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Research Article

Formulation and Evaluation of Niosomes Based Creams from Extract of Hibiscus Sabdariffa for Antibacterial Activity

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ABSTRACT

The formulation and evaluation of a niosomal-based cream that incorporates *Hibiscus sabdariffa* leaf extract for its antibacterial characteristics is the main focus of this work. *Hibiscus sabdariffa* has strong antibacterial properties and is well-known for its diverse phytochemical profile, which includes flavonoids, anthocyanins, and polyphenols. Its medicinal potential is hampered by issues like low stability and restricted absorption. The ethanol injection approach was used to encapsulate the extract into niosomes in order to get over these restrictions. Franz diffusion studies were used to characterise the formed niosomes for *in vitro* release, drug content, particle size, and entrapment efficiency. With a controlled drug release over six hours, NF2 had the highest drug content (73.2%) and entrapment efficiency (79.09%) among the three niosomal formulations (NF1, NF2, and NF3). After being added to a cream base, the optimised niosomal solution was assessed for stability, viscosity, spreadability, pH, physical appearance, and drug release. When compared to a standard formulation and a plain extract-based cream (F2), the niosomal cream (F1) performed better in terms of consistency, spreadability, and controlled drug release. The drug's compatibility with excipients was validated by FTIR investigations. When compared to the extract alone, the niosomal cream showed larger zones of inhibition against Staphylococcus aureus and Escherichia coli, showing increased antibacterial efficacy by niosomal encapsulation, according to antibacterial activity measured using the agar well diffusion method.

INTRODUCTION

Herbal extracts are widely explored for their medicinal properties. Hibiscus sabdariffa contains bioactive compounds like flavonoids and polyphenols, which exhibit antimicrobial activity. However, their stability and bioavailability remain a challenge [1]. Nanoparticles are commonly used to improve the bioavailability of several bioactive compounds. Niosomes non-ionic surfactant-based vesicles, are widely used for controlled drug release and enhanced permeability [2]. The prepared extract not have the ability and efficacy to permeate on produce the therapeutic activity and it is less stable, hence obtained extract was formulated as niosomes to increase its permeability and efficacy of formulation. Herbal technology disadvantages overcome by niosomes lesser size can be obtained. This study aims to formulate a niosomal cream containing *Hibiscus sabdariffa* extract and evaluate its antibacterial Activity [3].

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Plant Profile

Hibiscus sabdariffa

Roselle (*Hibiscus sabdariffa*) it is called roselle 'hemp" rosella and java jute Hibiscus sabdariffa, family Malvaceae, (*Hibiscus Sabdariffa*) is a perennial herbaceous shrub grown widely in India, west and east Africa. It is now widely grown in both tropical and subtropical countries for its leaf, fleshy calyx, seed and fiber [4]. It is an annual, perennial herb or woody sub shrub meant for delicacy and medicinal properties. It has tender young leaves, stems, roots and flowers having wide therapeutic applications as anti-inflammatory agent, antiseptic, antiscorbutic, astringent, antioxidant and anti-microbial properties [5].

Figure 1: Leaves of *H.sabdariffa*

Figure 2: Calyxes of *H.sabdariffa*

Figure 3: Flowers of *H.sabdariffa*

This plant has huge Phyto constituents such as alkaloids, terpenoids, flavonoids, phenolic compounds, Anthocyanins [6].

Table 1: Taxonomical Classification

Parameter	Details
Botanical Name	Hibiscus sabdariffa
Kingdom	Plantae
Class	Malvales
Family	Malvaceae
Genus	Hibiscus
Common name	Roselle, sorrel, Redsorrel

MATERIALS AND METHODOLOGY

Materials

Hibiscus sabdariffa leaves (rosella), rosella leaves extract power, surfactants (span 60, span 80), Stabilizer (cholesterol) Solvents (ethanol, water) Diethyl ether, phosphate buffer, Castor oil Glycerine, Stearic acid , Beeswax, Propyl paraben.

Extraction of Rosella Leaves

Hibiscus sabdariffa plant was collected from the local farm and it was subjected to Authentication at Botanical Survey of India. It was washed, shade dried and grounded to form the coarse powder. It was then subjected to Soxhlation process [7]. The Soxhlet apparatus was connected to the round bottom flask containing 96% Ethanol. And in to the thimble coarse powder was placed. The apparatus was heated using heating mantle until the leaf extract is obtained [8].

Preparation of Niosomes: Ethanol Injection Method

0.5g of drug is dissolved in 2mL of ethanol (aqueous phase), different concentration of surfactant (span 60) & 5g of stabilizer (cholesterol) dissolved in 3 mL of Diethyl ether (oil phase). Then the aqueous phase is added to oil phase by using orifice needle size 14 added drop-wise. The mixture is kept under stirring at 60°C to allow the evaporation of ethanol, After 20 min of stirring niosomes are formed due to amphiphilic nature of surfactant and cholesterol [9].

Table 2: Composition of niosomes (gm)

Ingredients	N1	N2	N3
Drug	0.5	0.5	0.5
Surfactant (span 60)	6	8	10
Cholesterol	5	5	5
Diethy ether	3mL	3mL	3mL
Ethanol	2mL	2mL	2mL

Evaluation of Niosomes

Entrapment Efficiency (%EE)

A measured quantity of the niosomal suspension was placed in centrifuge tube the mixture was then vortexed thoroughly and centrifuged at 2,000 rpm for 10 minutes to isolate the unentrapped drug in the supernatant from the niosomal suspension. The concentration of the free drug in the supernatant was quantified using UV-Visible spectrophotometry at the drug's characteristic wavelength of 270nm [10].

Entrapment efficiency was calculated using the following formula [11]:

$$EE = (\text{Drug content in the product obtained (mg)}) / (\text{Total amount of drug added(mg)}) \times 100g \quad (1)$$

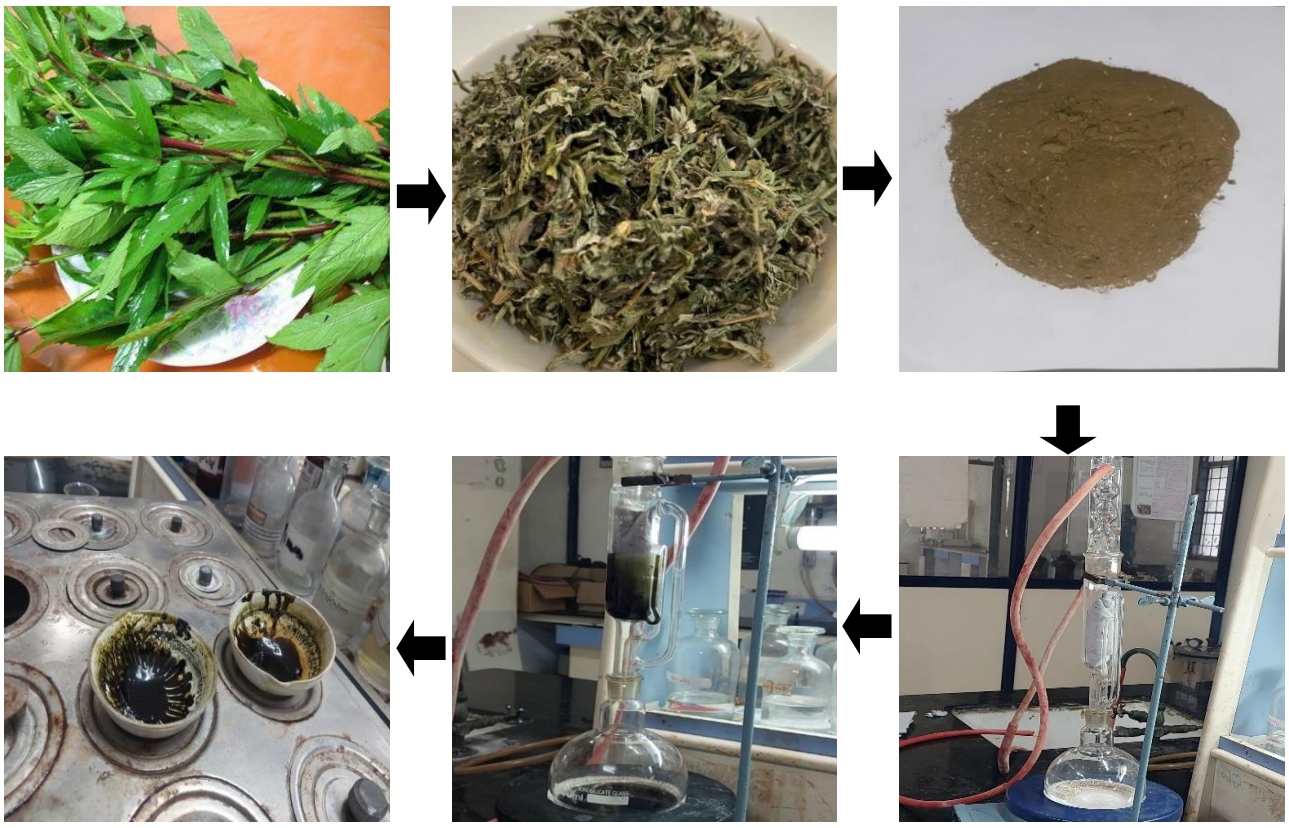


Figure 4: Extraction process of *Hibiscus sabdariffa*

Microscopic Study

Prepared niosomal suspension was placed out a microscopic analysis of niosomal cream, a small amount of the cream was placed on a clean glass slide. Spread it uniformly and cover it with a coverslip. Subsequently, examined the sample under a light microscope at an appropriate magnification to investigate the shape, size, and, distribution of the noisome within the cream [12, 13]. This procedure assists in confirming the presence of vesicles and identifying any aggregation or irregularities in the formulation.

Drug Content

The drug content within the niosomal formulation of Hibiscus sabdariffa was analysed to quantify the amount of extract that was successfully incorporated into the vesicles. A known volume of 1 mL of niosomal suspension was disrupted by the addition of an equal volume of ethanol, which broke the vesicles and released the encapsulated drug. The mixture was then filtered through a 0.45 µm membrane filter, and the absorbance of the filtrate was measured at 270 nm using a UV–Visible spectrophotometer [14].

Franz Diffusion Study

The *in vitro* diffusion studies can be performed by using Franz diffusion cell. Niosomes is placed in the donor chamber of a Franz diffusion cell fitted with a cellophane membrane. The niosomes is then dialyzed against a suitable dissolution medium at room temperature; the samples are withdrawn from the medium at suitable intervals, and analyzed for drug content using suitable method (U.V spectroscopy, HPLC, etc). The maintenance of sink condition is essential [15].

Preparation of Niosome Based Cream

Cream was prepared by emulsification techniques using aqueous and oily phase.Specified quantity of Glycerin and 0.2mL of Methyl paraben is added to distilled water (aqueous phase) [16]. 10g of Beeswax and 5g of stearic acid is added to 10mL liquid paraffin (oil phase). Both aqueous and oil phases are kept on water bath with temp 60°C for 20mins. Slowly add the aqueous phase into the oil phase while stirring it continuously [17]. Optimised Niosomal suspension was added into the cream base and stirred for certain period of time to ensure homogeneity [18]. Cream formulation is as shown in Table 4.

Evaluation of Cream Formulations

- ***Appearance***

The cream was evaluated for its color, texture, and smoothness.

Table 3: Composition of Niosomes Based Creams Contain *Hibiscus Sabdariffa* Extract

PHASE	INGREDIENTS	QUANTITY (g/100g)	FUNCTION
Aqueous phase	Glycerin	5	Humectant
	Propylene glycol	3	Penetration enhancer
	Distilled water	Quantity required	Base
Oil phase	Stearic acid	5	Emulsifier and thickener
	Beeswax	5 to 8	Emollient
	Liquid paraffin	3	Skin moisturizer
	Leaves extract	2	Antibacterial agent
	Propyl paraben	0.2	preservative
	Lavender oil	Quantity required	Fragrance

Table 4: Comparative Cream Formulations (F1-F2) Standard Cream

Ingredients	F1 (Niosomes+Cream)	F2 (Extract+Cream)
Niosomes	3.0gm	–
Hibiscus extract	–	3.0gm
Stearic acid	3gm	3gm
Cetyl alcohol	0.9gm	0.9gm
Mineral oil	2.7gm	2.7gm
Glycerine	3gm	3gm
Preservatives	0.15gm	0.15gm

- ***pH Test***

A small quantity of cream was combined with water, and the pH was assessed to ensure skin safety [19].

- ***Spreadability***

The cream was placed on the glass slide surface & other empty slide has placed on 1st slide at an angle of 45 subgated for spreadability nature [20, 21].

- ***Viscosity***

The viscosity of the cream was quantified using a viscometer [22].

- ***Drug Content***

A specified amount of cream was dissolved in a solvent, and the drug concentration was analyzed using a UV spectrophotometer [23].

- ***Drug Release***

The cream was placed in a dialysis bag within a buffer solution, and the drug release was monitored at regular intervals [24].

- ***Stability Study***

The cream was stored at various temperatures to observe any alterations in colour, pH, or drug content over time [25, 26].

Pre Formulation Studies

Fourier Transform Infrared Spectroscopy

Take a small amount of the pure drug, along with individual excipients like surfactant and cholesterol, and the niosomal cream that has been prepared. Each sample should be dried and finely powdered if possible [27]. Mix a small quantity of the powdered sample with potassium bromide (KBr) powder and compress it into a thin, transparent pellet using a hydraulic press. Then, place the pellet in the FTIR spectrophotometer and scan it over the wavelength range of 4000 to 400 cm^{-1}. The spectrum shows the various chemical bonds of drug, cholesterol, surfactant, and the combined physical mixture.

Calibration Curve

The calibration curve of hibiscus sabdariffa was observed by different standard solutions with known concentration of anthocyanins. The active ingredient was extracted from the plant material using ethanol, followed by filtration or centrifugation, and dilution if required. Both the standard solutions and the plant extract were analyzed using UV-Vis spectroscopy (measuring absorbance at 270 nm for anthocyanins) [28]. A calibration curve was constructed by plotting the concentration of the standards against their corresponding absorbance or peak area. The concentration of the target compound in the Hibiscus sabdariffa extract was subsequently determined by comparing its analytical response to the established calibration curve. The accuracy of the method was validated through repeatability tests and recovery studies to ensure dependable results [29].

Anti-bacterial Study

The agar disc diffusion method was employed for the determination of antimicrobial activities of the synthesized compounds and standard drug. 0.1 mL from 108 cfu/mL of different pathogenic bacteria suspension was spread on different plates nourished with LB (Luria-Bertani) media [21]. Filter paper discs (5 mm in diameter) were

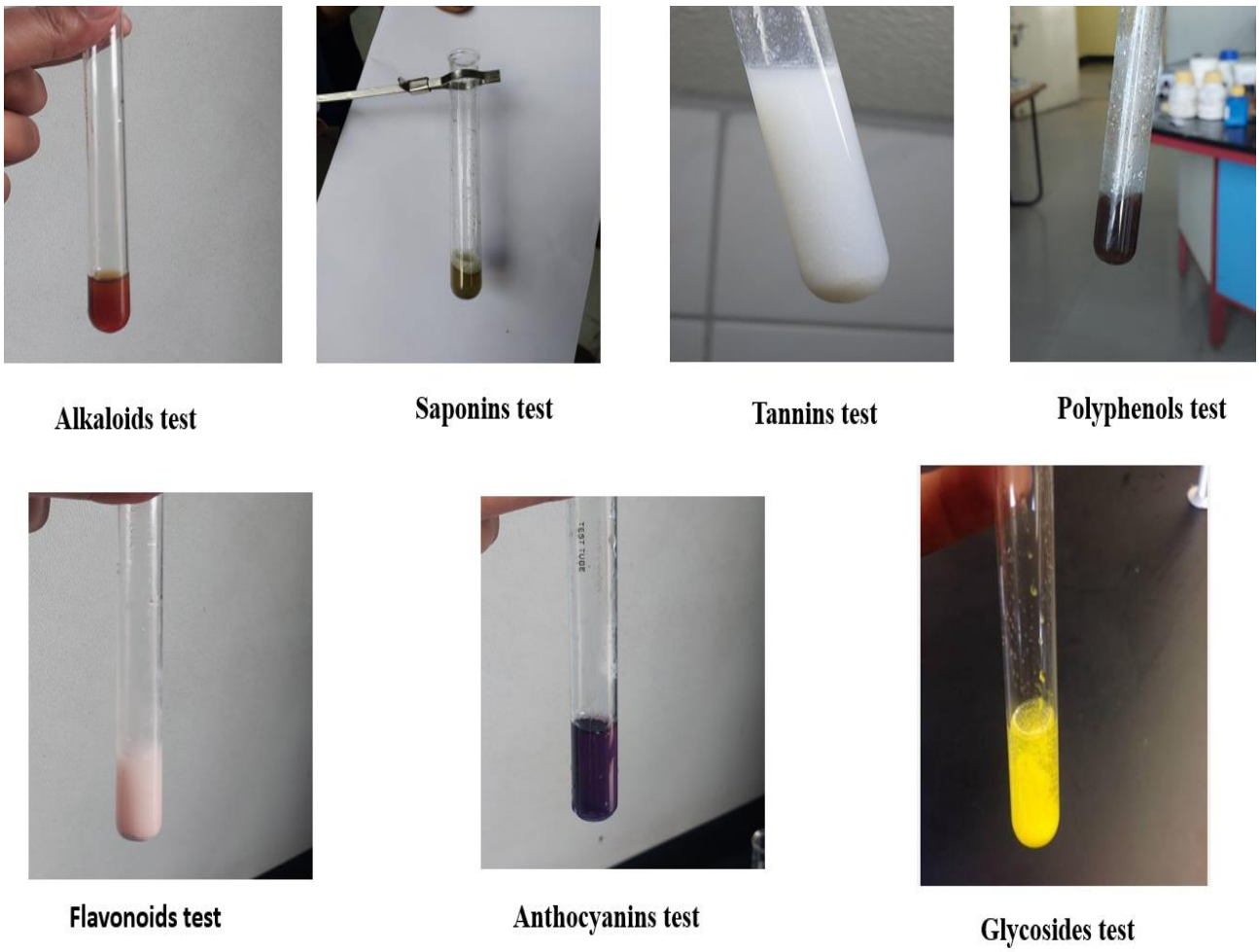


Figure 5: Pytochemical screnning of Hibiscus sabdariffa Extract

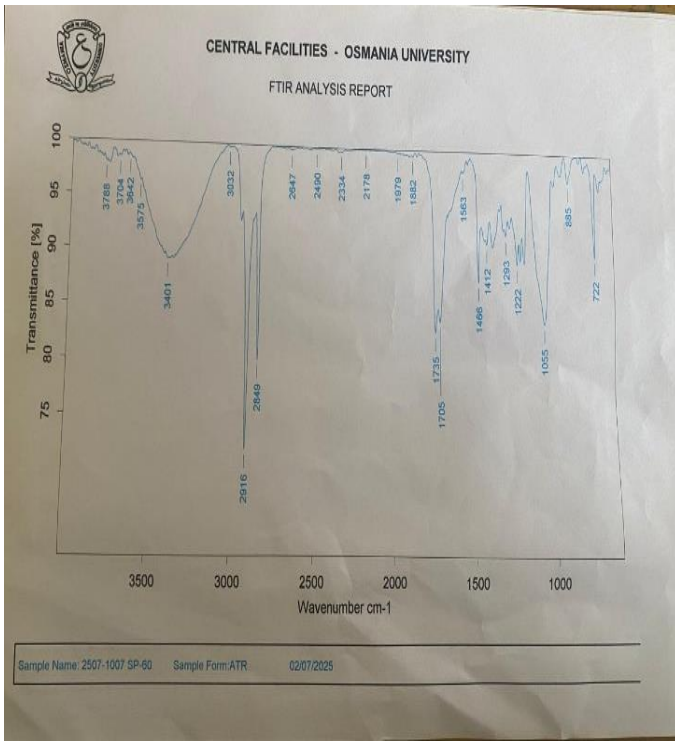


Figure 6: FTIR analysis of span 60

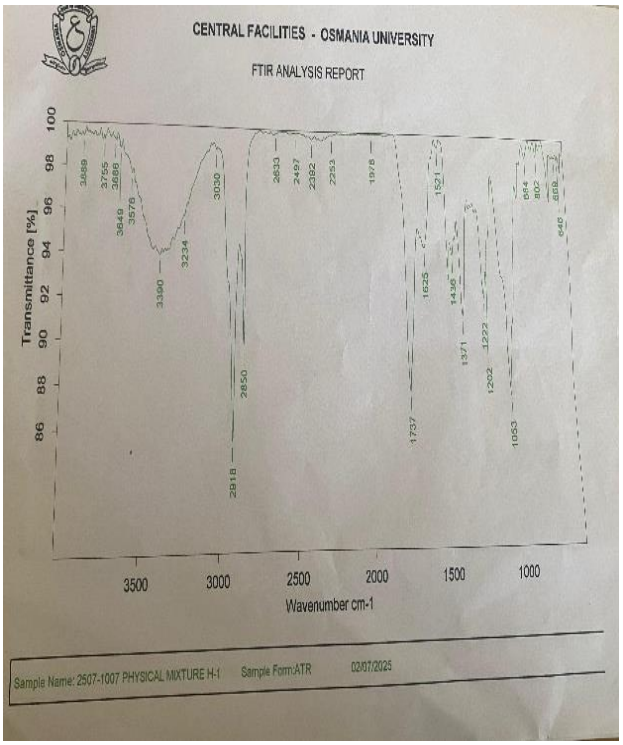


Figure 7: FTIR analysis of physical mixture

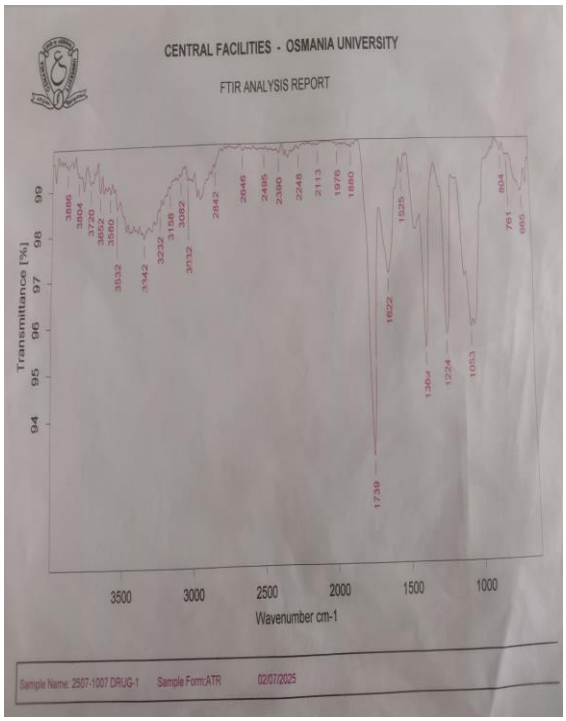


Figure 8: FTIR analysis of hybiscuss sabdariffa

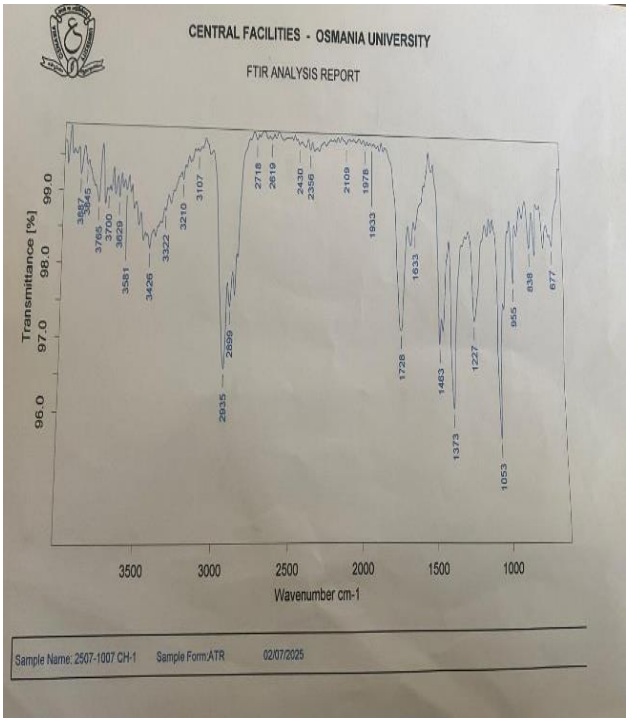


Figure 9: FTIR analysis of Cholesterol

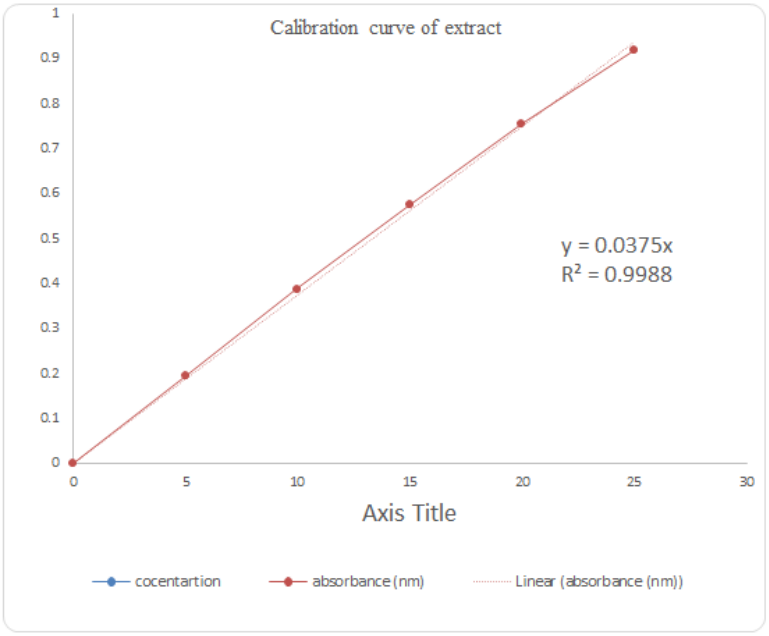


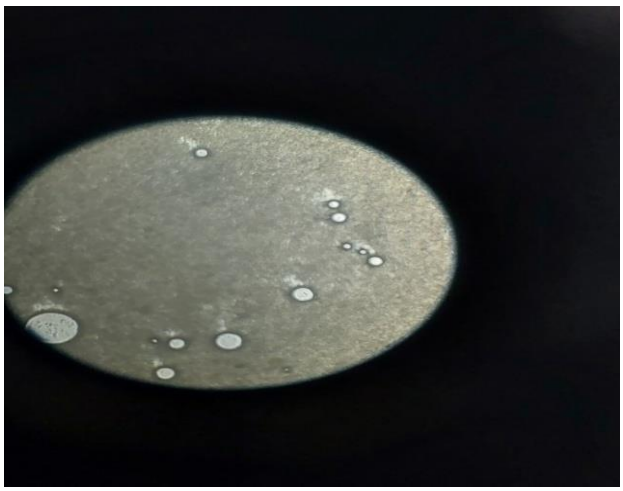
Figure 10: Calibration curve of extract

placed on the plates and then onto the discs synthesized compounds and standard drug were impregnated in different concentrations. Drug (20µg/µL concentration) served as the standard for measuring the antibacterial activity. The plates were then incubated at 37°C for 24h [30]. The Zones of inhibition were measured in mm. The measured values are given in the table no 11.

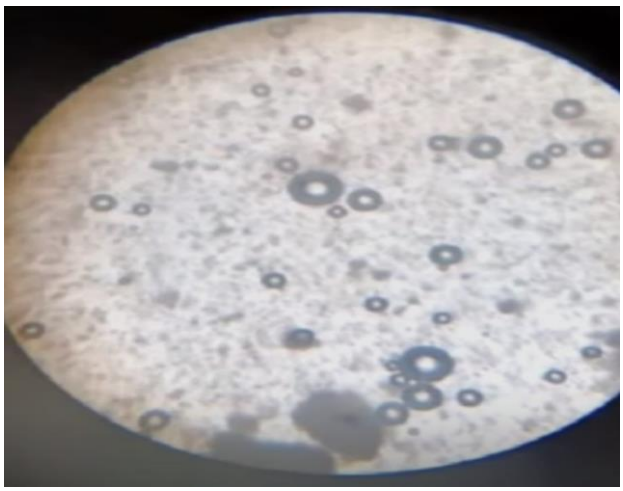
RESULT AND DISCUSSION

Table 5: Organoleptic characteristics of Hibiscus Sabdariffa extract

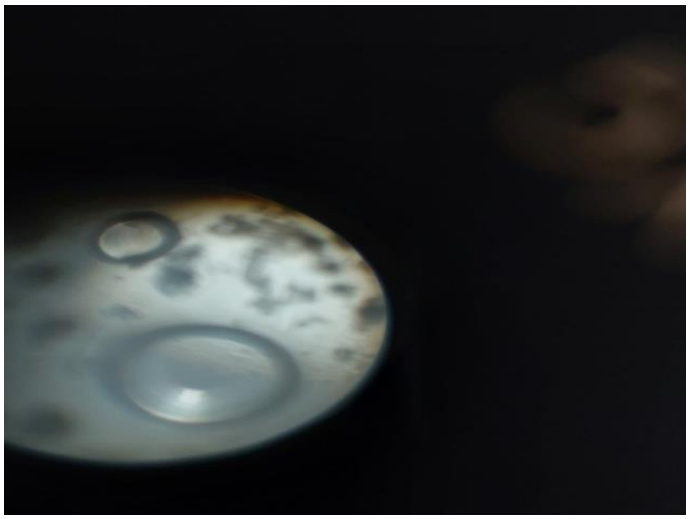
S.NO	Parameter	Observation
1	Colour	Dark green
2	odour	characteristic
3	Taste	characteristic
4	Solubility	Water, alcohol
5	ph	6.5



NF1



NF2



NF3

Figure 11: Microscopy study of Niosomes

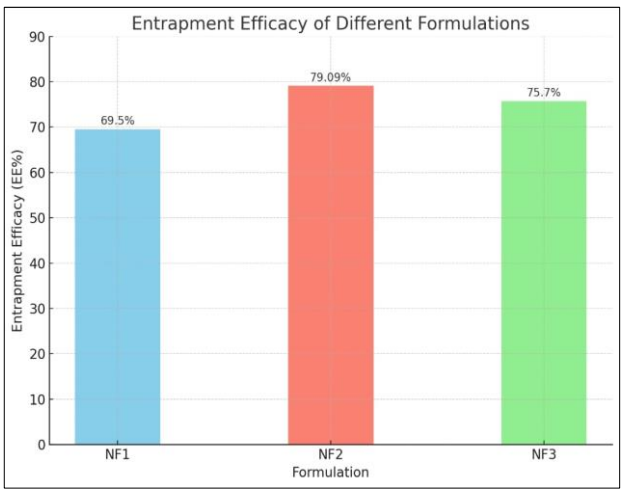


Figure 12: Entrapment efficacy

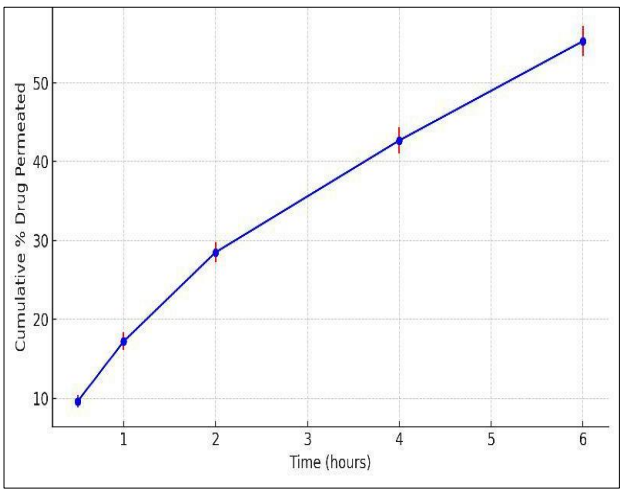


Figure 13: Franz diffusion study

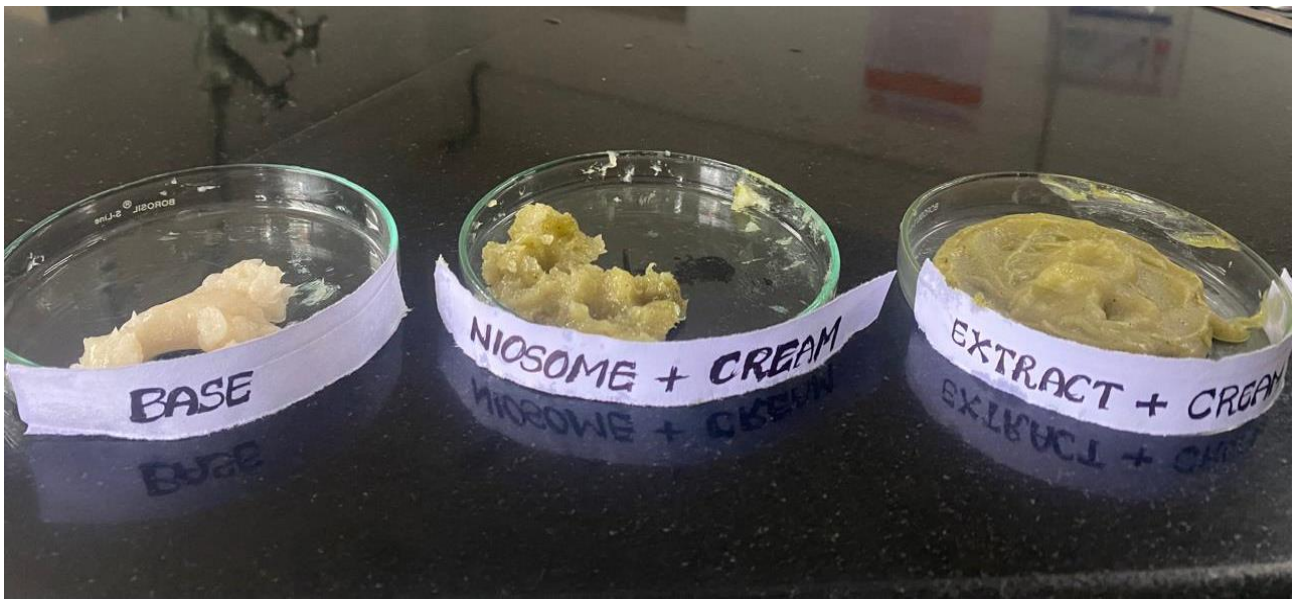


Figure 14: Comparative cream formulations

Table 6: Phytochemical screening of Hibiscus Sabdariffa Extract

Test	Ethanolic Extract	Observation
Alkaloids	(+)Tannic acid test	Reddish colour
Saponins	(+)Foam test	Persistent form
Tannins	(+)Lead acetate test	White or yellow ppt
Anthocyanins	(+)pH test	Red to purple
Flavonoids	(+)Shinoda test	Light pink
Glycosides	(+)NaOH test	Yellow ppt
Polyphenols	(+)Ferric chloride test	Blue – black colour

(+)Positive indicates the presence of Phytoconstituents

Table 7: Calibration curve

Concentration	Absorbance
0	0
5	0.195
10	0.388
15	0.575
20	0.755
25	0.92

Table 8: Drug Content and Entrapment efficacy of Hibiscus sabdariffa in formulation

Formulation	Drug content %w/w	EE% Entrapment efficacy
NF1	58.5%	69.5
NF2	73.2%	79.09
NF3	63.3%	75.7

Table 9: Franz diffusion study

Time (hours)	Cumulative % Drug Permeated (Mean ± SD)
0.5	9.6 ± 0.8
1	17.2 ± 1.1
2	28.5 ± 1.3
4	42.7 ± 1.7
6	55.3 ± 1.9

Table 10: Evaluation of Niosomes Based Cream

Characterisation	F1	F2	Standard
Colour	Green	Light green	White
Appearance	Semi solid	Semi solid	Semi solid
Consistency	Very good	Good	Very good
Homogeneity	Very good	Good	Very good
Greasiness	Non greasy	Non greasy	Non greasy
Washability	Washable	Washable	Poor

Table 11: Determination of pH, phase separation, Viscosity, Washability and Greasines

Formulations	F1	F2	F3
pH	6.7	6.6	6.7
Phase separation	No phase separation	No phase separation	No phase separation
Viscosity	18820cP	11810cP	21200cP
Irritant effect	Nil	Nil	Nil
Spreadability	10sec	7 sec	6 sec

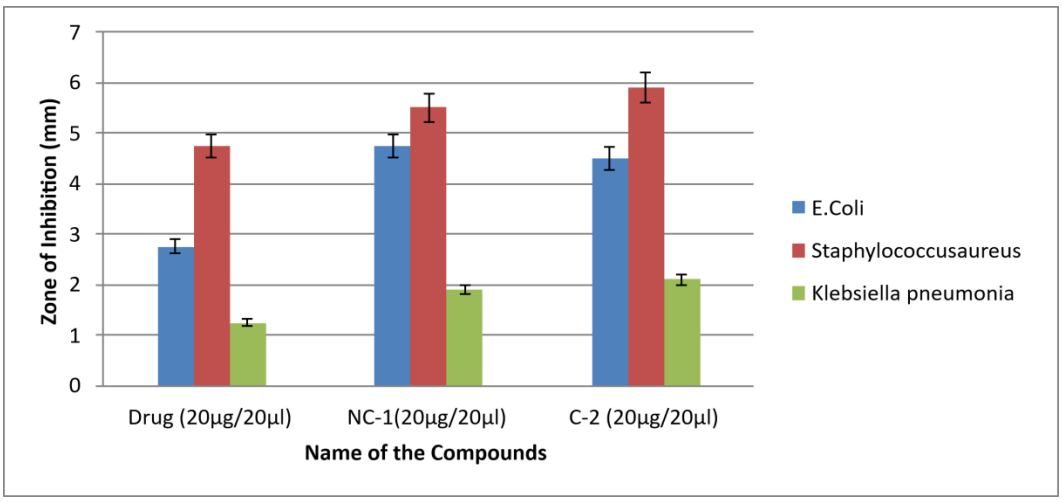
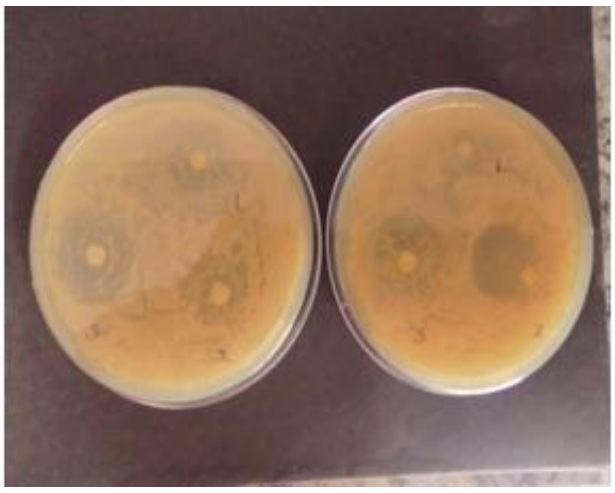
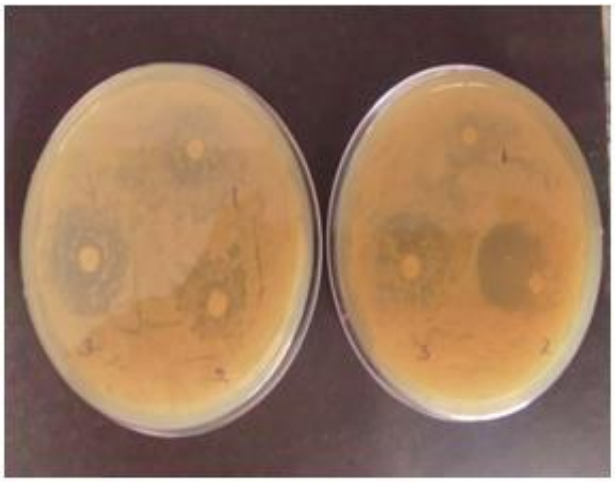
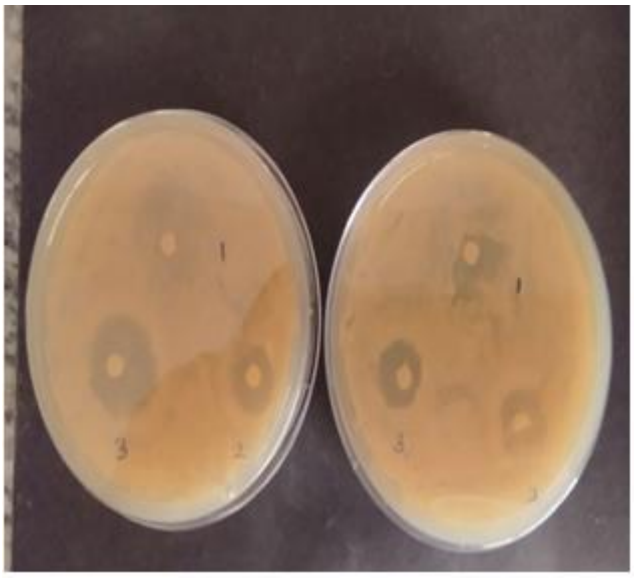


Figure 15: Effects of different formulations on bacterial growth inhibition





Staphylococcus aureus ***E.coli***



Klebsiella pneumonia

Figure 16: Antibacterial activity of comparative cream formulation

Table 12: Anti-bacterial activity

S.NO	Name of organism	Drug	NC-1	C-2
1	E.coli	2.75	4.75	4.5
2	Staphylococcus aureus	4.75	5.5	5.9
3	Klebsiella pneumonia	1.25	1.9	2.1

DISSCUSION

Organoleptic Characteristics of Hibiscus Sabdariffa Extract

The extract of *hibiscus sabdariffa* contains organoleptic features. It is dark geen in colour with unique odor and flavor. The exact is both water and alcohol soluble. The ph of extract is 6.7

Phytochemical Screening of Hibiscus Sabdariffa Extract

Phytochemical tests confirmed that the ethanolic extract of *hibiscus sabdariffa* contains various bioactive compounds like alkaloids, saponins, tannins, glycosides, flavonoids, anthocyanins and polyphenols. Which possess therapeutic benefits.

Pre Formulation Studies

Fourier Transform Infrared Spectroscopy

The physical mixture and separate drug, cholesterol and surfactant which are used in niosomes formulation were analysed by FTIR (Fourier Transform Infrared Spectroscopy) to evaluate the compatibility between the drug and excipients. The results of FTIR studies are shown in results.

Calibration Curve

The calibration curve is obtained from the concentration and absorbance of the extract. From the above table no.6 and graph indicating that absorbance is directly proportional to concentration. From this results and method the quantification of the drug is analysed.

Evaluation of Niosomes

Microscopic Study

The microscopic study of the three noisome formulations shows NF2 has shown desirable results with uniform shape and size compared to NF2 and NF3.

Drug Content

The results of drug content shows that NF2 has high concentration with 73.2% w/w were the NF1 has 58.5% w/w and NF3 has 63.3% w/w. As the concentration of the NF2 is high it gives the high therapeutic effects.

Entrapment Efficacy (EE %)

The entrapment efficacy of the three formulations shows that NF1 contain 69.5%, NF2 has 79.09%, and NF3 has 75.7%. The results show that NF2 has high entrapment efficacy compared to NF1 and NF2. The more the entrapment the more the therapeutic effect.

Franz Diffusion Study

The Franz diffusion study shows that results of the drug release with different time intervals, at 6 hours it reached to 55.3%. As the permeation of the drug increases the formulation allows controlled and sustained release of active ingredient. Through this study its shows that it is beneficial for therapeutic and enhancing efficacy. It also improves the bioavailability**.**

Evaluation of Niosomes Based Cream

The evaluation of cream carried out for the three formulations (F1, F2, and standard). They have differentially in colour, texture, consistency, homogeneity and washability. When compared to two formulations F1 and F2, F1 shows the favourable outcomes.

The pH of formulation has are comparable. The pH ranges from 6.6 to 6.7, which is suitable for all skin types without causing any irritation. Spreadability of the three formulations has different time intervals as per the results F1 has more spreadability and easy to use.

Anti-Bacterial Activity

The anti-bacterial activity of the three formulations is evaluated against three different bacterial strains *E. coli, staphylococcus aureus* and *klebsiella pneumonia*. From these three formulations different formulations are against different bacteria like NC-1 has highest efficacy against *E. coli* with the value of 4.75, C-2 is against *staphylococcus aureus* and *klebsiella pneumonia* it shows the potency of formulations against different bacteria so, the formulations are used as anti-bacterial.

CONCLUSION

The study carried out the formulation and evaluation of noisome based anti-bacterial cream with extract of *hibiscus sabdariffa*. The extract contains flavonoids, anthocyanins and polyphenols which are fight against bacteria. By using ethanol injection method with span 60 and cholesterol niosomes showed good entrapment efficiency (79.09%) and drug content (73.2%). The evaluated niosomal suspension was then

added into the cream and compared with extract based and standard cream. The niosomes contained formulation (F1) showed good results in physical properties as well as produced greater zone of inhibition against *E.coli, S.aureus*, *K.pneumoniae*. This proves that niosomal encapsulation enhances the topical application and antibacterial activity of hibiscus sabdariffa, which makes it an effective herb, based antibacterial treatment.

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