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DEVELOPMENT OF HERBAL NIOSOMES FOR WOUND HEALING

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Keywords:

Herbal niosomes, Extraction, *Psidum guajava*, Wound healing

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ABSTRACT: Traditionally *Psidum guajava* has been used for the treatment of several kinds of disorders. The component quercetin a flavonoid that is present in the guava leaves, is responsible of having wound healing properties, so the aim and objective of the study was to Develop Herbal loaded Niosomes for the treatment of Wound Healing. To enhance the transport and efficacy of many materials through stratum corneum one of various methods expanded nowadays is the usage of new drug delivery system such as Niosomal formulations. Niosomes are self assemblies of non ionic surfactants with or without cholesterol, which can be used for both hydrophilic and hydrophobic substances. The extraction of *Psidum guajava* is performed by Soxhlet apparatus, and the extract is evaluated for phytochemical screening, antimicrobial activity, and TLC. Materials used in the formulation are span 60, cholesterol, diethyl ether, chloroform, buffer and extract. The herbal drug-loaded niosomes are prepared by using reverse phase evaporation method. Total twelve formulations are prepared and the formulations (F1, F2, F3, F4, F5, F6) were prepared by varying drug to surfactant ratio and formulations (F7, F8, F9, F10, F11, F12) were prepared by varying cholesterol ratio. The evolved herbal niosomes are evaluated for drug content, entrapment efficiency, particle size, zeta potential, and *in-vitro* drug release. The final optimized batch F7 containing span 60, cholesterol of 2:2 ratio showed good drug content of 83.4%, the particle size of 919.5 nm, the zeta potential of -84.1 mV, entrapment efficiency of 90.4%, and *in-vitro* drug release of 85.32%. The formulation is successfully developed for wound healing.

INTRODUCTION:

Herbal Formulations: In traditional medicine, different natural treatments have been suggested for skin damage, like wound healing which mostly contains medicinal plants; one among such plants is *Psidium guajava*. In order to reduce the side effects associated with allopathic medicine, there is a need to develop herbal formulations of novel drug delivery system *i.e.* niosomal formulation.

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Herbal medicines are plant-derived materials and preparations with therapeutic or other human benefits. In Natural products, especially plants, have been used for the treatment of various diseases for thousands of years and number of modern drugs have been developed from them. These plants can either be sold raw or as extracts, where the plant is macerated with water, alcohol, or other solvents to extract some of the chemicals, including fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, *etc.*

***Psidium guajava* and its medicinal uses:** *Psidium guajava* (Guava) belongs to the family Myrtaceae, it is properly known as poor man's apple of the tropics. Medicinal uses of guava leaves are traditional, preparations of the leaves have been

used in folk medicine in several countries. Traditionally used in the treatment of several diseases like inflammation diabetes, hypertension, wounds, pains, and fever. The extracts of leaves, roots, bark of guava are used as folk medicine to treat wounds, ulcers, toothache, cough, sore throat, inflamed gums, gastroenteritis, vomiting, diarrhoea, dysentery. The leaves have anti-bacterial activity and have antiseptic properties and have strong anti-microbial action of guava leaves on gram-positive and gram-negative organisms. guava leaves have anti-inflammatory activity. The leaves of the guava tree in decoction is used for epilepsy, spasms, cerebral affections (CNS activity). The young leaves and shoots are used for inflammation of the kidney and kidney problems and used in the treatment of malaria.

Why Niosomes: Niosomes are self-assemblies of non-ionic Surfactants with or without cholesterol, which can be used for the encapsulation of both hydrophilic and hydrophobic substances. To enhance the transport and efficacy of many materials through stratum corneum, one of the various Method Expanded nowadays is the usage of new drug delivery systems such as niosomal formulations. Niosomes have been shown considerably increased transdermal drug delivery through the stratum corneum as the main barrier against drug transport through the skin, so niosomes can be used in targeted drug delivery through two mechanisms. Niosomes increase stratum corneum by hydration and changing the attributes of stratum corneum by reducing transepidermal water loss. Niosomes increase drug transfer through stratum corneum by acting such Surfactants as the matrix Niosomes prolong the release of drug in skin damage, thereby reducing the need for dressing change during the day.

Wound: A wound is a breakage in the epithelial integrity of the skin or a loss or breaking of cellular and anatomic or functional continuity of living tissue. Wounds are physical injuries and results in an opening or break of the skin that leads to the disturbance in the normal skin anatomy and function.

MATERIALS AND METHODS:

Preparation of Leaf Powder: The fresh leaves of guava were collected, cleaned and air dried for about three weeks; after dried, the leaves were then blended using a household electric blender. This fine leaf powder was passed through the sieve no. 44 and it should be stored in an air-tight container for further use.

FIG. 1: FRESH LEAVES

FIG. 2: DRIED LEAVES

FIG. 3: LEAF POWDER

Extraction: About 10 gms of powdered material was extracted with ethanol as a solvent by hot extraction method using Soxhlet apparatus. The extraction was continued until the solvent became clear then collected the extract. After each extraction solvent, the extract was evaporated to dryness under reduced pressure by using a rotary vacuum evaporator at 50-55 ºC, 85 rpm. After this extract was transferred into the china dish and kept on a hot plate for 1 h and air-dried for 2 days, then triturated the extract to become a fine powder. The following image is the extracted powder, Soxhlet apparatus.

FIG. 4: SOXHLET APPARATUS

FIG. 5: LEAF EXTRACT

Preliminary Phytochemical Analysis: The preliminary phytochemical tests were performed to test different chemical groups present in extracts. The ethanolic extract of guava leaves was dissolved in various solvents, and the phytochemical tests for alkaloids, flavonoids, carbohydrates, proteins, tannins, steroids, terpenoids, phenols, quinones, starch, fat, and oil were carried out. The physio-chemical investigation of *Psidium guajava* was carried out with standard procedures for determining the presence or absence of phytochemicals.

Test for Alkaloids:

Mayer's Reagent: To 1 ml of the extract, 2 ml of Mayer's reagent was added. Dull white precipitate indicates the presence of alkaloids.

Dragendroff's Test: Dissolve extract in chloroform and evaporate, acidify the residue by adding few drops of Dragendroff's reagent. Presence of alkaloids is indicated by the appearance of orange red color precipitate.

Wagner's Test: By adding Wagner's reagent gives reddish brown colour.

Test for Flavonoids:

Shinoda Test: To dry extract, add 5 ml. of 95% ethanol, few drops conc. HCL and 0.5 g magnesium turnings. The pink colour observed.

Zinc-hydrochloric Acid-reduction Test: To the test, solution add zinc dust and few drops of HCL it appears magenta-red colour.

Alkaline Reagent Test: To the test, solution add sodium hydroxide solution it results in an increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.

Lead Acetate Solution Test: Add few drops of lead acetate solution (10%) to the test solution is which gives yellow precipitate.

Ferric Chloride Test: To 1 ml of extract, 1 ml of neutral ferric chloride was added. Presence of flavonoids is confirmed by the formation of brown colour

Test for Steroids:

Liberman-Burchard's Test: the extract should be dissolved in 2 ml of chloroform, to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mixed. The presence of steroids is indicated by changing of red colour through blue to green colour

Test for Terpenoids:

Salkowski Test: Five ml of each extract was mixed in 2 ml of chloroform, and concentrated sulphuric acid was carefully added to form a layer. A reddish-brown precipitate of the interface indicated the presence of terpenoids

Test for Quinone: To 1 ml of extract, a few drops of concentrated hydrochloric acid were added. The appearance of yellowish-brown colour was noticed that indicates the presence of quinone

Test for Phenols: To 1 ml of extract, lead acetate solution was added, and the precipitate formation indicated the presence of phenolic compounds

Test for Starch: to 1 ml of extract, a few drops of iodine solution were added any characteristic colour change showed that the presence of starch.

Test for Anthocyanin:
NaOH: To the extract add 2 ml of NaOH and observed for the formation of blue green colour

Test for Proteins:
Ninhydrin Test: ninhydrin was dissolved in acetone. The leaf extract was treated with ninhydrin and observed with the formation of purple colour Biuret test: a) Add 2 ml of Biuret reagent to 2 ml of extract. Shake well and warm it on water bath. When red or violet colour appears, it indicates the presence of proteins. b) Add 4% NaOH and few drops of 1% $CuSO_4$ solution to the extract. Violate or pink colour appears. Million's test: Test solution treated with million's reagent and heated on a water bath, results in red colour. Xanthoprotein test: Test solution treated with conc. nitric acid and on boiling gives yellow precipitate.

Test for Carbohydrates:
Molisch' s Test: two drops of Molisch's reagent was added to an aqueous or hydrochloric acid solution of the extract, and two ml of concentrated sulphuric acid is added to the sides of test tube. The formation of a reddish-violet ring at the junction of the liquids indicated the presence of carbohydrates Fehling's test: Mix 1 ml. Fehling's A and 1 ml. Fehling's B solutions boil for one minute. Add equal volume of test extract solution. Heat in boiling water bath for 5-10 min. presence of carbohydrates is indicated by the appearance of orange red precipitate. Benedict's test: Equal volume of Benedict's reagent and test extract is taken in the test tube and mixed. Heat it for 5 min. Depending on the amount of reducing sugar present in the test solution, the solution appears red, yellow and green color.

Test for Cellulose: to 1 ml of extract, a few drops of iodine solution were added followed by a few drops of sulphuric acid. Dark brown or red colour observed. Showed the presence of cellulose

Test for Fixed Oils and Fat: to 1 ml of extract, a few drops of Sudan 3 solution were added. The presence of fixed oil and fat is indicated by the appearance of a shining orange colour

Test for Glycosides:
Bal Jets Test: Treat the extract with sodium picrate solution. Appearance of yellow to orange colour confirms presence of glycoside with lactone ring.

Keller-killiani Test: To the test solution, few drops of glacial acetic acid, 2 ml of ferric chloride solution, and conc. sulphuric acid is added to the sides of test tube it shows the separation between two layers, lower layer shows reddish-brown, and upper layer turns bluish-green. Bromine water test: Test solution dissolved in Bromine water, and it results in the formation of yellow precipitate.

Thin Layer Chromatography: The Thin glass plates are coated with silica gel G and dried at room temperature. The dried plates should be activated at 100 ºC for 30 min in an oven and cooled at room temperature. The glass plates were developed in an air tight chromatographic chamber containing about 100 ml of solvent mixture of n-butanol, acetic acid, water (5:4:1) for quercetin. The developed plates were dried and visualized under UV light, ammonia vapors and iodine vapors sprayed to observe the color of the spots. R_f values were calculated for isolated samples and compared with coinciding with standard reference compound.

Anti Microbial Activity: The organisms used for the test were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*. The anti-microbial study was performed using the agar diffusion method. The prepared nutrient broth was poured into sterile petri plates and kept for drying and cooling; then, by using micron wire loop, each bacterial culture was spread. To form 4 mm deep holes, a sterile cork borer was used. Then 0.5 gms of the extract was added into this hole. Plates are incubated at 37 ℃ for 48 h. The zone of inhibition developed was then measured for the particular compound with each microbial strength.

Preparation of Formulations:
Reverse Phase Evaporation Method:
Materials Used: Span 60, cholesterol, diethyl ether, chloroform, and phosphate buffer are used in the formulation. Total Twelve formulations were prepared by varying the drug to surfactant ratio (F1, F2, F3, F4, F5, F6) and by varying cholesterol ratio (F7, F8, F9, F10, F11, F12). Cholesterol and surfactant (1:1) are dissolved in a mixture of diethyl ether and chloroform. The aqueous phase (drug) is added to this. The resulting two phases are sonicated at 4-5 ℃ Clear gel formed is further sonicated after the addition of a small amount of phosphate buffer.

The organic phase is removed under low pressure. Then the niosomal suspension is diluted with phosphate buffer and heated on a water bath at 60 ℃ for 10 min to get niosomes. Then the obtained niosomes are observed under a projection micro-scope.

TABLE 1: COMPOSITION OF FORMULATION BY VARYING DRUG TO SURFACTANT RATIO

Formulation	Drug	surfactant	Cholesterol	Solvent (Diethyl ether, chloroform)	Buffer
F1	20 mg	20 mg	20 mg	7:3	5 ml
F2	20 mg	30 mg	20 mg	7:3	5 ml
F3	20 mg	40 mg	20 mg	7:3	5 ml
F4	20 mg	50 mg	20 mg	7:3	5 ml
F5	20 mg	60 mg	20 mg	7:3	5 ml
F6	20 mg	70 mg	20 mg	7:3	5 ml

TABLE 2: COMPOSITION OF FORMULATION BY VARYING CHOLESTEROL RATIO

Formulation	Drug	surfactant	cholesterol	Solvent (Diethylether, choloform)	Buffer
F7	20 mg	30 mg	30 mg	7:3	5 ml
F8	20 mg	30 mg	40 mg	7:3	5 ml
F9	20 mg	30 mg	50 mg	7:3	5 ml
F10	20 mg	30 mg	60 mg	7:3	5 ml
F11	20 mg	30 mg	70 mg	7:3	5 ml
F12	20 mg	30 mg	80 mg	7:3	5 ml

Evaluation of Niosomes:

Drug Content: Drug content of the niosomes are measured by taking 1 ml of niosomal formulation is dissolved The 10 ml of pH6.8 phosphate buffer. The amount of niosomal formulation

Entrapment Efficiency: Entrapment efficiency indicates the amount of drug encapsulated in the prepared formulation. 1ml of niosomal formulation was taken and dissolved in 10 ml of 6.8 pH buffer, then it was transferred to Ephendroff tubes, centrifuged at 15000 rpm, 4 °C for 15 min in two cycles to separate the unentrapped drug. For the determination of free drug the clear fraction is used.

The free drug content is calculated by using of equation given below from the portion. The absorbance was measured at 271 nm in UV spectrophotometer to calculate the entrapment efficiency.

Entrapment efficiency% = total drug - free drug / total drug × 100

***In-vitro* Drug Release:** The niosomal preparation is placed in the Franz diffusion cell in the donor chamber, cell fitted with a cell phase membrane. The niosomes are then analyzed against a buffer of pH 6.8 at room temperature. Then the samples are withdrawn from the medium at suitable intervals; aliquots are replaced and analyzed for drug release using a UV spectrophotometer

Particle Size: The mean diameter of niosomes in the formulation was determined by a nanoparticle analyzer (HORIBA SZ 100. Series). One drop of the sample was taken from each formulation and diluted in 10 ml of dispersion medium (distilled water). Dynamic light scattering (DLS), is used for measuring the size of the particles in the sub-micron range.

Measurement of Zeta Potential: The zeta potential is a physical property exhibited by all particles in the preparation. It is an important factor to be considered in understanding the electric double layer repulsion and it can be measured by phase analysis light scattering when the electric field is applied, charged particles present in the preparation are attracted towards the electrode of opposite charge while viscous forces acting on the particle tend to oppose the movement. When equilibrium is reached, the particles move with constant velocity, also known as electrophoretic mobility, and zeta potential can be measure.

RESULTS:

Preliminary Phytochemical Analysis: Phyto-chemical screening of ethanolic extract of guava leaves was tested for alkaloids, flavonoids, tannins, carbohydrates, proteins, quinones, steroids, phenols. These tests were revealed the presence of alkaloids, flavonoids, terpenoids, tannins, steroids, phenols, carbohydrates, proteins, quinones, anthocyanins, fat

and oil, by positive reaction with the respective reagent. Phytochemical screening showed the maximum presence of phytoconstituents in ethanolic extract of guava given in the table.

TABLE 3: PHYTOCHEMICAL ANALYSIS OF GUAVA LEAVES EXTRACT

S. no.	Chemical test	Observation	inference
1	Test for alkaloids		
	Dragendroff's reagent	Orange-red	+
	Mayer's reagent	Dull white	+
2	Test for flavonoids	Formation of greenish brown	+
3	Test for tannins	Formation of brown colour	+
4	Test for steroids	Red Color solution to green colour	+
5	Test for carbohydrates Molisch' reagent	Reddish violet ring at the junction	+
6	Test for phenols	White precipitate	+
7	Test for terpenoids	Reddish-brown precipitate	+
8	Test for quinones	Yellowish-brown colour	+
9	Test for proteins Ninhydrin test	Purple colour	+
10	Test for anthocyanin	Blue colour solution to green colour	+
11	Test for fat and oil	Orange colour	+
12	Test for starch	Any characteristic colour	--

Thin Layer Chromatography: To confirm the presence of major compound flavonoid (quercetin). Mobile phase is taken as n butanol, acetic acid, water in different ratios. 5:4:1 (n-butanol: acetic acid: water) showed R_f value 0.93 indicated the presence of major flavonoid quercetin.

FIG. 6: TLC OF EXTRACT

Anti-microbial Activity: Anti-microbial activity was checked against different microorganism and zone of inhibition was identified by using Vernier callipers. The assay showed prominent activity. The ethanolic extract of leaves of guava was highly effective against *Staphylococcus aureus* with a zone of inhibition 25 mm, *Bacillus subtilis*, and *E. coli* with a zone of inhibition 23 mm and 31 mm respectively. It has been compared with streptomycin disc. The zone of inhibition of this disc was found to be 27 mm. So the guava leaves, when compare to streptomycin disc, extract has shown good activity extract was exhibiting good antimicrobial activity against micro-organism than streptomycin disc.

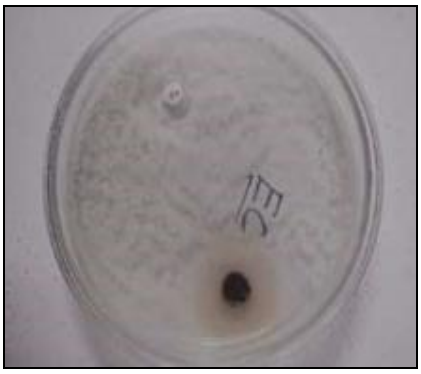


FIG. 7: ANTIMICROBIAL ACTIVITY OF EXTRACT

Herbal Niosomes are prepared by the Reverse phase Evaporation method. Total twelve formulations were prepared by changing drug to Surfactants ratio (F1, F2, F3, F4, F5, F6), and other six Formulations are prepared by changing cholesterol ratio (F7, F8, F9, F10, F11, F12) and evaluated for particle size, zeta potential, drug content, Entrapment efficiency, Drug Release.

Optical Microscopy: Morphology was determined for all twelve formulations by using optical microscopy (S-3700N, Hitachi, Japan). The photomicrography pictures of the preparation were obtained from the microscope by using a digital SLR camera.

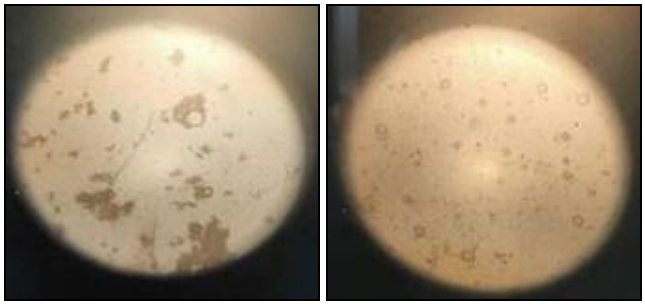


FIG. 8: HERBAL NIOSOMAL IMAGES OF FORMULATION F1 & F7

Herbal Niosomes by Changing Drug to Surfactant Ratio:

Drug Content: The drug content was measured for the formulations by changing the drug to surfactant ratio.

The drug content obtained for F1 formulation is 87% for F2 is 74%, F3 is 67%, F4 is 55% F5 is 60% and for F6 is 54%. Among all six formulations, F1 is found to have good drug content of 87%. The absorbance was measured and drug content was calculated. The percentage of drug content of various formulations was found to be in the range of 54-87%.

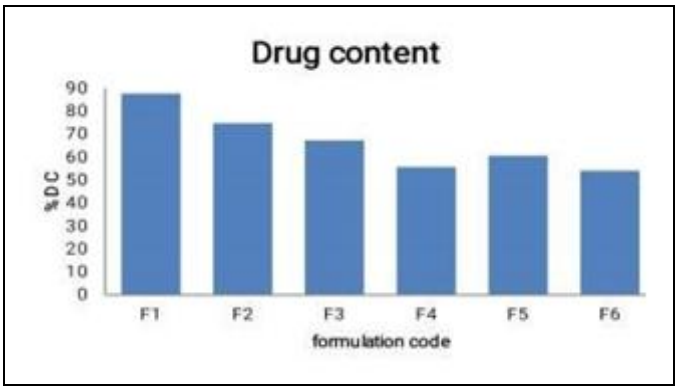


FIG. 9: DRUG CONTENT PROFILE OF FORMULATIONS CHANGING DRUG TO SURFACTANT RATIO

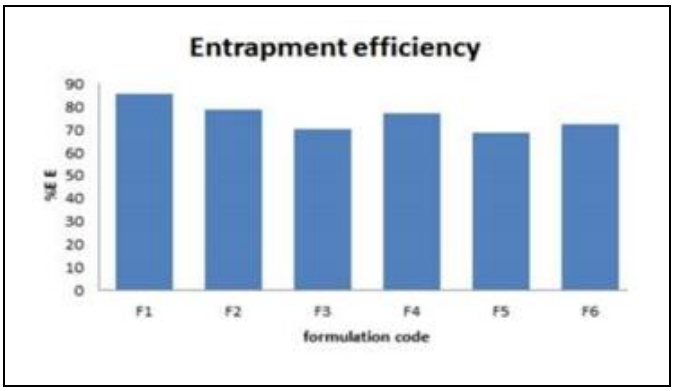


FIG. 10: ENTRAPMENT EFFICIENCY PROFILE OF FORMULATIONS CHANGING CHOLESTEROL RATIO

Entrapment Efficiency: The entrapment efficiency of Formulations F1 is 85%, F2 is 78%, F3 is 70%, F4 is 76%, F5 is 68% and F6 is 72%. Among all six formulations entrapment, efficiency was found to be good in F1 formulation of 85%. The entrapment efficiency of Formulations was found to be within the range of 68%-85%.

***In-vitro* Drug Release:** The drug release for F1 formulation is 84% for F2 is 81% and for F3 to F5 is 65-72% F6-80% for six hours. Among all six formulations F1 showed a good drug release of 84%. The drug release for Formulations was found within the range of 65% to 84% of drug release

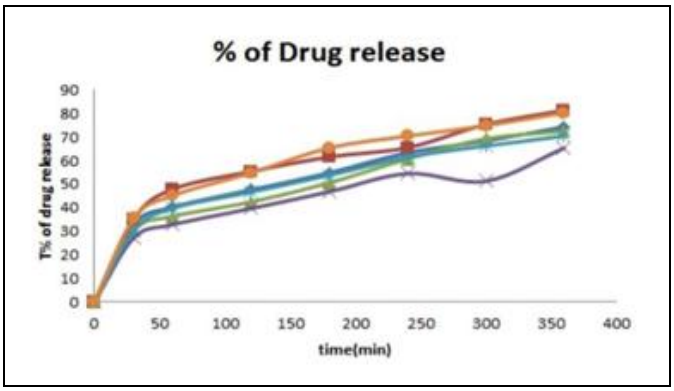
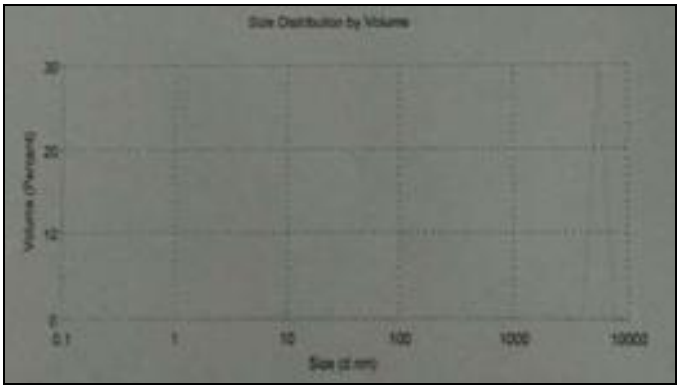


FIG. 11: *IN-VITRO* DRUG RELEASE OF FORMULATIONS VARYING DRUG TO SURFACTANTS RATIO



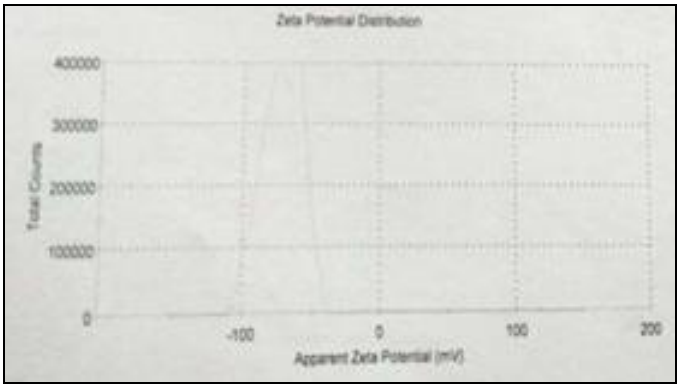


FIG. 12: IMAGE OF FORMULATION 1 SIZE DISTRIBUTION AND ZETA POTENTIAL

Particle Size and Zeta Potential: Among all the Formulations of varying drug to Surfactants ratio, the particle size of the best formulation is F1 formulation with the range of 816.3nm with zeta potential of -39.7 mV.

Herbal Niosomes by Changing Cholesterol Ratio:

Drug Content: The drug content was measured for the Formulations by changing cholesterol ratio. The drug content obtained for Formulations F7 is 87%, F8 is 77%, F9 is 71%, F10 is 60%, F11 is 56%, F12 is 75%. Among all six formulations, the drug content was found to be good for the formulation. F7 is 87%. Percentage of drug content of various formulations was found to be in the range of 60-87%

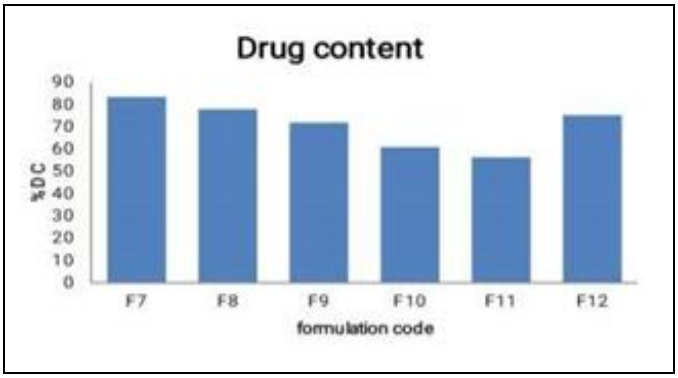


FIG. 13: DRUG CONTENT OF FORMULATIONS BY CHANGING CHOLESTEROL

Entrapment Efficiency: The entrapment efficiency of Formulations varying cholesterol ratio was found to be for F7 is 90%, F8 is 85%, F9 is 72%, F10 is 69%, F11 is 54%, F12 is 45%. Among all six Formulations. F7 formulation was found to have good entrapment efficiency of 90%. The entrapment efficiency of Formulations was found within the range of 45 to 90%.

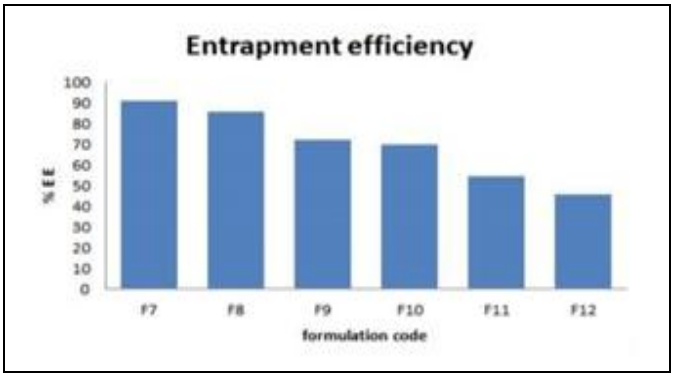
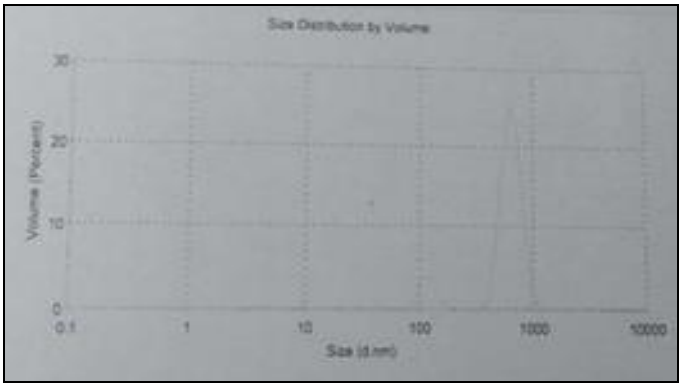


FIG. 14: ENTRAPMENT EFFICIENCY OF FORMULATIONS VARYING CHOLESTEROL

Particle Size and Zeta Potential: Among all the Formulations varying cholesterol ratio, the particle size of the best formulation is F7 formulation with the range of 919.5 nm and with a zeta potential of -81.1 mV.



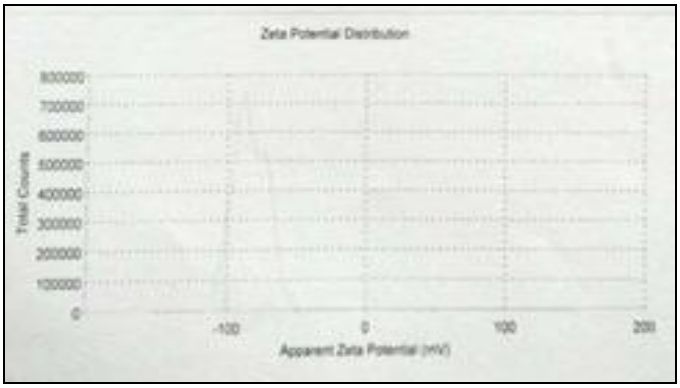


FIG. 15: IMAGE OF FORMULATION 7 SIZE DISTRIBUTION AND ZETA POTENTIAL

***In-vitro* Drug Release:** The *in-vitro* drug release for the formulations F7- 87%, F8 -75%, F9- 72%, F10 -73% F11- 69%, F12- 74%.

For all Formulations drug release was found to be within the range of 69-85% for six hours.

Among all six formulations F7 formulation showed good *in-vitro* drug release of 85%

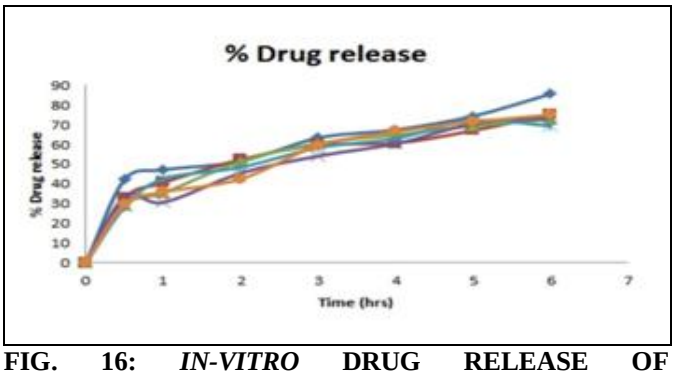


FIG. 16: *IN-VITRO* DRUG RELEASE OF FORMULATIONS VARYING CHOLESTEROL RATIO

Comparative Study among the Best Formulations of Herbal Niosomes: Niosomes were prepared by Reverse phase Evaporation method by varying drug to surfactant and cholesterol ratio.

By comparison, Niosomal formulation (F7) of changing Cholesterol ratio was considered the best formulation because of its highest drug content of 87% ± 2, entrapment efficiency of 90.1% ± 3 and drug release of 87% ± 3.

TABLE 4: KINETIC DATA OF F7 NIOSOMAL FORMULATION

Time (h)	Cumulative drug Release (%) n=3	Drug remaining (%)	Log drug Remaining (%)	$\sqrt{T}$	Log T	Log Cumulative Drug release (%)
0.5	42.1%	57.9%	1.76	0.70	0.15	1.62
1	46.9%	53.1%	1.72	1	0	1.67
2	51.2%	48.8%	1.68	1.41	0.14	1.70
3	63.1%	36.9%	1.56	1.73	0.23	1.80
4	67.2%	32.8%	1.51	2	0.30	1.82
5	74.1%	25.9%	1.41	2.23	0.34	1.86
6	85.3%	14.7%	1.16	2.4	0.38	1.93

Kinetic Models for Optimized Formulation: Several plots like zero order plot, first-order plot, Higuchi plot, and Peppas plot were drawn for the optimized formulation in order to know the release kinetic and drug release mechanism **Table 4** & **5**, **Fig. 8**.

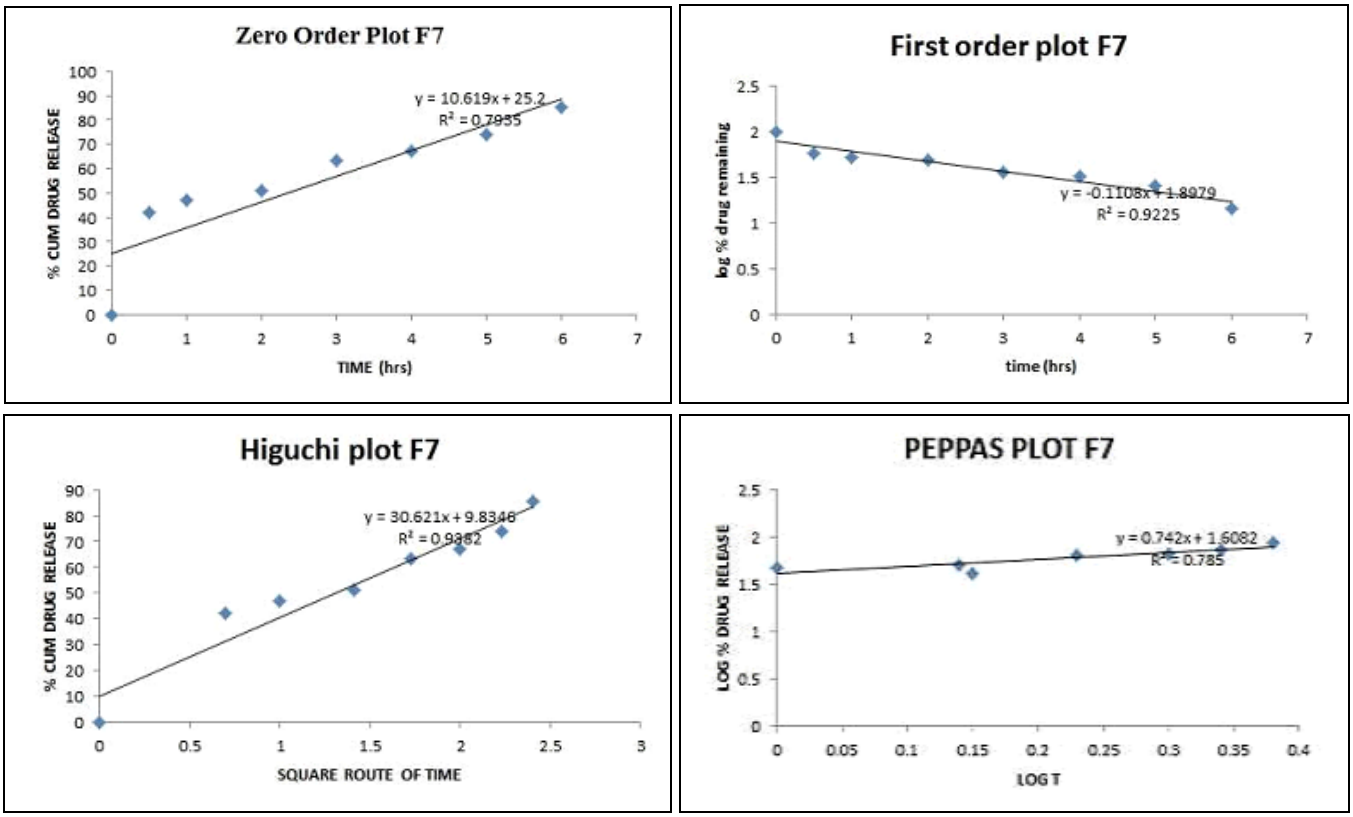


FIG. 17: *IN-VITRO* KINETIC PLOTS OF HERBAL NIOSOMAL FORMULATION

TABLE 5: KINETIC DATA OF F7 FORMULATION

Formulation	Zero order plot (R^2)	First order plot (R^2)	Higuchi plot (R^2)	Peppa's plot (R^2)
F 7	0.79	0.92	0.93	0.78

From the result, it was concluded that the drug release was following Higuchi and first order.

DISCUSSIONS AND CONCLUSION: P*sidium guajava* is used in the treatment of wound healing as it has the wound healing properties like anti-inflammatory activity, antioxidant activity, anti-microbial activity. By using Soxhlet apparatus, 10 gms of powdered material was extracted with ethanol as a solvent by hot extraction method In the

present study; a preliminary phytochemical analysis was carried out to identify the active constituents such as alkaloids, flavonoids, sterols, terpenoids, proteins, cellulose, quinone, oil and fat, phenol, starch, anthocyanin, carbohydrate present in the leaves of guava plant. Work has revealed the presence of alkaloids, flavonoids, glycosides, polyphenols, reducing compounds, saponins, and tannins in the ethanolic extract of *Psidium guajava* leaves. The antimicrobial studies showed that ethanolic extract of *P. guajava* L had inhibitory effects on *E. coli*, with zones of inhibition of 25 mm, respectively. This shows that the plant could be used to treat skin infections and other infections caused by these microorganisms. From the results, it was revealed that the guajava leaf extracts show different degree of inhibition against different microorganisms. TLC is performed for both standard and test using a solvent mixture of toluene, ethyl acetate, methanol, formic acid (7:5:2:1).

The sample and standard solution were spotted by using a capillary tube on the TLC plate, then TLC plates are placed in the developing chamber of the mobile phase. Then allow the mobile phase to travel along with the sample. Then visualize the spot and calculated the R_f values; they found to be for test is 0.90 and standard is 0.93 indicates identified as quercetin. *Psidium guajava* extract-loaded niosomes were prepared by a reverse-phase evaporation method. Total twelve formulations were prepared by varying drug Surfactant ratio (F1, F2, F3, F4, F5, F6) and by varying lipid concentration (F7, F8, F9, F10, F11, F12) The first six formulations (F1, F2, F3, F4, F5, F6) were prepared by varying drug Surfactant ratio.

Among the six formulations F1 the better result with drug content of 87.7%, entrapment efficiency of 85.4%, *in-vitro* drug release 84.1%, particle size of 816.3nm and zeta potential of -71.8mV. The next six formulations (F7, F8, F9, F10, F11, F12)) were prepared by varying lipid concentration ratio. Among six formulations, F7 is giving the better results with drug content of 83.4%, entrapment efficiency of 90.4% *In-vitro* drug release 85.3%, the particle size of 919.5nm zeta potential of -71.8 mV. An increase in concentration of Surfactant level showed a decrease in entrapment efficiency, possibly due to the formation of mixed micelles along with the niosomal vesicles with a high concentration of Surfactants, which may lead to lower entrapment efficiency. As the amount of Surfactant increases, drug release decreases because the permeability of the encapsulated drug is decreased. The vesicle size of niosomes increased proportionally with increased HLB of the Surfactant such as span 60 (HLB 4.7) because of the decreased surface free energy with increased Surfactant hydrophobicity. An increase in the cholesterol content of the bilayers resulted in a decrease in the release date of encapsulated material and therefore an increase in the rigidity of the resulting bilayers.

The reduction in drug entrapment when cholesterol content was further increased may be due to two major factors with increased cholesterol, the bilayers hydrophobicity and stability increased, permeability decreased, which may lead to efficiently trapping the hydrophobic drugs into bilayers as Vesicles formed. IN contrast, higher amounts of cholesterol may compete with the drug to orientate space within the bilayers, remove the drug from bilayers and hence excluding the drug as the amphiphiles assemble into the Vesicles. The zeta potential of spanniosomal formulations increases with the hydrophobicity of the surfactant increased. This could be due to the fact that the surface free energy of span surfactants increases with increased HLB value. On comparison of all the prepared niosomal formulations F7 formulation is showing the good result of having drug content of 83.4% and entrapment efficiency of 90.4% and *in-vitro* drug release of 85.3%, the particle size of 919.5 nm Zeta potential of -84.1 mV

Scope: optimization of various parameters is required for effective scale-up of formulation. *In-vivo* studies are recommended to find the therapeutic benefit of developed niosomal formulations.

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