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Formulation and Evaluation of Green Tea Emulgel for Treatment of ACNE

Mahaveer Singh[1], Shiva Mishra[2], Harshrajsinh Ajaysinh Rana[3], Prashant Sharma[4], Dr. Anandkumar K. Patel[5], Sudha Unmesh Puri[6], Dr Sandeep Gupta[7], Dr Sribhavani K. R[8*]

[1]Associate professor, Department of pharmaceutics, DR Karigowda college of Pharmacy, Kuvempunagar, Hassan
[2]Associate Professor, Lloyd School of Pharmacy, Greater Noida
[3]Assistant Professor, Pharmaceutics Department Parul College of Pharmacy and Research, Parul University
[4]Assistant professor, K.R. Mangalam University, Sohna Road, Gurugram
[5]Professor, Saraswati Institute of Pharmaceutical Sciences, Gandhinagar
[6]Assistant Professor, Department of Pharmacology at JSPM's Jayawantrao Sawant College of Pharmacy and Research, Hadapsar, Pune, Maharashtra.
[7]Principal, LCIT College of Pharmacy, Bodri, Bilaspur (CG) 495220
[8*]Assistant Professor in the Department of Pharmacology, Indrayani Vidya Mandir's Krishnarao Bhegade Institute of Pharmaceutical Education and Research, Talegaon Dabhade, Pune District, Maharashtra

Corresponding Email: [8*]mailtosribhavani@gmail.com
[8*]Assistant Professor in the Department of Pharmacology, Indrayani Vidya Mandir's Krishnarao Bhegade Institute of Pharmaceutical Education and Research, Talegaon Dabhade, Pune District, Maharashtra

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ABSTRACT:

Emulgel is most trendsetting innovation is this 21st century. In this new technology, for the administration of hydrophobic drugs, emulgels have emerged as a possible drug delivery technology. The goal of the study was to make a allopolyherbal emulgel utilising Carbapol 940 as a gelling agent. As penetration enhancers resveratrol, green tea and chamomile oil were employed. The emulsion was made and put into the gel basis. The formulations were evaluated for Physical Parameter **SPF identification antioxidant activity, Viscosity, Estimation of pH**, Spread ability, Centrifugation Freeze thaw, HPLC ultraviolet spectroscopy, fourier transform infrared spectroscopy, ZETA potential, particle size, microscope

1. Introduction

Since the dawn of humanity, the sun has been the source of life and energy. Recent research, on the other hand, says that the sun has one of the most detrimental effects on the human body. Because the skin is the body's outermost covering, it is particularly vulnerable to harm from radiation released in the environment. UV exposure is recognised to be one of the primary causes of nonmelanoma skin cancer, sunburn, photo ageing, drug-induced photo toxicity, pigmentation, immunological suppression, and other skin cancers.Daylight allows for continuous electromagnetic radiation that includes ultraviolet (UV 200–400 nm), visibility (400–800 nm), and thermal (IR) (beyond 800 nm) bands, among others. UV light is classified into three wavelength ranges: UV-A (320–400 nm), UV-B (290–320 nm), and UV-C (200–290 nm), with UVC being totally intercepted by the ozone layer and so not reaching the earth's surface. UV-A and UV-B rays induce erythema, edoema, immunological suppression, photo ageing, DNA damage, and melanogenesis, among other things. Even though UV-B makes up less than 1% of the sun's energy, it is one of the major causes of skin ageing, skin cancer, and sunburn, which can be seen after 12–24 hours of exposure to these rays.UV-A rays are thought to be more hazardous than UV-B rays because they are more numerous and may penetrate deeper into the skin. They can also change the structure of elastin and fibrin, as well as cause damage to blood vessels and DNA. Sunscreen formulations are designed to protect skin from the detrimental effects of UV radiation by either establishing a protective barrier on the skin surface or absorbing the harmful rays. The aforementioned effect of sunscreen formulations is due to sunscreen agents. Physical and chemical sunscreens work in distinct ways.

Chemical filters absorb UV rays, but physical filters reflect them back. Because UV filters often require an oily substrate for breakdown, oily bases are widely used in sunscreen formulations. Oil-in-water or water-in-oil was stated to be a problem. For sunscreen formulation, the in-oil approach was most popular. However, the emulsion system's oily vehicle can make the face feel greasy, which isn't ideal for acne-prone skin. Emulgel, a formulation that combines emulsion with a gel foundation, solves the previous problem by making the formulation more water-based and less oily. Many sunscreen formulations with FDA-approved sunscreen compounds including zinc oxide, titanium dioxide, oxybenzone, avobenzone, octacrylene, and others are available on the market. The majority of the formulations are expensive to produce, and the usage of physical and chemical sunscreen agents has a number of negative side effects. Furthermore, none of these ingredients have antioxidant properties that help protect the skin from UV ray-induced free radical damage. Several natural compounds have been studied for their antioxidant and photoprotective properties in the literature. Natural compounds have recently been evaluated as viable sunscreen ingredients due to their photoprotective and antioxidant properties. Furthermore, they are plentiful and quite affordable. The sunscreen compounds currently employed in UV sunscreens are ineffective against reactive oxygen species (ROS). As a result, antioxidants are included in these formulations to neutralise ROS. Trans-resveratrol is a potent regulator of lipid peroxidation and a peroxyl radical scavenger, making it a strong free radical scavenger. Additionally, the use of resveratrol for photoprotective properties is widely documented. Polyphenols found in nature have been shown to provide a variety of health advantages. They have been shown to neutralise free radicals and to aid in the prevention of free radical damage. As a result, using polyphenols to generate formulations with antioxidant and photoprotective action is a very appealing technique. Green tea is a good source of natural polyphenols, which come mostly from the Camellia sinensis plant. As a result, resveratrol and green tea were chosen as ingredients in a herbal sunscreen formulation. The current work aims to create a hydrogel-based sunscreen formulation including phytochemicals, taking into account the limits and issues associated with currently commercialised sunscreen formulations.

Components and Procedures: -

COMPONENT

Resveratrol and Chamomile oil was purchase from vital herbal. Green tea dry leaves purchase from market, DPPH purchase from sigma Aldrich, Polyacrylic acid,glycerin,methyl paraben.propelen glycol,span 80, tween 80 provided by PSIT ,KANPUR

Procedures

Preformulation Study:

All the drugs were identified by various physical parameters, spectroscopic studies and chromatographic studies. Various confirmatory chemical tests performed to identify purchased chemicals. And thus selected phy to chemicals further processed to know SPF values and antioxidant activity. Resveratrol, green tea, chamomile oil were analyzed by using high-performance liquid chromatography (HPLC).The HPLC system consisted of following components:- Shimadzu HPLC is use for quntative and qualitative analysis. Solvent was use methanol, acetonitrile, and potassium dihydrogen phosphste buffer(pH6.8). c18(5µm)column is use (dimesion:4.6×250mm) in Isocratic method as shown is table number 2.

Study of the UV Spectrum: -

A UV–visible spectrophotometer was used to investigate the UV spectrum of resveratrol and green tea (Shimadzu UV-1700). In ethanol, a 20mg/ml standard solution of each phytoconstituent was produced, and the UV absorption spectrum in the 200–400 nm wavelength range was recorded.

Prepration of Dried Green Tea Ethanolic Extract: -

100 gram of dry green tea leaf was taken in 800ml ethanol and extraction was done by soxhlet apparatus at 40º-50ºc.Obtain aqueous extract concentrated under vacuum using rotator evaporator at 60º-80ºc for 2hours in Oder to remove ethanol content and obtain the extract in dry solid form. The extract was then standardized by HPLC, WHO guidelines of quality standrsization and ayurvedic pharmacopoeia.

Formulation Development

The emulsification method was used to make the cream bases. In a nutshell, there's an oil phase with lipophilic chemicals and an aqueous phase with hydrophilic molecules. In oil phase green tea, Chamomile oil with span80 and in aqueous phase Resveratrol, tween 80, ethanol adds. After that in oil, all ingredients add and vortex for 5 minutes. Like that aqueous is also ready. The oil phase was kept on a magnetic stirrer and the aqueous phase dispersed, in the small droplets into a continuous oil phase with temperature 40-60°c.

Ingredients	**F1**	**F2**	**F3**
Resveratrol	**1%**	5%	1%
Green Tea	**0.5%**	0.3%	-
Chamomile oil	**0.5%**	0.5%	0.5%
Tween 80	**0.1%**	0.2%	-
Span80	**0.5%**	.10%	0.5%
Ethanol	**2%**	2%	2%
Carbomer	**3%**	3%	3%
Glycerin	**0.2%**	0.2%	0.2%
Methyl paraben	**0.3%**	0.3%	0.3%

Proplen glycole	**6%**	6%	6%
Deionized water	**qs**	qs	qs

Table: - formulation for emulgel

Formulation of gel:
Formulation of gel takes deionized water and adds glycerin and propylene glycol in it. Then add carbomer and start using a magnetic stirrer for 15 minutes. Sure that there is no globule in it. Keep for 60 minutes until all carbomer is devolved. Use pH paper to know the pH. For gel, pH should be at 6 pH with continuous stirring add triethanolamine, and adjust pH6. When solution stir until the transparent gel appears to come. Use Ph paper to check pH.

Formulation of Emulgel:
Emulgel was created in a two-step process. The first step is to make oil in water emulsion and a gel basis. Second, in a 1:1 molar ratio, blend the emulsified and gel base.

Evaluation of Emulgel:
SPF identification: -
The initial stock solution was prepared by taking 1% w/v formulation of cream in ethanol. After then, a 0.1 percent stock solution was made from this stock solution. After that, each aliquot's absorbance readings were measured at 5 nm intervals between 290-320 nm. Using a Shimadzu UV-Spectrophotometer with ethanol as a blank.
SPF=CF (λ) ×I (λ) ×Abs (λ)
The aliquots were scanned between 290 and 320 nm, and the absorbance value obtained was multiplied by the corresponding EE (λ) and I (λ) values. After that, they added together and multiplied by the4 adjustment factors (10)

The DPPH method was used to determine antioxidant activity in vitro:-
In various vials, 1 ml of varied concentrations of active medication and standard were collected. 5ml of DPPH ethanol solution was added to this, shaken well, and incubated at 37°C for 20 minutes. At 516 nm, the absorbance was measured against ethanol as a blank. The DPPH absorbance was used as a control. The following calculation was used to compute the antiradical activity of percent
% Anti Radical activity= Control Absorbance – Sample Absorbance*100
Control Absorbance

Interpretation Qualities:
The characteristics of consistency, application feel, and irritation are established.

Thermal Permanence:
The lipid separation from the cream was examined in a humidity chamber at 60-70± percent RH and 37°±2º C. The beaker was stored in a humid room for 8 hours at 60-70 percent relative humidity and 37°±2°C. There should be no oil separation in the cream to pass the test.

Estimation of pH:
The pH of cream can vary; however, it usually falls between 5 and 9. The pH of the cream ranges from 6 to 9. According to Hazelton, there isn't much of a link between pH and irritancy. To ensure accurate readings, the sensor must be cleansed and devoid of any acid or alkaline residue. Procedure: Oil in water lyophilized emulsions has been used in all of the formulations. Because the pH of the emulsion could not have been directly measured, 10 percent mixtures

with distilled water were produced and the pH of the ensuing mixture was determined that used a pH meter.

Viscosity:
The Brookfield viscometer DVII+ Pro was used to determine the viscosity of creams. The operating condition was set after selecting the proper spindle (T-BAR SPINDLE) for the provided product. The viscosity was then measured directly at 50 rpm. Full-scale viscosity range for any DV-II+Pro model and spindle may be calculated using the equation:
Full scale viscosity range [Cp]= TK*SMC*10000
RPM

Stability by Centrifugation
Emulgel were spun at 3500- 4000rpm at intervals of 500 rpm for 10 minutes for the centrifugation trials. The phase separation of the formulations was observed.

Microscope: -
For microscope of emulgel use Trinocular microscope.

Skin Irritation Study
Experimental Animals:
Preparation of Animals for Testing: Each RABBIT back skin was shaved in a 2cm2 region before the experiment
Control animal: No formulation was used on the control animal.
Test Animals (Gel Formulation Animal): Active substances were used in the formulation.
The trial ingredient was 0.5 gm of herbs gel formulation, which was applied to a 2cm2 area of skin and covered with a gauze patch. The patch was kept loosely in contact with the skin for 1 hour with a semi-occlusive dressing before the gauze was removed. The remaining test chemical was eliminated after the exposure period (72 hour) without affecting the current reaction or epidermal integrity. Following the removal of the patch, observations were made. The animals were given 0.5 gm of base formulation, which was a cream created with all components except the active drug materials, and observations were performed in the same way as the test animals.

Dissolution study: - Disslution profile of emulgel formulation containing allopolyherbal was determined according to USP dissolution tear apparatus vertical diffusion cell. The vertical diffusion cell technique is a simple, dependable, and repeatable method of evaluating drug release from semisolid dosage forms (cream, ointment, and gel). An appropriate synthetic, inert support membrane is distributed uniformly with 200-400mg cream, ointment, or gel. At 32, the deployment experimental investigation was carried out. The volume removed is replenished with new receptor media after sampling, which usually takes 4-8 hours. A dissolving medium of phosphate- buffered saline (pH= 6.8) was used, and the temperature was set to 37°C. A 1ml aliquot of material was extracted at regular intervals, diluted, and spectrophotometrically analysed. In the meanwhile, an equal volume of buffer was added to keep the volume constant. For the formulation from the previously established calibration cutve, the cumulative percent release was computed.

Antibacterial activity:- Standardized inoculums are inoculated in the plates prepared earlier (aseptically) by dipping a sterile in the inoculums, removing the excess by pressing and rotating the swab firmly against the side of the culture tube above the lavel of the liquid, and finally streaking the swab all over the surface of the medium three times, ritating the plate through an

angle of 60 after each application. Finally, run the swab around the agar's edge. Close the cover and let the inoculums dry at room temperature. Each petri dish is separated into two halves, with each portion containing a sample disc, such as standard tetracycline hydrochloride, which is soaked in sample solution overnight and test emulgel are dissolving and make solution of 10 µg/ml. after that standard and test placed in the plate with help of forceps which is sterile.For diffusion, the petri plates are kept at room temperature for 1 hour. After that, incubate for 24 hours at 37 degrees Celsius. Observe the zone of inhibition created by the sample and note the average of two diameters of each zone of inhibition using a measuring scale.

Particle- size and zeta- potential measurement: - The particle size and zeta potential of emulgel were reported by Anton paar (model number: 500) particle size and zeta potential meter. From oniosome healthcare Pvt.Ltd.

TEM: - Transmission electron microscopy of emulgel were reported by Hitachi Japan(model:H-7500) From oniosome healthcare Pvt.Ltd.

2. Result

Physical parameter:

All physical evalution like colour, oder, solubility, melting point for drug were performed as shown in table number 2 and 3

DRUG	COLOUR	ODOUR	SOLUBILITY	MELTING POINT
RESVERATROL	White with yellow tinge	Characteristic	Ethanol	261-263°c
GREEN TEA	Olive jupiter		Ethanol/Water	140-148°c
CHAMOMILE OIL	pale yellow	Sweetly smoky	Ethanol	161°c boiling point

Table;2 Physical Parameter of crud drug

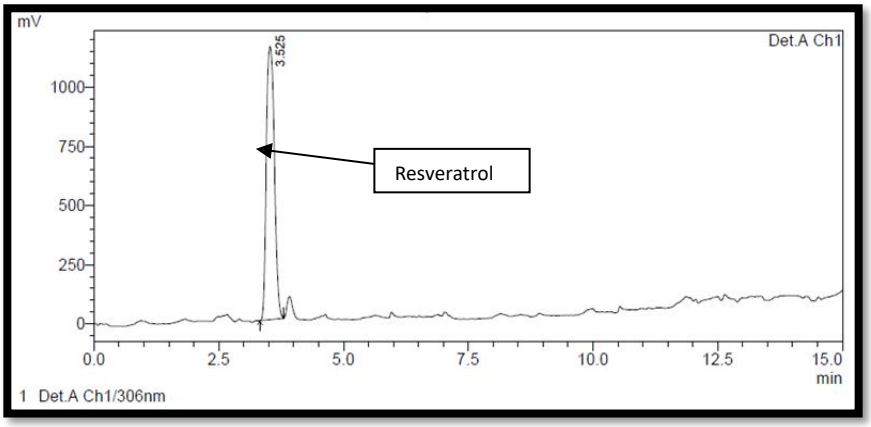
Figure:-1 Formulation1,Formulation2, Formulation3

Formulation no.	colour	Homogeneity	Consistency	Phase separation
F1	Green	Excellent	Excellent	No
F2	Green	Excellent	Excellent	No
F3	White	Good	Good	No

Table: 3 Physical parameter of formulation

Identification by HPLC: - In chromatogram of Resvertrol we use mobile phase methanol:buffer:ACN in ratio of 63:30:7.flow rate is 1ml/min.At 306 wavelength resveratrol found and retention time is 3.525 min.

For HPLC of green tea mobile phase is use methanol:ACN in ratio of 10:90in this flow rate is 1ml/min. At 280 nm wavelength 3pick are found they are following a. Epigallocatechin gallate (EGCG) retention time is 3.355, b. Epicatechin-3-gallatc (ECG) retention time is 1.824, c.Epigallocatechin (EGC) retention time is 2.207.
For HPLC of Chamomile oil mobile phase is use ACN:WATER in ratio of 30:70. Flow rate was 0.6ml/ min , at 340 nm wavelength 3 picks are found they are following a. Apigenin-7-o-(4''- acetyl-6-malonyl)-glucopyranaside retention 3.816, b. Apigenin 9.273, c.Cosmosiin 13.927.

Parameters	**Drug name**
	Resveratrol
Chromatogram of resveratrol	
Flow rate	1ml/min
Stationary phase/ Column	5µm C18 (dimesion:4.6×250mm) column:shimadzu
Mobile phase	Methanol:potassium dihydrogen phosphste buffer(pH6.8):Acetonitrile (63:30:7)
Method	Isocratic
wavelength	306nm
Retention time	3.52
	Green tea
Chromatogram of green tea	

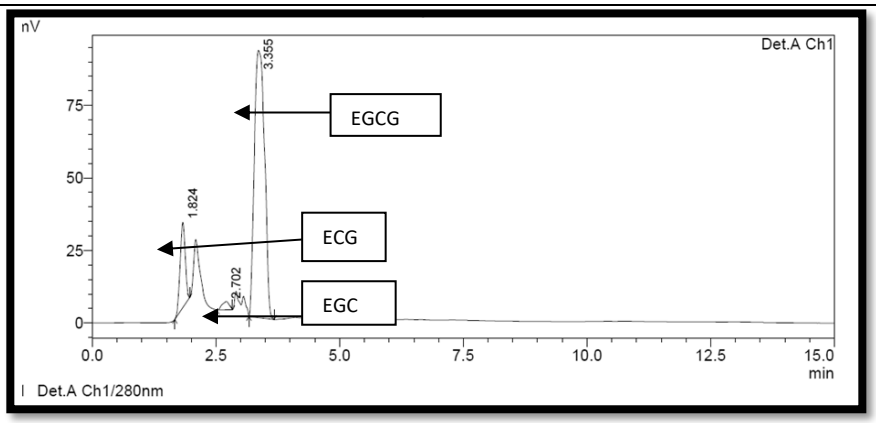
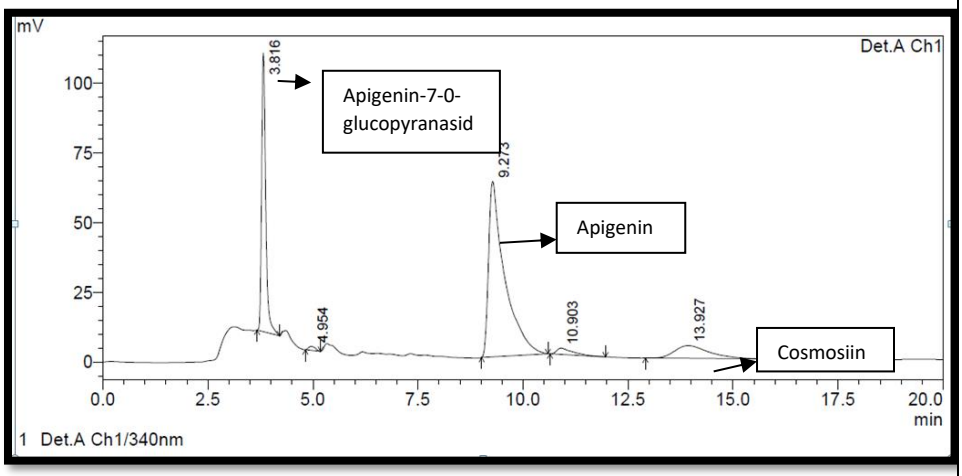
	
Flow rate	1ml/min
Stationary phase/ Column	5µm C18 (dimesion:4.6×250mm) column:shimadzu
Mobile phase	Methanol:Acetonitrile(10:90)
Method	Isocratic
wavelength	280nm
Retention time	3.35
	Chamomile oil
Chromatogram of Chamomile oil	
Flow rate	0.6ml/ min
Stationary phase/ Column	5µm C18 (dimesion:4.6×250mm) column:shimadzu
Mobile phase	Acetonitrile:Water(30:70)
Method	Isocratic
wavelength	340nm
Retention time	3.46

Table 3: -method development for HPLC

Study of the UV spectrum: - During uv spectrum(2µ,4µ,6µ,8µ,10µ) found that λ max of Resveratrol at 296λ and for green tea 274λ. Standard curve shown in figure number 1and 2

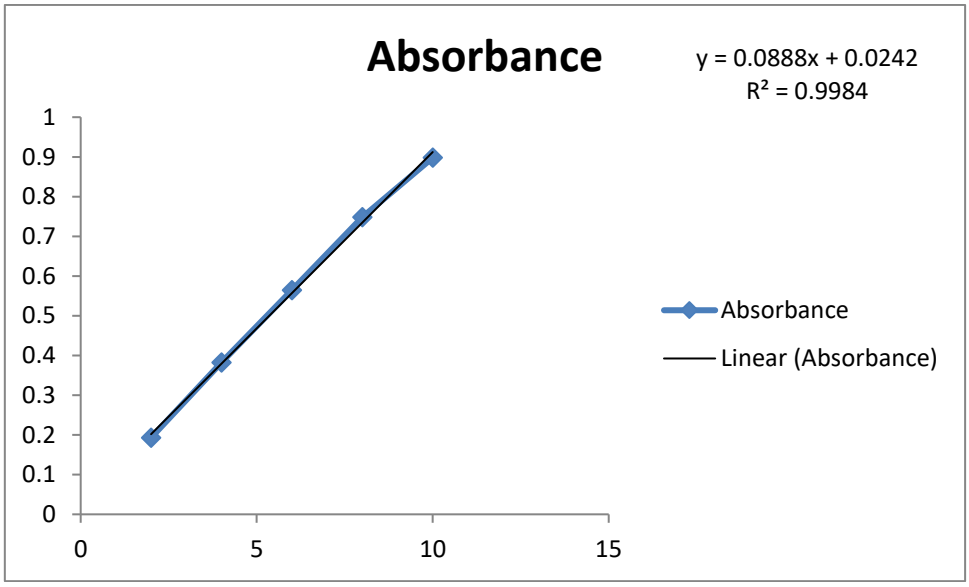


Fig1: Standard curve of Resveratrol at-λ- max 296

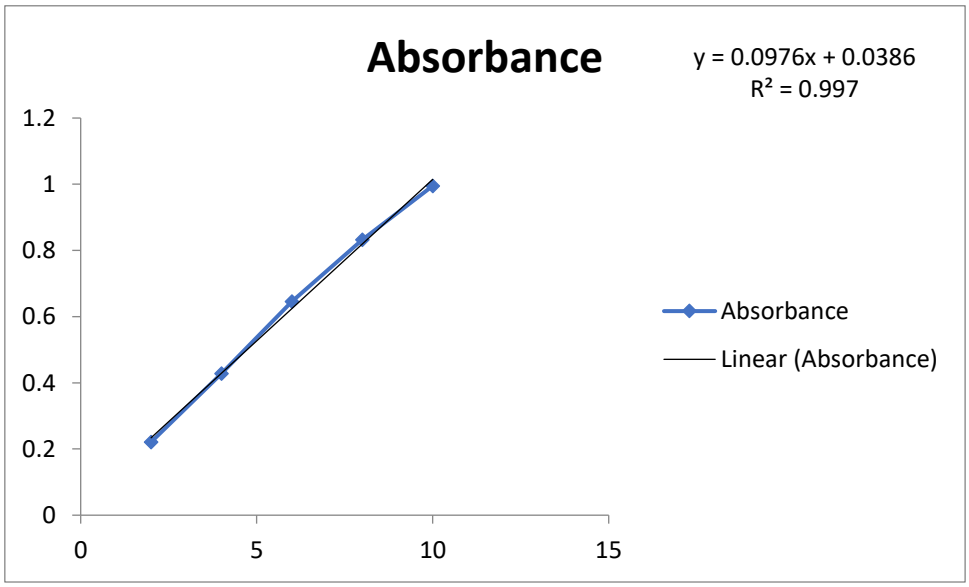


Fig 2: Standard curve of green tea

Formulation development: - All data give in table number 1

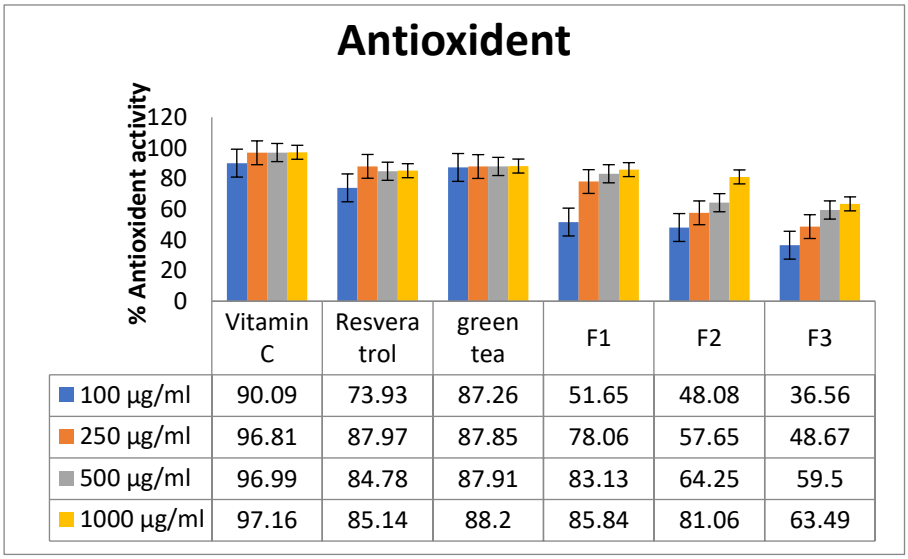
Evaluation of emulgel: -

SPF identification: - Here we identify SPF value by DPPH method of formulations and find F1 formulation have high SPF value and *** give to them. In formulation 2 and 3 have been find SPF value 11, and 10 we give ** to f2 and * to f3 formulation. Here we see that when herbal drug is lesser in amount than the SPF value is lower as shown in table 1and 4.

			FORMULATION F1		FORMULATION F2		FORUMULATION F3
Wavelength (nm)	EE(λ)*I(λ)	Absorbance (λ)	EE(λ)*I(λ)*ABS(λ)	Absorbance (λ)	EE(λ)*I(λ)*ABS(λ)	Absorbance (λ)	EE(λ)*I(λ)*ABS(λ)
290	0.015	1.744	0.02616	0.943	0.02466888	1.023	0.025236264
295	0.0817	1.856	0.1516352	1.077	0.16331111	1.07	0.174742888
300	0.2874	2	0.5748	1.179	0.6776892	1.077	0.729871268
305	0.3278	2.129	0.6978862	1.232	0.859795798	1.072	0.921701096
310	0.1864	2.001	0.3729864	1.15	0.42893436	1.002	0.429792229
315	0.0837	1.938	0.1622106	1.139	0.184757873	0.971	0.179399895
320	0.018	1.87	0.03366	1.104	0.03716064	0.982	0.036491748
	Totel=1		2.0193384		1.174396		1.049417
			SPF=20.193384		SPF=11.74396		SPF=10.49417

Table: -4 SPF determination of emulge

The DPPH method was used to determine antioxidant activity in vitro: - Exogenous causes such as UV light and pollution, as well as endogenous creation of radicals from cellular metabolism, can harm the skin at the cellular and tissue levels. Although the body has a sophisticated defence system to avoid radical damage, this system can be overburdened, resulting in oxidative stress, immunological suppression, and even cancer. Antioxidant supplements applied topically can help to neutralise reactive oxygen species produced by both endogenous and exogenous sources.1, 1-diphenyl-2-picrylhydrazyl (,-diphenyl—picrylhydrazyl; DPPH) is a stable free radical capable of delocalizing free radicals and causing ethanaol to turn violet. However, after reacting with antioxidant molecules, there is a reduction in violet colour intensity, which may be detected at 517nm.Absorbance before reaction-absorbance after reaction/ absorbance before reaction *100 = percent suppression of the DPPH radical. 2 mL DPPH 0.5 milimolar solution + 0.2 mL ethanolic extract. Measure the sample and standard at 517 nm after reaction of 30 minute. The anti-radical property of similarly samples obtained was assessed using the DPPH method, and activity was shown to be highly potent and here we compare the crud drug and formulations with ascorbic acid (vitamin C) and find 1000µg/ml of formulations gives % ANTIOXIDENT activity following; 85.84% of formulation F1,81.06% formulation F2, and 63.49% of formulation F3. Such as formulation F1 give higher %antioxidant activity and near to ascorbic acid% as compare to other formulation.



	Vitamin C	Resveratrol	green tea	F1	F2	F3
100 µg/ml	90.09	73.93	87.26	51.65	48.08	36.56
250 µg/ml	96.81	87.97	87.85	78.06	57.65	48.67
500 µg/ml	96.99	84.78	87.91	83.13	64.25	59.5
1000 µg/ml	97.16	85.14	88.2	85.84	81.06	63.49

Table 5: - antioxidant activity

Interpretation Qualities: - In the interpretation of qualities of emulgel following are found given in table number 5

Parameters	Formulation 1	Formulation 2	Formulation 3
Appearance	EmulGel like	EmulGel like	EmulGel like
Colour	Green	Green	White
Homogeneity	Uniform and homogeneous	Uniform and homogeneous	Uniform and homogeneous
Consistency	Good	Good	Average
Texture	Smooth	Smooth	Smooth
Irritation	NO	NO	NO

Table: -6 Interpretation qualities of emulgel

Thermal Permanence: - The lipid separation from the cream was examined in a humidity chamber at 60-70± percent RH and 37°±2º C for 8 hours.

Formulation	F1	F2	F3
Observation	No phase seperation	No phase saparation	No phase saparation
Inference	Stable	Stable	Stable

Table: -7 thermal permanence

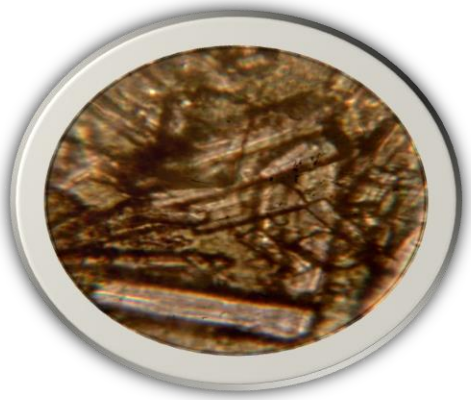
Estimation of pH: - The pH of cream can vary; however, it usually falls between 5 and 9. The pH of the cream ranges from 6 to 9. According to Hazelton, there isn't much of a link between pH and irritancy. To ensure accurate readings, the sensor must be cleansed and devoid of any acid or alkaline residue. The pH of emulgel was getting 6.3±2. For determination of pH digital meter was use.

Viscosity:

For viscosity Brookfield viscometer DVII+ Pro was used to determine the viscosity of emulgel. The operating condition was set after selecting the proper spindle (T-BAR SPINDLE) for the provided product. The viscosity was then measured directly at 50 rpm of formulation F1. After solving formula viscosity get 40000.

Stability by Centrifugation: - Emulgel were spun at 3500- 4000rpm at intervals of 500 rpm for 10 minutes. There is no phase sepration were found.

Microscopy: - For microscopy of gel Zoom Ratio 6.4 : 1, 7x to 45x magnifications set in Trinocular microscope and cylindrical rod like structure is found. Image is shown in figure 1



Fugure1:-microscopy of emulgel

Skin Irritation Study: - Each RABBIT back skin was shaved in a 2cm2 region before the experiment. The trial ingredient was 0.5 gm of herbs gel formulation, which was applied to a 2cm2 area of skin and covered with a gauze patch. The patch was kept loosely in contact with the skin for 1 hour with a semi-occlusive dressing before the gauze was removed. The remaining test chemical was eliminated after the exposure period (72 hour) without affecting the current reaction or epidermal integrity. 1 rabbit take in control group and 1 for test group. For Patch test only f1 formulation is use and as observed that there was not erythema (redness of skin).And in control group no formulation apply as shown in figure 2,3 and 4

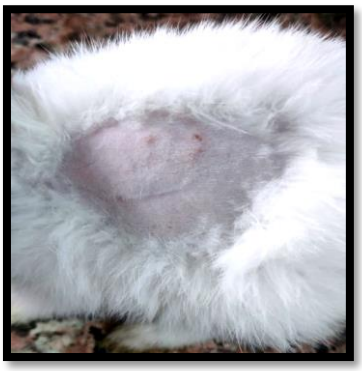


Figure 2:-Control group

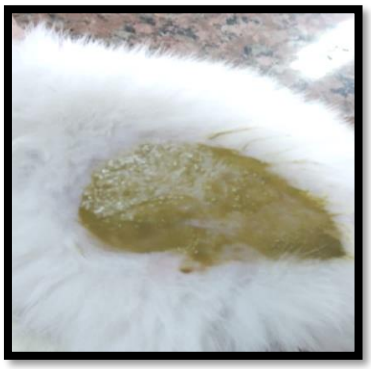


Figure 3:-Test group

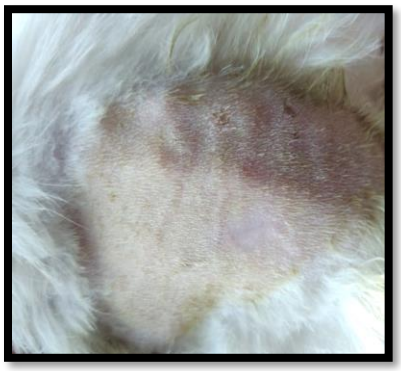


Figure 4:-After 72 (no erythema found)

In vitro Dissolution study: - Disslution profile of emulgel formulation containing allopolyherbal was determined according to USP dissolution tear apparatus vertical diffusion cell (franz deffusion cell). The cumulative % of emulgel dissolution profile is shown in figure number 5. The result explains the drug released of 8 hour by using of Synthetic membranes with 6.8 pH phosphate buffer and found herbal drug relies as faster to synthetic drug. Here we indicate Resveratrol as rvs, green tea as GT. The cumuiative present of drug relies is calculate by using both drugs standard curve .

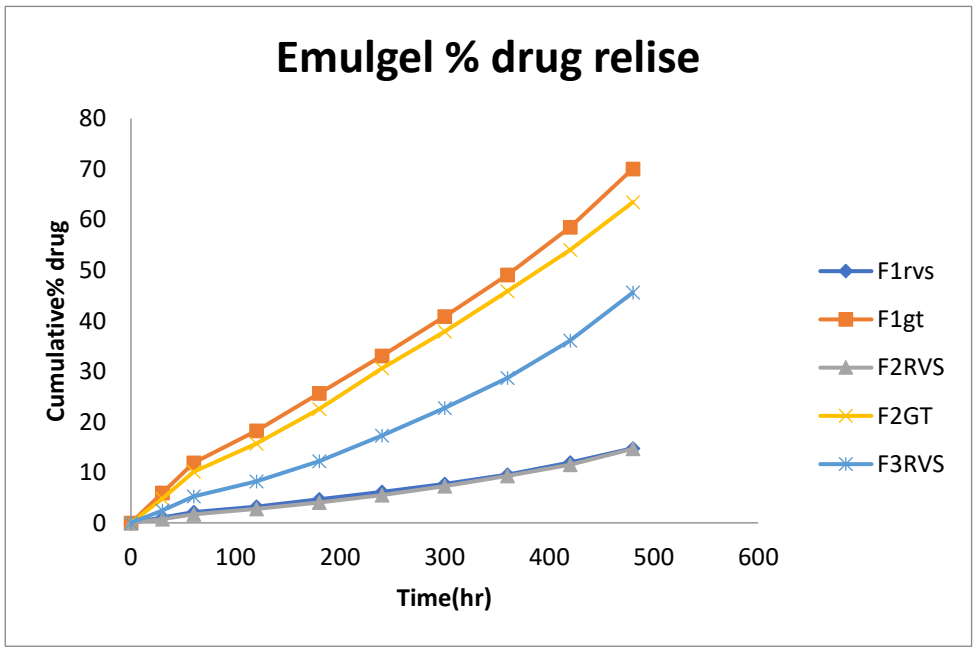
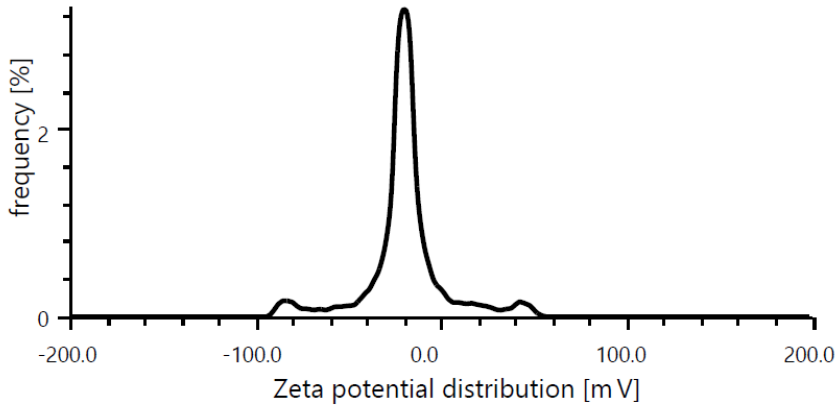


Figure:-5 cumulative % of emulgel drug relies

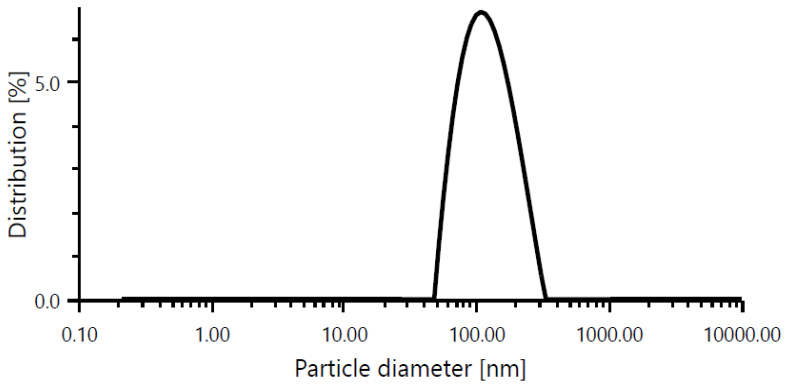
S.NO	ORGANISMS	ZONE OF INHIBITION (mm)		
		Standard Tetracycline hydrochloride (10µg/disc)	Sample (100µg/disc)	
			Emulgel	
1.	Staphylococcus	26mm	1.5mm	

Antibacterial activity: -

Particle- size and zeta- potential measurement: - The zeta potential of emulgel done by oniosome healthcare Pvt.Ltd. As result we found that zita potential(of emulgel -21.5mV and transmittance was 73.4% at 25°c temperature. During zita potential emulgel dissolve in water that time density was found 3.2825 and the solvent viscoscity was 0.0008903 pa.s along with 1.3303 refractive index. As shown in figure



Particle size:- The particle size of emulgel was find in NANO range result are following D10 =64.38 nm, D50= 110.75 nm , D90 = 201.3 nm. That means D10 percent of emulgel is smaller than 64.38 nm, D50 present of emulgel is smaller than 110.75 nm, and D90 present of emulgel is smaller than 201.3 nm As shown is figure



Transmission electron microscopy: - The Transmission electron microscopy of emulgel done by oniosome healthcare Pvt.Ltd. As result we found that all the partical size under NANO range

as shown in figure as well as histogram of particle the mean range is 100.729 and the max range is 255.

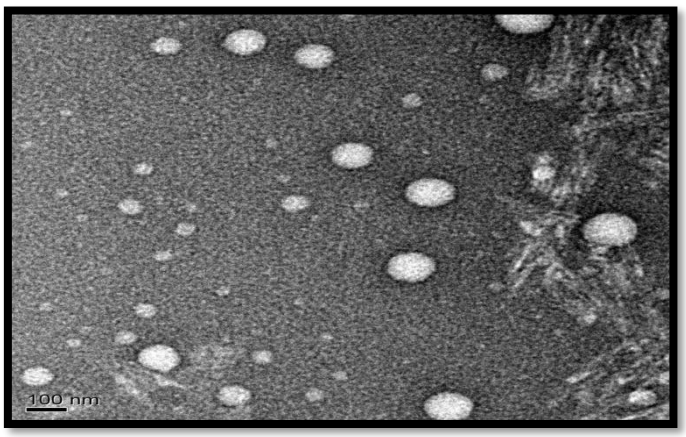
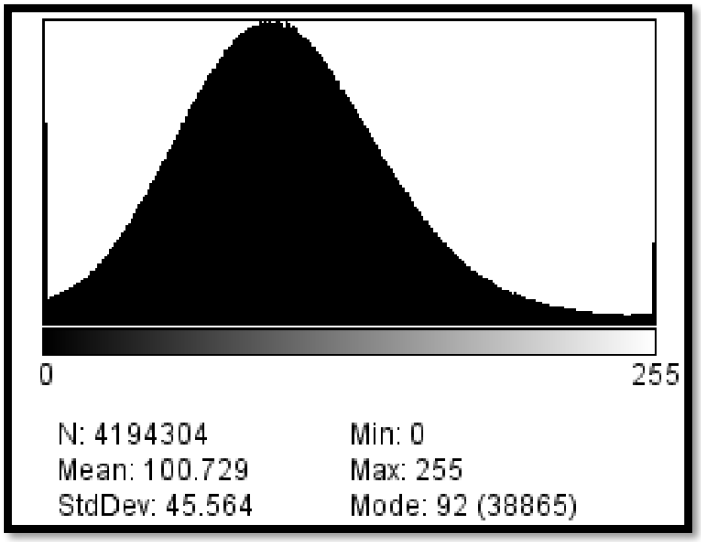


Figure of Transmission electron microscopy



Histogram of Transmission electron microscopy

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