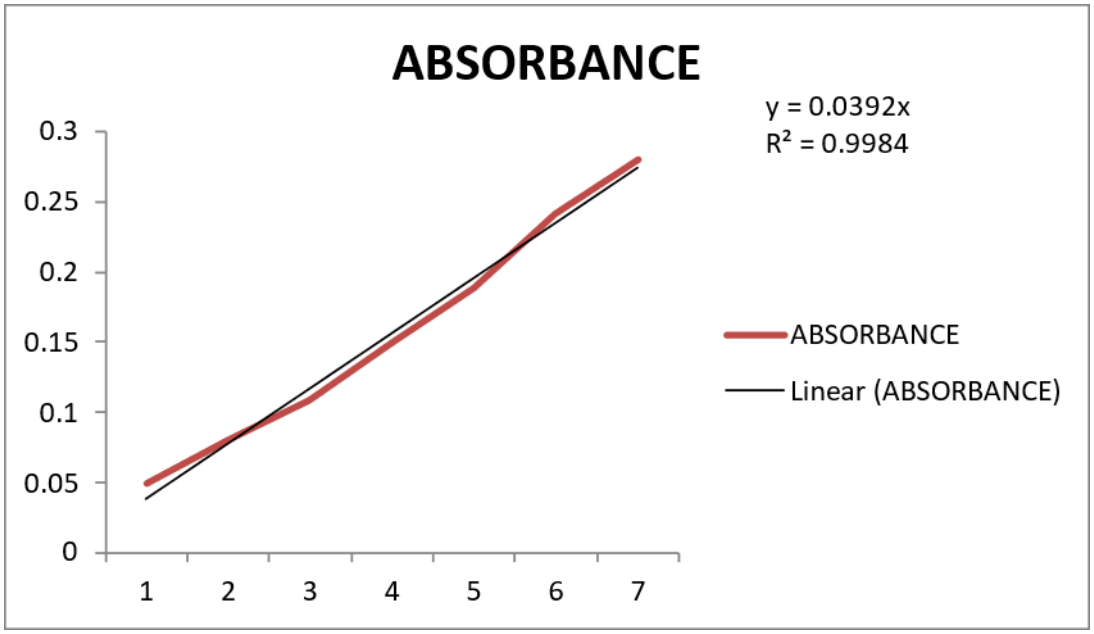

Figure no.8: Graph of Standard Curve of Thyme

PREPARATION OF STANDARD CURVE OF CLOVE:

To determine standard curve of clove we have to find out the absorbance of clove. Take standard solution which can be prepared by adding a drop of clove oil into 2ml of ether as a co-solvent, then add into buffer solution of pH 7.4 & make volume up to 10ml. Now, take 7 test tubes where first test tube contains 0.2ml of standard solution & make volume up to 5ml by standard buffer solution of pH 7.4 than in next test tube add 0.4ml of standard solution & volume makeup by buffer solution of pH 7.4 up to 5ml, follow above procedure for preceding next test tube of varying concentration (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4ml).Then each 7 test tube test under U.V Spectrophotometer & on the basis of absorption. The following Standard curve shows lambda maximum at 282nm.

Table no.4: Standard curve of Clove

Sr no.	Sample Concentration(µg/ml)	Lambda max value(λ)nm	Absorbance(nm)
1	0.2	282	0.05
2	0.4	282	0.08
3	0.6	282	0.109
4	0.8	282	0.15
5	1.0	282	0.189
6	1.2	282	0.241
7	1.4	282	0.328

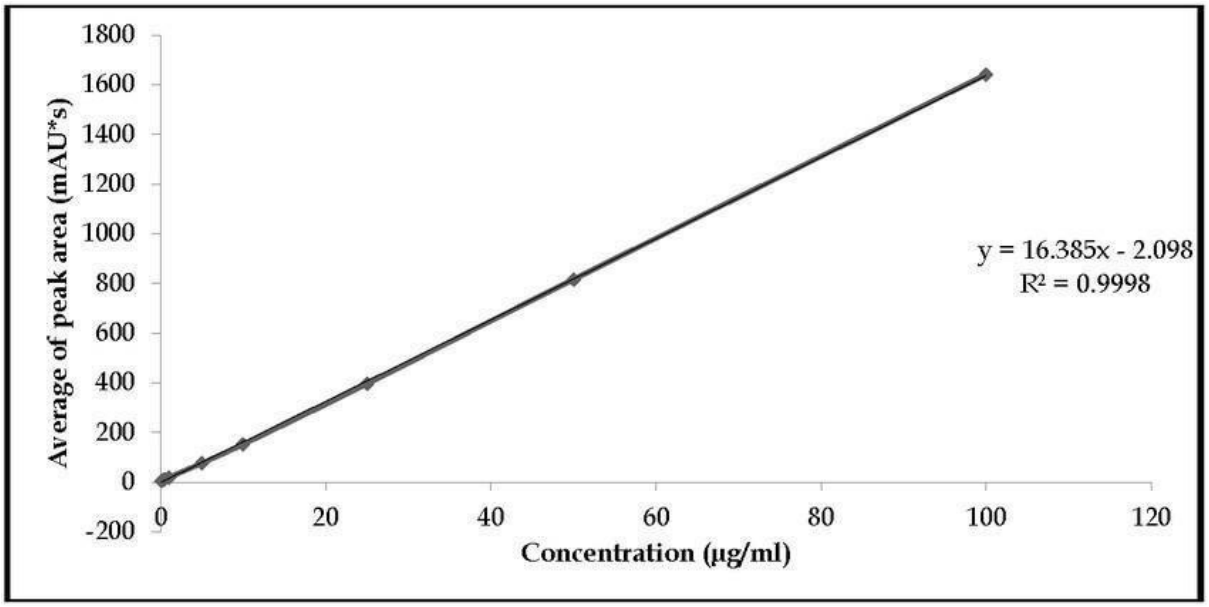

Figure no.9: Graph of Standard Curve of Clove

PREPARATION OF STANDARD CURVE OF TEA TREE OIL:

To determine standard curve of tea tree oil we have to find out the absorbance of tea tree oil. [19] Take standard solution which can be prepared by adding a drop of tea tree oil into 2ml of ether as a co-solvent, then add into buffer solution of PH 7.4 & volume makeup up to 10ml. Now, take 7 test tubes where first test tube contains 0.2ml of standard solution & make volume up to 5ml by standard buffer solution of pH 7.4 than in next test tube add 0.4ml of standard solution & volume makeup by buffer solution of pH 7.4 up to 5ml, follow above procedure for preceding next test tube of varying concentration (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4ml). Then each 7 test tube test under U.V Spectrophotometer& on the basis of absorption The following Standard curve shows lambda maximum at 237nm. [20]

Table no.5: Standard Curve of Tea Tree Oil

Sr no.	Sample Concentration(µg/ml)	Lambda max value(λ) nm	Absorbance(nm)
1	0.2	237	0.145
2	0.4	237	0.255
3	0.6	237	0.395
4	0.8	237	0.587
5	1.0	237	0.725
6	1.2	237	0.855
7	1.4	237	0.935

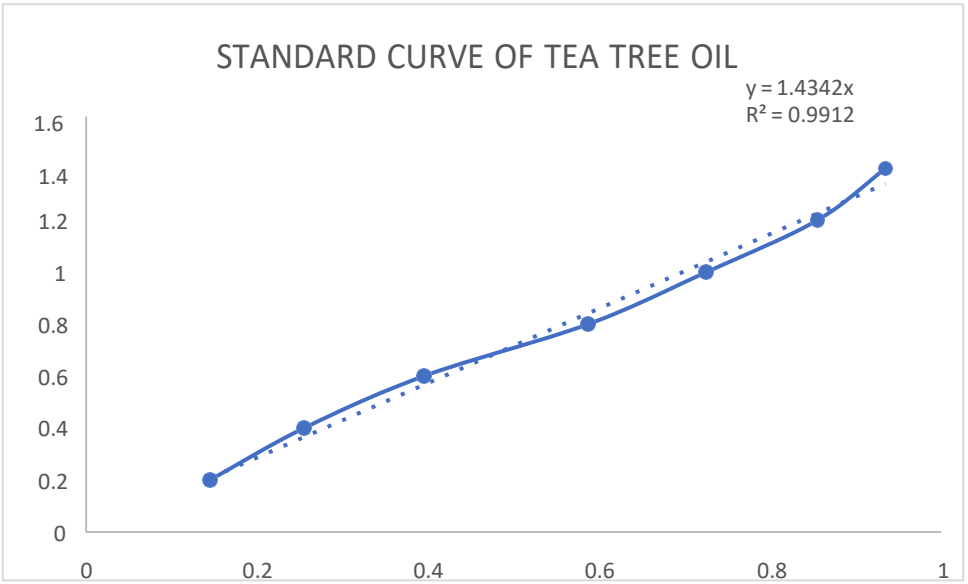

Figure no.10: Graph of Standard Curve of Tea Tree Oil

RESULT AND DISCUSSIONS:

Solubility Study:

Thymol showed solubility of more than 100 mg/g in isopropyl myristate with maximum solubility of more than 1000 mg/g was found in clove oil. Tea tree oil showed maximum solubility more than 100 mg/g in thyme oil. After considering required biopharmaceutical and therapeutic properties of thyme oil in isopropyl myristate and clove oil along with tea tree oil in a ratio of 2:1:1:1 was chosen as oil phase to develop topical nanoemulgel of thymol and eugenol.Tween 80 revealed the highest emulsification efficiency for selected oil phase. PEG 400 showed highest nano- emulsification efficiency in a phase diagram study for selected oil and surfactant system.

EVALUATION PARAMETERS

Table no.6: Optimized Nanoemulgel Evaluation. (F3 Batch)

Sr.no.	Evaluation Parameter	Result / Observation
01	Appearance	Yellowish (Transparent in Nature)
02	Fragrance Efficiency	Pleasant odor
03	pH of gel	7.30
04	Homogenicity	Good
05	Viscosity	46,328±135cp
06	Extrudability	12.12±0.25gm

Drug Content Uniformity:

Table no.7: Result of Drug Content Uniformity of Nanoemulgel

Sr.no.	Batch Code	Amount of Drug	Absorbance at 278nm	Drug Content
1	F1	100 mg	0.214 A	45.55
2	F2	100 mg	0.372 A	67.30
3	F3	100 mg	0.421 A	80.63

The batch F3 shows a maximum absorbance at 278 nm and F3 show maximum drug content. The results are given in above table.

ANTIBACTERIAL ACTIVITY:

Table no.8: Antibacterial activity

Sr.no.	Marketed formulation Tetracycline	Pure thyme + clove + tea tree oil	Prepared nanoemulgel	Control saline water
F1	2.5	2.1	1.9	No Zone of inhibition
F2	3.2	2.8	2.1	No Zone of inhibition
F3	2.7	2.5	2.4	No Zone of inhibition
F4	2.9	3.1	2.9	No Zone of Inhibition

DRUG RELEASE DATA:

Table no.9: Result of Drug Release of Nanoemulgel

Time	Absorbance	Microgram /ml	Microgram/20ml	Correction factor	% drug release
10 min	0.034	0.076	0.265	0.265	35.53
20 min	0.156	0.087	0.789	0.209	45.56
30 min	0.180	0.098	1.897	0.396	51.32
40 min	0.215	0.123	1.987	0.787	53.34
50 min	0.289	0.154	2.98	2.35	60.55
60 min	0.300	0.189	3.13	7.35	71.45
2 hr	0.317	0.200	3.54	26.01	73.65
3 hr	0.356	0.225	3.68	95.72	80.27
4 hr	0.397	0.276	4.1	392.45	82.93
5 hr	0.412	0.287	5.6	2197.73	90.64
6 hr	0.465	0.291	5.98	9876.87	98.23

CONCLUSION

From recent work it was assured that thymol,eugenol,tea tree oil loaded nanoemulgel can be considered a reliable delivery system for anti-acne treatment.A nanoemulgel is porous in nature release the drug for prolong period of time therefore it shows better and prolong pharmacological action for treatment of acne (acne vulgaris).

Topical Nanoemulgel have proven as better option for effective and convenient drug delivery system. Gel and non-greasy like properties are giving more patient compliacne and lacks of oily as a base provides better drug release compared to other formulations. Incorporation of Nanoemulsion into gel matrix makes formulation dually control released system, Problems like creaming and phase separation which is associated with classical emulsion gets resolved with improved sparedability.Nanoemulsion loaded gel gives higher effectiveness in some topical disorders.

Future of Nanomulsion-Gel based formulations may provide a better and reliable solution for delivery of hydrophobic drugs. A considerable lot of medications utilized as a part of treatment of skin infection are hydrophobic natured and such medications can be conveyed successfully as Nanoemulgel where drug is incorporated into oil phase of Nanoemulsion and then merged with gel base. In spite of having couples of impediments, nanoemulgel has the likelihood to possess the focal place for topical conveyacne for lipophilic drugs in future.

REFFERENCE

1) Ak, M., 2019. A comprehensive review of acne vulgaris. J. Clin. Pharm, 1(1), pp.17- 45.
2) Ravindran, P.N., 2017. The encyclopedia of herbs and spices. CABI.
3) Talianu, M.T., Dinu-Pîrvu, C.E., Ghica, M.V., Anuţa, V., Jinga, V. and Popa, L., 2020. Foray into concepts of design and evaluation of microemulsions as a modern approach for topical applications in acne pathology. Nanomaterials, 10(11), p.2292.
4) Kotnala, A., Verma, K., Sharma, A., Parashar, S., Rathi, B., Kumar, R., Chhikara, B.S. and Singh, J., 2019. Indian Medicinal Plants for skin care and cosmeceuticals: A review. Journal of Biomedical and Therapeutic Sciences, 6(2), pp.24-60.
5) Merr, L.M Perry 2021. Kew Science, Plants of the World Online. Retrieved 28 February 2021.
6) "Clove". Drugs.com. 5 March 2018. Retrieved 9 November 2018.
7) Kamatou, G.P., Vermaak, I. and Viljoen, A.M., 2012. Eugenol—from the remote Maluku Islands to the international market place: a review of a remarkable and versatile molecule. Molecules, 17(6), pp.6953-6981.
8) Balch, P.A., 2006. Prescription for nutritional healing. Penguin.
9) Essential oil of Melaleuca, terpene-4-ol (tea tree oil): ISO 4730: 2017 (E). International Organization for Standardization (ISO), Geneva, Switzerland. 2017. Retrieved 2 February 2019.
10) Shafi SK, Duraivel S, Bhowmik D, Kumar KS 2013 Microsponge drug delivery system. Indian Journal of Research in Pharmacy and Biotechnology Mar 1;1(2):206.
11) Joshi, B., Singh, G., Rana, A.C., Saini, S. and Singla, V., 2011. Emulgel: a comprehensive review on the recent advacnes in topical drug delivery. Int Res J Pharm, 2(11), pp.66-70.
12) Verma, S., Singh, A.K. and Mukerjee, A., 2016. Formulation and evaluation of ketoconazole nanoemulgel. World journal of pharmacy and pharmaceutical sciences, 5(2), pp.899-911.
13) Aiswarya, G., 2015. Development, evaluation, and optimization of flurbiprofen nanoemulsions gel using quality by design concept. Asian Journal of Pharmaceutics (AJP), pp.35-43.
14) Vats, S., Saxena, C., Easwari, T.S. and Shukla, V.K., 2014. Emulsion based gel technique: Novel approach for enhancing topical drug delivery of hydrophobic drugs. IJPRS, 3, pp.649-60.
15) Mohamed, M.I., 2004. Optimization of chlorphenesinemulgel formulation. The AAPS journal, 6, pp.81-87.
16) Kumar, L. and Verma, R., 2010. In vitro evaluation of topical gel prepared using natural polymer. International journal of drug delivery, 2(1).
17) Anand, K., Ray, S., Rahman, M., Shaharyar, A., Bhowmik, R., Bera, R. and Karmakar, S., 2019. Nano-emulgel: emerging as a smarter topical lipidic emulsion- based nanocarrier for skin healthcare applications. Recent patents on anti-infective drug discovery, 14(1), pp.16- 35.
18) Ahmad, J., Kohli, K., Mir, S.R. and Amin, S., 2011. Formulation of self- nanoemulsifying drug delivery system for telmisartan with improved dissolution and oral bioavailability. Journal of Dispersion Science

and Technology, 32(7), pp.958- 968.

19) Ahmad, J., Mir, S.R., Kohli, K. and Amin, S., 2014. Effect of oil and co-surfactant on the formation of Solutol HS 15 based colloidal drug carrier by Box–Behnken statistical design. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 453, pp.68-77.

20) Stojanovic, R., Belscak-Cvitanovic, A., Manojlovic, V., Komes, D., Nedovic, V. and Bugarski, B., 2012. Encapsulation of thyme (Thymus serpyllum L.) aqueous extract in calcium alginate beads. Journal of the Science of Food and Agriculture, 92(3), pp.685-696.