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Formulation and Evaluation of Antimicrobial Nail Lacquer for the Treatment of Paronychia and Onychomycosıs

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Abstract: Onychomycosis and Paronychia are nail infections caused by fungal and bacterial species respectively. In the present work, a medicated antimicrobial nail lacquer containing Oleuropein has been developed. The nail lacquer formulation was prepared by simple mixing and it was analyzed for non-volatile content, gloss, smoothness, viscosity, water resistance, drug diffusion studies, antimicrobial susceptibility studies against common fungal and bacterial organisms. Among all formulation, nail lacquer prepared with 3.5% Oleuropein, 7% ethyl cellulose, 15 % salicylic acid, 10% ethyl acetate exhibited better non-volatile content, drug release, and zone of inhibition. The drug shows complete release of 96.40% at 3 hours. FTIR studies revealed that drug and excipients are compatible. Formulation and usage of these systems are safe, without any complication. So, we conclude that the antifungal nail lacquer may be one of the novel dosage forms that can revolutionize the pharmaceutical and health care sector.

Keywords: Oleuropein; nail lacquer; Onychomycosis; drug release; evaluation.

1. Introduction

Nail polish or nail varnish is applied to human fingernails or toenails to decorate and/or protect the nail plate. Conventional nail lacquers have been used as cosmetics since a long time for beautification and protection of nails. Topical nail preparations like lacquers, enamel and varnish are an integral part of today's beauty treatments. It protects the nail plate, but more importantly it enhances their beauty, imparting color, and gloss. [1-5] Onychomycosis accounts for one third of integumentary fungal infections and one half of all nail disease. Tinea unguium is more than a cosmetic problem, although persons with this infection are often embarrassed about their nail disfigurement. Because it can sometimes limit mobility, onychomycosis may indirectly decrease peripheral circulation, thereby worsening conditions such as venous stasis and diabetic foot ulcers. Fungal infections of the nails can also spread to other areas of the body and, perhaps, to other persons [5-10]

Candidal onychomycosis can be divided into three general categories.

- Infection beginning as a paronychia (infection of the structures surrounding the nail; also called a "whitlow"), the most common type of Candida onychomycosis.
- Patients with chronic mucocutaneous candidiasis are at risk for the second type of Candida onychomycosis, called Candida granuloma, which accounts for less than 1% of

onychomycosis. This condition is seen in immune compromised patients and involves direct invasion of the nail plate.

❖ Candida onycholysis can occur when the nail plate has separated from the nail bed. Distal subungual hyperkeratosis can be seen as a yellowish gray mass lifts off the nail plate. [11-20]

The penetration of drug into nail is quite difficult due to various factors such as the molecular size of the drug, hydrophilicity, pH, solute charge. Consequently, researches are currently being undertaken to design novel in vitro methods to assess the ability of compounds to penetrate the nail plate. The goal of treatment of Onychomycosis is drug penetration into deep nail stratums at amounts above the minimal inhibitory concentration (MIC). Effective penetration remains challenging as the nail is composed of approximately 25 layers of tightly bound keratinized cells which in comparison to stratum corneum is 100- fold thicker. [20-33] Keratolytic agents such as (papain, urea, and salicylic acid) enhance the permeability of three imidazole antifungal drugs (Miconazole, Ketoconazole, and Itraconazole) Urea and salicylic acid hydrate and soften nail plates. Urea and salicylic acid also damage the surface of nail plates, resulting in a fractured surface. [33-42]

In this study, we intended to formulate and evaluate an antifungal nail lacquer using Oleuropein as active substance and salicylic acid as penetrating agent.

2. Results and Discussion

2.1 FTIR Compatibility studies

IR spectra of ethyl cellulose, Oleuropein and final formulation were measured and the results are depicted below

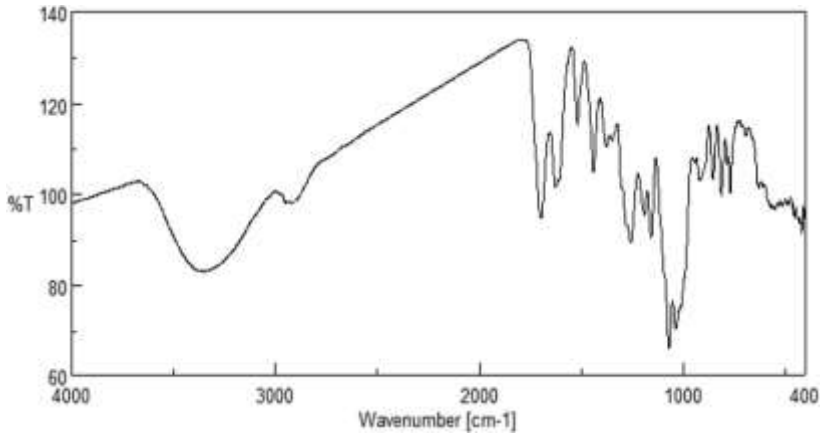

Figure 1. FTIR spectrum of Oleuropein

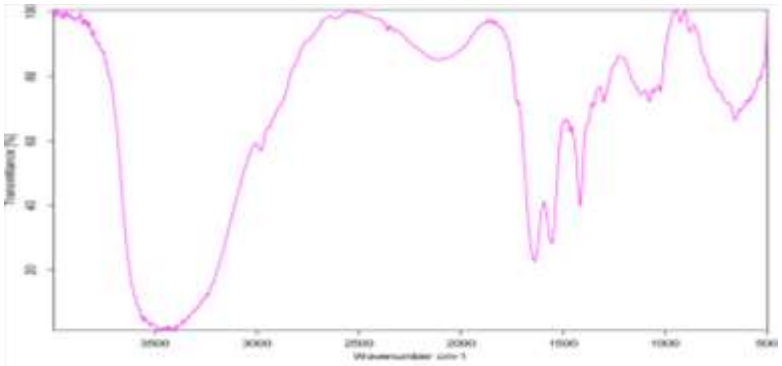

Figure 2. FTIR spectrum of Ethyl Cellulose

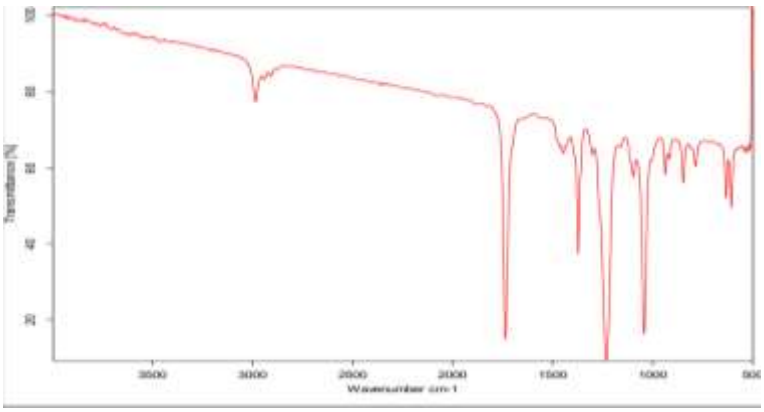

Figure 3. FTIR spectra of final formulation (F7)

Table 1 FTIR Drug Excipient compatibility

Functional group	**Standard wavenumber (cm-1)**	**Oleuropein**	**Ethyl cellulose**	**Formulation F7**
O-H str	3400	3350	3400	3100
O-H bend	1400	1350	1200	1500
C-O str	1150	1150	1150	1100
=C-H str	3100	3200	-	3100
C=C str	1650	1700	-	1620
C=O str	1720	1750	-	1710

After spectral comparison it was confirmed that no incompatibility reaction took place between drug and excipients, as all major characteristic IR peaks of Oleuropein are present in the physical mixture with individual excipients and in the final optimized formulation, F7. The drug peak and all the excipient peaks were found to be intact indicating good compatibility.

2.2 Evaluation of nail lacquer

Table 2. Non-volatile content

Formulation code	Non-volatile content (% w/w)	Formulation code	Non-volatile content (% w/w)
F1	42.8 ± 0.2	F6	30.6 ± 0.6
F2	41.6 ± 0.2	F7	44.3 ± 0.2
F3	38.2 ± 0.3	F8	29.7 ± 0.1
F4	39.5 ± 0.4	F9	32.2 ± 0.7
F5	43.8 ± 0.2	IS limit*	>20

*Indian Standards (9245-1994) for nail enamel

All formulations showed desired amount of non-volatile content (30.6 – 44.3%) with complete evaporation of volatile matter leaving a thin film. It was found to comply with the Indian Standards (IS: 9245-1994) for nail enamel's limits, that is non-volatile content greater than 20% w/w. Only formulations F5 and F7 showed higher amounts of non-volatile contents.

Table 3. Drying time

Formulation code	Drying time (sec)	Formulation code	Drying time (sec)
F1	80 ± 0.5	F6	90 ± 0.3
F2	90 ± 0.2	F7	99 ± 0.4
F3	75 ± 0.5	F8	85 ± 0.5
F4	80 ± 0.2	F9	82 ± 0.3
F5	98 ± 0.2	IS limit	<360

Drying time was found within 360 seconds, IS limit for nail enamel. All formulations showed rapid drying rate. i.e., less than 100 seconds. Rapid drying rate improves patient compliance and quickens up film forming.

Table 4. Viscosity

Formulation code	Viscosity (cP)	Formulation code	Viscosity (cP)
F1	140	F6	150
F2	145	F7	182
F3	142	F8	160
F4	140	F9	165
F5	180	IS limit	120-200

The viscosity of the sample ranged from 120 to 200 centipoise and it was observed that between 140 to 185 centipoise the product was clear and glossy, so all formulations were well within limits. More over this viscosity range provided good flow property. Viscosity outside this range produces clouding and decreases gloss which will not be cosmetically acceptable. Formulations F5 and F7 showed better gloss as their viscosity were on the higher side, 180 and 182 respectively

Table 5. Thickness and Folding Endurance

F. code	Thickness (mm)	Folding Endurance	F. code	Thickness (mm)	Folding Endurance
F1	0.058	118	F6	0.046	148

F2	0.054	114	F7	0.045	155
F3	0.052	122	F8	0.049	123
F4	0.054	124	F9	0.050	134
F5	0.048	152	IS Limit	<0.080	>120

Thickness of all the films measured by using a vernier caliper. Results showed that thickness of all formulations varied from 45 to 58 µm. Folding endurance indicates the flexibility of the polymer film. In order to evaluate the flexibility, the prepared films were subjected to folding endurance studies. The number of folds a film can sustain without break will dictate its folding endurance. The values obtained were above 125 in all the developed films. Irrespective of polymer concentration used, all the films showed good folding endurance, revealed that the prepared films were having the capacity to withstand the mechanical pressure along with good flexibility. The folding endurance is an important evaluation, which ensures the flexibility of the developed films. This is the measure of the resistance towards water permeability of the films. This was done by applying a continuous film on a surface and immersing it in water.

Table 6. Water resistance

Formulation code	**W1(mg)**	**W2(mg)**	**Formulation code**	**W1(mg)**	**W2(mg)**
F1	5	7	F6	5	10
F2	7	10	F7	6	8
F3	5	12	F8	5	9
F4	6	10	F9	7	12
F5	5	6	Marketed	5	10

The weight before and after immersion was noted and increase in weight was calculated. Higher the increase in weight lowers the water resistance. Here, formulation F5 and F7 has comparatively low weight and has the better water resistance. Both these formulations were like the marketed nail lacquer, which also has higher water resistance as compared to most of the formulations

Table 7. Smoothness and Gloss of all formulations

Formulation code	**Smoothness**	**Gloss**	**Formulation code**	**Smoothness**	**Gloss**
F1	++	++	F6	++	++
F2	++	++	F7	+++	+++
F3	++	++	F8	++	+++
F4	++	++	F9	++	++
F5	+++	+++	Marketed Nail Polish	+++	+++

+ - Average, ++ Good, +++ Excellent

Figure 4. Smoothness and Gloss of Formulation 5 and 7

Both these parameters were found to be satisfactory as can be observed from **Figure 6.11**. The nail lacquer poured onto the glass plate was found to spread and result in a uniform smooth film. The gloss of the applied lacquer was comparable with marketed cosmetic sample proving the cosmetic acceptance. These parameters also comply with the Indian Standards for nail enamel.

2.3 Determination of antimicrobial activity

Table 8. Antimicrobial activity

F.Code	Zone of Inhibition (mm)					
	C.albicans	**E.coli**	**K.pnemoniae**	**P.aeuroginosa**	**S.aureus**	**E.faecalis**
F5	20.5	11.5	10.5	16	15.5	12.5
F7	25.2	13.2	18.2	16.5	17.4	13.2
PC**	27.3*	10.2	20	18	18	16
NC$	0	0	0	0	0	0

*Ketoconazole **Linezolid $Acetone: Ethyl Acetate (1:1)

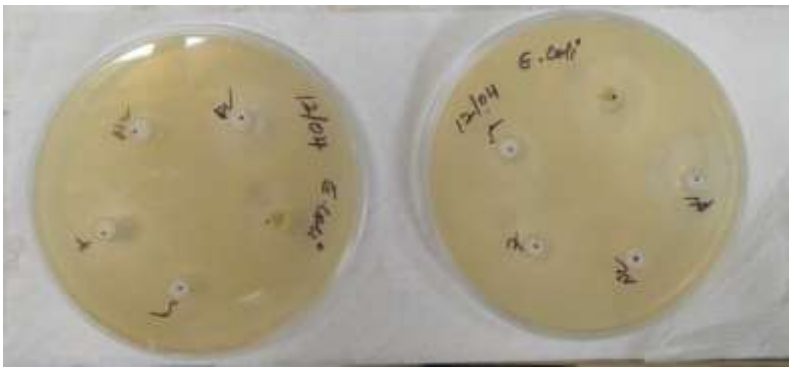

Figure 5. Figure showing zone of inhibition of F5 & F7 against E. coli

Figure 6. Figure showing zone of inhibition of F5 & F7 against S. aureus

Figure 7. Figure showing zone of inhibition of F5 & F7 against E. faecalis

Figure 8. Figure showing zone of inhibition of F5 & F7 against K. pneumonia

Figure 9. Figure showing zone of inhibition of F5 & F7 against

P. aeruginosa

Figure 10. Figure showing zone of inhibition of F5 & F7 against C. albicans

The zone of inhibition for the various formulations was determined, and it was found to range from 11.5-25.2 mm, which is comparable with that of standard with 27 mm. This indicates that all the formulations were sensitive to Candida albicans, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis. This shows the formulation can be effectively used against both bacterial and fungal infections.

6.3 Diffusion studies across artificial membrane

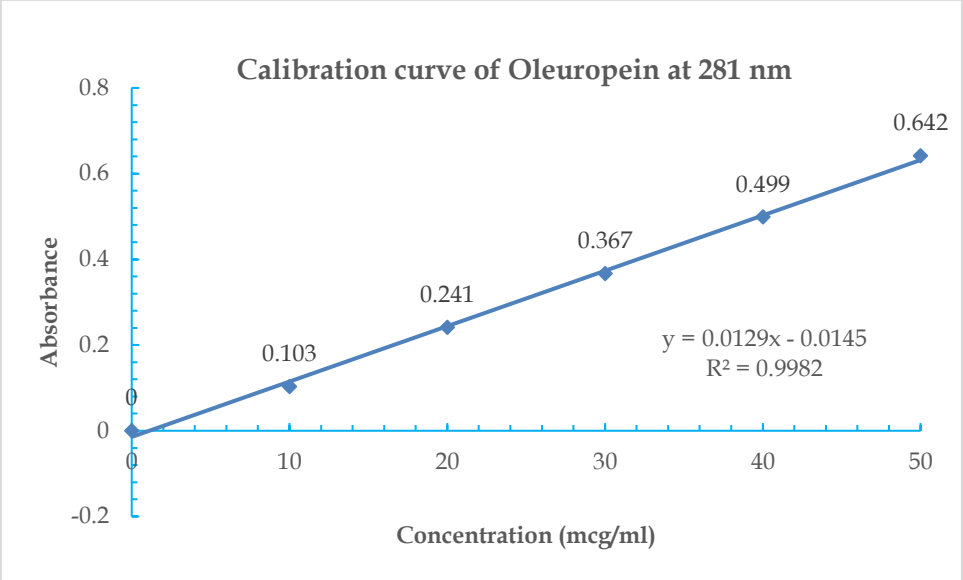

Figure 11. Calibration curve of oleuropein

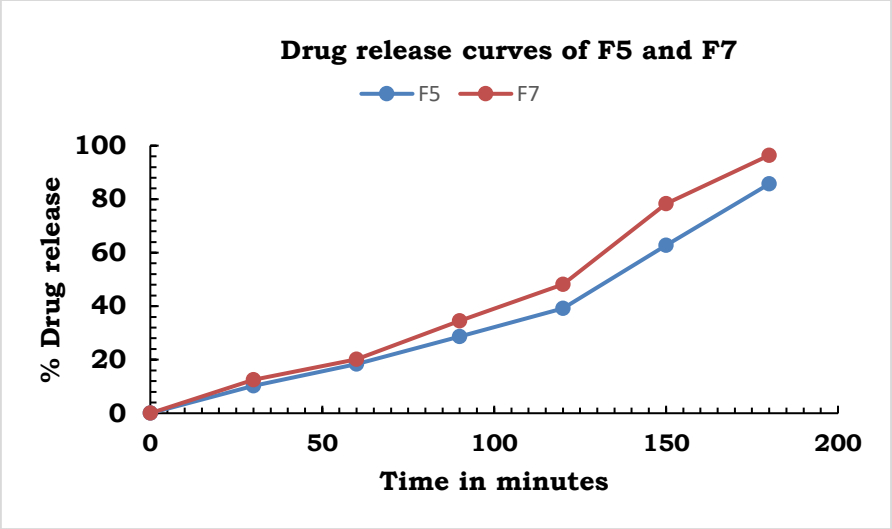

Figure 12. Drug release curve of oleuropein

The diffusion studies revealed that only 85.7% and 96.4% respectively was released in 3 hours. It was clear that salicylic acid has improved the drug permeation due to its keratolytic activity. This clearly indicates that F7 is better release profile as compared to F5 and therefore F7 is selected as the optimized formulation

3. Conclusıon

This study aimed to develop and evaluate Oleuropein-infused nail lacquer as a novel ungual drug delivery system for treating onychomycosis and paronychia. Oleuropein was selected as the model drug, and the formulations were enhanced with castor oil (a permeation enhancer) and salicylic acid (a keratolytic agent). The nail lacquers underwent comparative analysis based on drying time, non-volatile content, drug content, drug diffusion, and antimicrobial efficacy.
The FTIR studies confirmed the compatibility of the drug with the chosen excipients. All formulations exhibited satisfactory film formation, appropriate drying time, smooth flow, and the desired volatile content. The antimicrobial studies demonstrated the formulations' effectiveness against fungi, gram-positive, and gram-negative bacteria. The diffusion studies suggested a strong in vitro-in vivo correlation.
Notably, formulation F7 stood out, showing complete drug release sustained over 6 hours, indicating that the combination of the permeation enhancer and keratolytic agent significantly improved the permeation rate. Based on the drug diffusion studies, F7 was identified as the optimized nail lacquer formulation.
In conclusion, medicated nail lacquers have proven to be an effective topical treatment for microbial diseases affecting nails. The incorporation of permeation enhancers and proteolytic biocompatible polymers in nail lacquers presents a promising strategy to combat ungual infections more efficiently. Previous research supports the use of Quality by Design (QbD) and experimental designs, particularly 2^3 and 3^2 factorial designs, as foundational for such innovative formulations.
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4. Materıals and Methods

4.1 Equipments:

Following are the list of instruments used for the study

Table 9. Instruments which were used in the study

S.No	NAME OF THE INSTRUMENT	MODEL
1	UV-Visible spectroscopy	SHIMADZU 1800
2	FTIR	JASCO
3	Franz diffusion cell	Borosil®
4	Brookfield viscometer	DVE
6	Tray dryer	Rami International

4.2 Chemicals and Reagents:

Following are the list of chemicals used for the study

Table 10. List of chemicals used in the study

S.No	CHEMICAL/REAGENTS	MANUFACTURER
1	Oleuropein	Kshipra Biotech, Indore
2	Acetone	LR, SRL Chemicals, Mumbai
3	Ethyl acetate	LR, SRL Chemicals, Mumbai
4	Ethyl cellulose	LR, SRL Chemicals, Mumbai
5	Castor oil	LR, SRL Chemicals, Mumbai
6	Salicylic acid	LR, SRL Chemicals, Mumbai

4.3 Formulation of nail lacquer [43]

The formulation trials were done as per formula given in **Table 5.3** Ethyl cellulose was dissolved in required quantity of ethyl acetate in a beaker and stirred well using a glass rod. To above clear solution required quantity salicylic acid, castor oil along with oleuropein in acetone, were mixed thoroughly for about 20 minutes and made up to the volume to 10ml & was transferred into a suitable air tight container.

Table 11. Formula table for different formulations

Ingredients (g or ml)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Ethyl cellulose	5	4.5	4.0	3.5	3.0	2.5	2.5	2.5	2.5
Oleuropein	0.25	0.25	0.25	0.25	0.25	0.35	0.35	0.35	0.35
Ethyl acetate	12	12	12	12	12	12	12	12	12
Salicylic acid	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.5
Castor oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.5	2.5
Acetone (q.s)	10	10	10	10	10	10	10	10	10

4.4 FTIR Compatibility Studies [44]

FT-IR spectral analysis of pure drug and polymer were carried out individually and as mixtures. The compatibility between Oleuropein, ethyl cellulose, and prepared formulation were carried out in the ratio 1:1. The samples were analysed in FT-IR after mixing and triturating with potassium bromide.

4.5 Evaluation of Nail Lacquer [45,46]

4.5.1 Non-volatile content

10 ml of sample was taken in a petri dish and initial weights were recorded. The dish was placed in the oven at 105°C for 1hr, the petri dish was removed, cooled, and weighed. The difference in weights was recorded. Average of triplicate readings was noted and tabulated.

4.5.2 Drying time

A film of sample was applied on a petri dish with the help of a brush. The time (seconds) to form a dry-to-touch film was noted with the help of stop watch. The average of three readings is taken.

4.5.3 Viscosity

Viscosity was determined using Brookfield Viscometer, model DVE at room temperature using spindle no. 3 at 20 rpm. The experiment is repeated thrice and the mean values were tabulated.

4.5.4 Film thickness and folding endurance

The thickness of the film was measured by using Vernier calliper with a least count of 0.01 mm at different spots of the films. The thickness was measured at five different spots of the film and average was taken. Folding endurance of the films was determined by repeatedly folding a small strip of the film (approximately 2x2 cm) at the same place till it broke. The

number of times film could be folded at the same place, without breaking gives the value of folding endurance.

4.5.5 Water resistance

This is the measure of the resistance towards water permeability of the film. This was done by applying a continuous film on a surface and immersing it in water. The weight before and after immersion was noted and increase in weight was calculated. Higher the increase in weight lowers the water resistance.

4.5.6 Smoothness and Gloss

The sample was poured from a height of 1.5 inches into a glass plate and spread on a glass plate and made to rise vertically and visually observed for smoothness of film. Sample of nail lacquer was applied over the nail and gloss was visually seen, compared with marketed cosmetic nail lacquer.

4.6 Determination of antimicrobial activity [47]

One fungus (Candida albicans), three-gram negative (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa), two-gram positive (Staphylococcus aureus, Enterococcus faecalis) organisms were employed for testing antimicrobial activity using the well-diffusion method. The culture was maintained on Mueller Hinton agar. 0.5 McFarland standard suspension of all organisms were inoculated in the petri dish. The wells (8mm diameter) were dug in the Petri dish and filled with 100 µl of the sample. The plates were kept for diffusion at 40°C for 1hr, and followed by incubation at 30ºC for 48 hrs. After the completion of incubation period the zone of inhibition in millimeter were measured. Along with the test solution in each petri dish one well was filled up with solvent, which act as control. The zone of inhibition was recorded and compared with control. The readings were taken in duplicate and the average was taken.

4.7 Diffusion studies across artificial membrane

4.7.1 Construction of Calibration curve for Oleuropein [48]

100mg of Oleuropein pure drug was accurately weighed and transferred into a 100ml volumetric flask. Then the volume was made up to 100ml with distilled water, to obtain standard stock solution of Oleuropein, having concentration 1000mcg/ml. From the above solution aliquots of 1ml, 2ml, 3ml, 4ml, 5ml was pipetted out into another 100ml volumetric flask and made up to 100ml with distilled water to obtain a concentration range of 10µg/ml, 20µg/ml, 30 µg/ml, 40 µg/ml, and 50µg/ml solution. This solution was analyzed at 279 nm by using UV- Visible spectrophotometer. A graph of concentration vs absorbance was plotted. Drug content estimation in diffusion studies was based on this calibration curve.

4.7.2 Diffusion study [49-52]

Diffusion studies were performed by Franz diffusion cell using artificial membrane (cellophane) of 0.8µm. The membrane was soaked for 24hrs in solvent system and the receptor compartment was filled with solvent. Nail lacquer equivalent to 200mg was applied evenly on the surface of the membrane. The prepared membrane was mounted between the cells carefully to avoid entrapment of air bubbles under the membrane. The whole assembly was maintained at 37°C, and the speed of stirring was kept constant for 3 hrs. The 5ml aliquot of drug sample was taken at time intervals of 30 mins, 60 mins, 90 mins, 120 mins, 150 mins, and 180 mins and was replaced by the fresh solvent. Samples were analyzed by double-beam UV

spectrophotometer using standard calibration curve of Oleuropein in water at 281 nm Each experiment was repeated thrice.

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