Formulation and evaluation of topical anti-microbial herbal cream

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Abstract

Background: The aim of this study was to determine the antimicrobial effect of neem oil with liquid paraffin and coconut oil and their combination on the bacteria E. coli, S. aureus and Candida albicans, which causes skin diseases, increasingly resistant to many commonly used antibiotics as a result of colonization of the skin.

Methods: Nine different emulsions were prepared and evaluation were carried out including Homogeneity, FTIR, Phytochemical tests, Drug content and In-vitro drug release and Anti-Microbial studies were performed.

Results: The results revealed that the Phyto chemical Screening of Neem oil found to be presence of Alkaloids, glycosides, Saponins, flavonoids pH of the cream was found to be in the range of 5.8 - 6.7 which is good for the skin pH. All the formulation were near to the skin pH. The viscosity of the cream was found 28008 - 28932 cps.

Conclusion: Phyto chemical Screening of Neem extract were done and found to be presence of Alkaloids, glycosides, Saponins, flavonoids etc were present. IR studies revealed that there was no drug Excipients interaction. During our physicochemical evaluation studies all the formulation were found to have good pH and Viscosity, diffusion. In-vitro drug release studies showed that, the formulation F9 showed optimum drug release 97.17±2.000 in 3 hours. The tested organisms, particularly gram positive, gram-negative organisms and Fungi Candida albicans had shown high resistance towards different Antibiotics, Thus neem oil is effective against drug resistant organisms.

Keywords: Antimicrobial; Skin Cream; Neem oil; Azadirachtin; Mineral Oil; Coconut oil

1. Introduction

The use of plant materials for medical purposes, such as seeds, berries, roots, leaves, bark, or flowers, is known as herbal medicine, sometimes known as botanical medicine or phytochemical. To live a healthy life free from illness and as reliable sources of medication in the conventional healthcare system, medicinal plants are nature's gift to humans. Nearly a quarter of all medications contain plant components. Long before recorded history, the plants were employed for medical purposes, and traditional medicine is still used widely today. According to archaeological evidence, people have been using medicinal herbs at least since the Paleolithic, around 60,000 years ago. Traditional herbal remedies, which have been used to treat illnesses all throughout the world, are made from naturally derived plant materials that have undergone technological processing (1).
The pharmaceutical term Emulsion is defined as a thermodynamic-ally unstable system consisting of at least two immiscible liquids, one dispersed as fine spheres, known as the dispersed or internal phase, and the other phase known as the continuous or external phase. The system is stabilized by adding a third substance known as an emulsifier, which stabilizes the emulsion by forming a thin layer around the disperse phase and by increasing the viscosity of continuous phase. Stable emulsions are excellent and interesting vehicles for the delivery of active substances from natural and synthetic sources and offer promising applications in various fields due to the complete protection of the active substance in the internal phase.

1.1. Mechanism of Action

- Cytoplasmic membrane rupture,
- Interaction with membrane proteins (ATPases and others),
- Disruption of gram-negative bacteria’s outer membrane due to the release of lipopolysaccharides.
- Coagulation of the cell’s contents,
- Destabilization of the proton motive force with ion leakage,
- Inhibition of enzyme synthesis.

2. Material and methods

2.1. Materials and equipment

Neem oil procured from Yarrow chemicals. In the present study, the laboratory chemicals other than mentioned above used in the study were of analytical reagents grade, and several types of equipment employed in the formulation of herbal cream were electronic balance, pH meter, ultraviolet-visible spectrophotometer, and hot air oven.

2.2. Methodology

2.2.1. Solubility of Neem oil

Solubility is an important consideration in formulations as clarity of the solution is an essential requirement. A quantity of 5ml of Neem oil was measured and transferred into different volumetric flask containing 5ml of distilled water, Phosphate buffer of pH 5.5, Methanol, Dichloromethane and Chloroform as solvents respectively. Samples of these solutions were then collected and the drug concentration was determined spectrophotometrically at 220 nm against a suitable blank using an ultraviolet visible spectrophotometer.

2.2.2. Determination of $\lambda_{\text{max}}$ of Neem oil

NSO standard solution was prepared by dissolving 1gm i.e 1.16ml of NSO in 100ml of the 54% dichloromethane (DCM) & 46% methanol to get a stock solution of 10mg/ml of NSO. This solution was subjected to scanning between 200 – 400 nm in UV-Visible spectrophotometer and the absorption maximum ($\lambda_{\text{max}}$) was determined. The $\lambda_{\text{max}}$ of Neem oil is found to be at 220 nm.

2.2.3. Standard Calibration Curve of Neem oil

Preparation of Standard solution

A standard solution containing 10 mg/ml solution of pure drug was prepared by dissolving 1 gm i.e 1.16 ml of Neem oil in 100 ml of the 54% dichloromethane (DCM) & 46% methanol solution in a volumetric flask.

Preparation of Stock solution

From the standard solution 2, 4, 6, 8, 10 ml was pi-petted into 10 ml volumetric flasks separately and diluted to 10ml with dichloromethane & methanol to produce concentration of 20, 40, 60, 80 & 100 mg/ml respectively. The solutions were scanned by UV - Visible spectrophotometer at 220nm and results were recorded as shown in Table no -8 and in Figure no -

2.2.4. Phytochemical test of Neem oil

Neem seed oil were subjected to qualitative tests for the identification of various active constituents like alkaloids, flavonoids, steroids, glycosides, phenols, terpenoids, tannins, carbohydrates, proteins, fats, etc.
Test for Carbohydrates

- Molish's test: 2 ml of Molish reagent is added to a few drops of extract. After thoroughly shaking the mixture, 2.0 ml of concentrated sulfuric acid is gently added along the test tube's walls and allowed to stand. At the intersection of two solutions, a crimson ring that forms signifies the presence of carbohydrates.
- Fehling's test: 2 ml of Fehling's reagent are added to a few drops of extract. After thoroughly shaking the mixture, it was placed in a bath of boiling water for five minutes. Brick red precipitate formation denotes the presence of sugar.
- Mayes's test: Two drops of Mayes' reagent are put by the side of the test tube to a few drops of extract. The test is confirmed as positive when a green precipitate forms.
- Wagner's test: Two drops of Wagner's reagent are introduced by the side of the test tube to a few drops of extract. The test is confirmed to be positive by a reddish-brown coloured precipitate.

Test for Saponins

- Foam test: To a few ml of extract, 2 ml of distilled water was added in the test tube and the test tube is continuously shaken for 10 minutes. The formation of foam confirmed the presence of saponins.

Test for Flavonoids

- Acid test: To a few ml of extract, few drops of diluted sulphuric acid is added. Orange colour develops which indicates the presence of flavonoids.

Test for Glycosides

- Libermann's test: To 2 ml of extract, 2ml of chloroform and 2 ml of acetic anhydride is added. Formation of violet to blue to green reddish brown ring indicates the presence of glycosides.

Test for Amino Acids

- Ninhydrin test: To a few drops of extract, few drop of Ninhydrin solution is added in a test tube. A characteristic blue colour indicates the presence of amino acids

Test for Terpenoids

- Acetic anhydride test: To 2 ml of extract, 2 ml of acetic anhydride and concentrated SULPHURIC ACID is added. Formation of blue, green rings indicate the presence of terpenoids.

Test for Proteins

- Millon's test: To a few ml of extract, few drop of Millon's reagent is added. White precipitate indicates the presence of Proteins.

Tests for triterpenoids

- Salkowski's test The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layer turns red, sterol is present. Presence of golden yellow layer at the bottom indicates the presence of triterpenes.

Test for Tannins

- Lead Acetate Test: To a few ml of extract, add few drops of 1% lead acetate. The mixture is shaken well. A yellowish precipitate indicates the presence tannins.

Test for Steroids

- To 2 ml of extract, 2ml of chloroform and 2 ml of acetic anhydride is added reddish brown colour is formed. To this added 1 ml of concentrated sulfuric acid. Formation of violet to blue green colour indicates the presence of Steroids.

2.2.5. Drug Excipients compatibility studies

The spectra of samples were recorded over the wave number 4000 to 400 cm$^{-1}$ by using a Bruker Alpha II FTIR spectrometer. IR spectral studies of Pure Neem oil & Excipients and its physical mixture of it were carried out,
interpreted, and compared with each other. If there was no change in peaks of the mixture when compared to a pure drug, it indicates the absence of interactions.

2.2.6. Formulation development of Anti-Microbial Herbal Cream

Procedure: Total 9 different Formulations were prepared containing (F1-F9) Neem seed oil and with other ingredients in various concentrations. These emulsion based formulations contain an aqueous phase and an oil phase. The ingredients of the oil phase (A) were mixed by melting in a porcelain dish at 70 °C. in a water bath with constant stirring. The components of the water phase (B) were mixed separately in a beaker and heated in a water bath to approximately the same temperature as the oil phase. The aqueous phase was added drop-wise to the oil phase with continuous stirring using an emulsifier. Therapeutically active neem oil is dissolved and added to the above mixture and continuously stirred until a cream is formed. Preservatives Sodium benzoate was added after cooling to 40°C.

Table 1 Composition and ingredients to make 30gm of Anti-microbial herbal cream

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Ingredients</th>
<th>Quantity IN gm (30 gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OIL PHASE (A)</td>
<td>F1</td>
</tr>
<tr>
<td>1.</td>
<td>Neem oil</td>
<td>1 ml</td>
</tr>
<tr>
<td>2.</td>
<td>Liquid paraffin</td>
<td>9 ml</td>
</tr>
<tr>
<td>3.</td>
<td>Coconut oil</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Stearic acid</td>
<td>3 gm</td>
</tr>
<tr>
<td>5.</td>
<td>Sorbitan Monoleate</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>6.</td>
<td>Lanolin</td>
<td>0.5 ml</td>
</tr>
<tr>
<td></td>
<td>AQUEOUS PHASE (B)</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Sorbitol Solution</td>
<td>1 ml</td>
</tr>
<tr>
<td>8.</td>
<td>Poly sorbate</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>9.</td>
<td>Sodium Benzoate</td>
<td>0.1 gm</td>
</tr>
<tr>
<td>10.</td>
<td>Water</td>
<td>q. s to 30 gm</td>
</tr>
</tbody>
</table>

2.2.7. Evaluation of herbal cream

Visual examination (9,10)
This test was considered for visual characteristics, which include homogeneity, clarity, and consistency.

- Homogeneity: The formulation was tested for homogeneity by visual appearance and touch.
- Appearance: The appearance of the cream can be observed by colour.

Determination of type of smear, and emolliency (11)
This was determined by applying the cream to the skin surface of a human volunteer. After applying the cream, it was checked what kind of film or stain appeared on the skin.
Determination of pH (12)

The pH of the creams was determined at room temperature with the electrode set to a depth of 0.5 cm in a beaker containing the cream. Weigh about 5g of the cream and dispersed in 45 ml of water in a 100 ml beaker. The pH was determined at 27 °C using the pH meter.

Dye solubility test (13)

In this test, a small sample is mixed with a Scarlet red dye and observed under the microscope. If the continuous phase appears red, the cream is O/W (Oil in Water) type as the water is in the external phase, and the dye will dissolve in it to give color.

Viscosity (14)

The viscosity of the herbal cream was determined by Brookfield viscometer using RV spindle no 96 at 20 rpm at temperature 25 ºC. About 15ml of the was taken in beaker and spindle was immersed in the formulation. The reading was recorded at initial and after rotation at different temperature. The reading was recorded thrice.

Drug content determination (15)

The content of the herbal cream was estimated using UV-Visible spectrophotometer. Near about 1g of the formulation was taken in 50 ml of volumetric flask. The solution was make up to mark with phosphate buffer 5.5. Then, the whole solution was stirred. The solution was shaken and filtered through what man filter paper. The 0.1ml of the filtrate was further diluted to 10ml with solvent and estimated at suitable wavelength.

\[
\text{Drug content} \times \text{Concentration} \times \text{Dilution factor} \times \frac{1000}{\text{Theoretical yield}}
\]

\[
\text{%Drug content} = \frac{\text{Practical yield} \times 100}{\text{Theoretical yield}}
\]

In-vitro Drug diffusion (16)

For the study of drug release profile, various membranes are used which can be natural or synthetic. These natural membranes such as inner layer of egg. Egg membrane is collected by placing an egg in a concentrated 3 M solution of HCL. Wait until the bubbling stops and the foam disappear. The leftover substance is eggshell with yolk and remove the egg-shell membrane from the HCL solution and washed the membrane with PBS (pH 5.5).

A glass cylinder with both ends open, 10cm height, 3.7 cm outer diameter and 3.1 cm inner diameter was used as permeation cell. A egg membrane was fixed to one end of the cylinder with the aid of an adhesive to result in permeation. One gram of semisolid formulation was taken in the cell (donor compartment) and the cell was attached to a beaker containing 140ml of drug free pH 5.5 phosphate buffer as receptor compartment. The medium in the receptor compartment was agitated using a magnetic stirrer and temperature of 37ºC±1ºC was maintained. Samples of 2ml from the receptor compartment were taken at various intervals over a period of 3 hours with replacement of equal amount of drug free buffer (5.5 Phosphate buffer). The samples were estimated by measuring the absorbance at 220nm in a UV-1700 Shimadzu spectrophotometer.

Determination of Microbial growth

- In-vitro Anti- Bacterial studies (17)

Preparation of Nutrient media- Suspend 28g of nutrient agar powder in 1L of distilled water. Mix and dissolve them completely. Sterilize by autoclave at 121 ºC for 15 minutes. Pour the liquid into the petridish and wait for the medium to solidify. Prepare the agar in clean environment to prevent any contamination.

Determination of zone of inhibition: In all the plates well was made using the hole punch apparatus. Place 1gm of formulated cream were added in to the prepared plates containing micro-organism E. coli and S. aureus containing petri plates. Similarly the marketed product was added in 2 plates. These plates were in to the stored in incubator for 24 to 48hr. the next day, plates were observed for the zone of inhibition.
In-vitro Antifungal studies\(^{(18,19)}\)

Preparation of Sabouraud dextrose Agar Plates: Suspend 65.0 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Autoclave at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates or test tubes. Store prepared SDA plates or tubes at 2-8°C until any defects appear on them.

Determination of zone of inhibition: For the Prepared Sabouraud dextrose Agar Plates, place 1 gm of Formulated Creams. For the same plate inoculate *Candida albicans* and allow it to grow for 6 days and measure its zone of inhibition with Zone reader and note the values.

3. Results and discussion

3.1. Solubility of Neem oil

Neem oil is soluble in Methanol, Dicloromethane & Phosphate buffer 5.5. Freely soluble in chloroform and Slightly soluble in Ethanol.

3.2. Determination of \(\lambda_{\text{max}}\) of Neem oil

The solution of Neem oil (10 mg/ml) in the 54% dichloromethane (DCM) & 46% methanol was scanned between 400nm to 200nm in a UV Visible spectrophotometer, the maximum wavelength for the sample of Neem oil was found to be at 220nm shown in fig 1.

![Figure 1 \(\lambda_{\text{max}}\) of Neem oil](image)

3.3. Construction Calibration Curve of Neem oil

The standard solution 2,4,6,8,10 ml was pi-petted into 10 ml volumetric flasks separately and diluted to 10 ml with dichloromethane & methanol to produce concentration of 20,40,60,80&100 mg/ml respectively. The solutions were scanned by UV - Visible spectrophotometer at 220nm. The standard curve of neem oil is shown in fig 2.

![Figure 2 Calibration curve of Neem oil](image)
3.4. Phytochemical test of Neem oil

Table 2 Phytochemicals tests

<table>
<thead>
<tr>
<th>SL.NO</th>
<th>Test for Phytochemicals</th>
<th>Observation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbohydrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Molish's test</td>
<td>Violet ring at junction of two liquids</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Fehling's test</td>
<td>Brick red precipitate</td>
<td>Positive</td>
</tr>
<tr>
<td>2.</td>
<td>Alkaloids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mayer's test</td>
<td>Precipitate</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Wagner's test</td>
<td>Reddish brown precipitate</td>
<td>Negative</td>
</tr>
<tr>
<td>3.</td>
<td>Saponins</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Foam test</td>
<td>Foam lasting for 1 min</td>
<td>Positive</td>
</tr>
<tr>
<td>4.</td>
<td>Flavanoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acid Test</td>
<td>Orange, pink, red to purple color</td>
<td>Negative</td>
</tr>
<tr>
<td>5.</td>
<td>Glycosides</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Libermann's test</td>
<td>Pink color to the ammonical layer</td>
<td>Negative</td>
</tr>
<tr>
<td>6.</td>
<td>Aminoacids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nihydrin test</td>
<td>Blue color</td>
<td>Negative</td>
</tr>
<tr>
<td>7.</td>
<td>Test for Proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Millon's test</td>
<td>White precipitate</td>
<td>Positive</td>
</tr>
<tr>
<td>8.</td>
<td>Triterpenoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salkowski's Test</td>
<td>Golden yellow layer</td>
<td>Negative</td>
</tr>
<tr>
<td>9.</td>
<td>Tannins</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>White precipitate</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Steroids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>violet to blue green colour</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

3.5. Drug Excipients compatibility studies

IR Spectrum of pure drug Neem oil with other Excipients (Liquid Paraffin) exhibited its characteristic absorption bonds in the following IR region and Compounds of acidic group, Alcohol group, Ester group may be present. The absorption bands of Neem oil are shown in fig 1743 (C=O group may be present), 1162 (Peak indicates C-O group may be present), 1650 (C=O group may be present), 2922 (SP³ C-H stretch may be present), 3006 (Methyl groups have characteristic bending absorption), 722 (Presence of long chain hydrocarbon).

The FTIR spectrum of Neem oil with other Excipients shows 1744 (C=O group may be present), 1162 (Peak indicates C-O group may be present), 1650 (C=O group may be present), 2921 (SP³ C-H stretch may be present), 3005 (SP² C-H Stretch may be present), 714 (Presence of long chain hydrocarbon).
3.6. Evaluation of herbal cream

Table 3 Evaluation of Physical parameters of cream

<table>
<thead>
<tr>
<th>SL NO</th>
<th>Formula- tion code</th>
<th>Color</th>
<th>Visual examination</th>
<th>Type of Smear</th>
<th>pH</th>
<th>Dye solubility test</th>
<th>Viscosity (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Homogeneity</td>
<td>Appearance</td>
<td>type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>Pale brown</td>
<td>Homogeneous</td>
<td>Smooth</td>
<td>Slightly greasy</td>
<td>6.7</td>
<td>O/W type</td>
<td>28932</td>
</tr>
<tr>
<td>F2</td>
<td>Pale brown</td>
<td>Homogenous</td>
<td>Smooth</td>
<td>Slightly greasy</td>
<td>6.5</td>
<td>O/W type</td>
<td>28496</td>
</tr>
<tr>
<td>F3</td>
<td>Pale brown</td>
<td>Homogenous</td>
<td>Smooth</td>
<td>Non-greasy</td>
<td>6.7</td>
<td>O/W type</td>
<td>28932</td>
</tr>
<tr>
<td>F4</td>
<td>Pale brown</td>
<td>Homogenous</td>
<td>Smooth</td>
<td>Slightly greasy</td>
<td>5.8</td>
<td>O/W type</td>
<td>28945</td>
</tr>
<tr>
<td>F5</td>
<td>White</td>
<td>Homogenous</td>
<td>Smooth</td>
<td>Non-greasy</td>
<td>6.4</td>
<td>O/W type</td>
<td>28008</td>
</tr>
<tr>
<td>F6</td>
<td>White</td>
<td>Homogenous</td>
<td>Smooth</td>
<td>Non-greasy</td>
<td>6.2</td>
<td>O/W type</td>
<td>28932</td>
</tr>
<tr>
<td>F7</td>
<td>White</td>
<td>Homogenous</td>
<td>Smooth</td>
<td>Non-greasy</td>
<td>6.4</td>
<td>O/W type</td>
<td>28869</td>
</tr>
<tr>
<td>F8</td>
<td>White</td>
<td>Homogenous</td>
<td>Smooth</td>
<td>Non-greasy</td>
<td>6.1</td>
<td>O/W type</td>
<td>28496</td>
</tr>
<tr>
<td>F9</td>
<td>White</td>
<td>Homogenous</td>
<td>Smooth</td>
<td>Non-greasy</td>
<td>5.8</td>
<td>O/W type</td>
<td>28059</td>
</tr>
</tbody>
</table>
The cream was successfully prepared and evaluated. The various different formulation of cream containing Liquid paraffin and Coconut oil, shows the different physicochemical parameters. The pH of the cream was found to be in the range of 5.8-6.7 which is good for the skin pH. All the formulation were near to the skin pH. The viscosity of the cream was found 28008-28932 cps which shows easy spreadable with small shear rate. Where F4,F8,F9 are shows good spreadability, But F9 shows shown good zone of inhibition in compare to others. The pH of the cream was found to be in the range of 5.8-6.7 which is good for the skin pH. All the formulation were near to the skin pH. A high pH value indicating alkalinity could affect the pH balance of the skin, thereby causing negative skin reactions such as rashes while a pH value lower than that of the skin would be termed too acidic for the skin. This can also lead to sensitivity problems and hyper reaction. A pH value of 5.5 is the ideal pH for pharmaceutical products for skin application.

3.6.1. Viscosity

The viscosity of the cream was found 28008-28932 cps which shows easy spreadable with small shear rate. Where F4,F8,F9 are shows good spreadability are shown in Table 3.

3.6.2. Drug content

The data of Drug content analysis from the 9 prepared formulations range between (91.28-96.58%), as shown in Table 4, Fig 4 indicating the homogenous distribution within the prepared creams.

Table 4 Viscosity of cream

<table>
<thead>
<tr>
<th>SL No</th>
<th>Formulation Code</th>
<th>Drug Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>F1</td>
<td>93.55± 0.313</td>
</tr>
<tr>
<td>2.</td>
<td>F2</td>
<td>91.7%± 0.152</td>
</tr>
<tr>
<td>3.</td>
<td>F3</td>
<td>94.28%±0.3</td>
</tr>
<tr>
<td>4.</td>
<td>F4</td>
<td>95.7%±0.13</td>
</tr>
<tr>
<td>5.</td>
<td>F5</td>
<td>94.25%±0.213</td>
</tr>
<tr>
<td>6.</td>
<td>F6</td>
<td>93.27%±0.254</td>
</tr>
<tr>
<td>7.</td>
<td>F7</td>
<td>94.86%±0.123</td>
</tr>
<tr>
<td>8.</td>
<td>F8</td>
<td>93.24%±0.043</td>
</tr>
<tr>
<td>9.</td>
<td>F9</td>
<td>96.58%±0.012</td>
</tr>
</tbody>
</table>

Figure 5 Drug content
3.6.3. In-vitro Drug Diffusion

*In-vitro* studies are carried using Franz diffusion by using egg membrane. *In vitro* release studies showed that release was 91.11±0.522, 94.41 ±0.702, 97.17±2.000. From the results, it is clear that the best release profile was obtained with formulation F9.

**Table 5 In-vitro Drug Diffusion of cream**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>F4</th>
<th>F8</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>14.76±2.818</td>
<td>15.36±0.155</td>
<td>19.36±1.255</td>
</tr>
<tr>
<td>60</td>
<td>25.13±2.696</td>
<td>25.77±2.130</td>
<td>27.77±2.630</td>
</tr>
<tr>
<td>90</td>
<td>42.49±3.004</td>
<td>42.05±1.435</td>
<td>44.03±1.326</td>
</tr>
<tr>
<td>120</td>
<td>60.97±1.525</td>
<td>60.81±0.919</td>
<td>55.22±0.269</td>
</tr>
<tr>
<td>150</td>
<td>89.97±3.081</td>
<td>88.97±0.538</td>
<td>83.97±0.538</td>
</tr>
<tr>
<td>180</td>
<td>91.11±0.522</td>
<td>94.41±0.702</td>
<td>97.17±2.000</td>
</tr>
</tbody>
</table>

**Figure 6** Graph representing % drug release of the formulations (F4,F8,F9)

3.6.4. In-vitro Anti-Microbial studies

The Zone of inhibition of the Formulation 4, Formulation 8, Formulation 9 were compared. The formulation 9 showed the zone of inhibition 4, 2.7, 3.5cm respectively. The formulation 9 showed an acceptable zone of inhibition when compared to formulation 4,8.

<table>
<thead>
<tr>
<th>A) Escherichia coli</th>
<th>B) Staphylococcus aureus</th>
</tr>
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</table>
4. Conclusion

The herbal medicines are used in the ancient time itself for the treatment of different types of diseases. The neem is one of the medicinal plant having antibacterial activity against the different bacteria and Fungi. Plant extracts with antibacterial activity have been formulated as topical creams. It has been previously reported that formulation of Neem oil as topical cream may lead to enhancement of stability and acceptability of the active ingredient, while the antimicrobial activity remains considerable. Topical route of application has a great potential as an effective and safe way to administer drug for its Anti-Microbial in effect.

The o/w cream preparations of Neem oil are designed using different bases for the treatment of Microbial infection. Phytochemical Screening of Neem extract were done and found to be presence of Alkaloids, glycosides, Saponins, flavonoids etc were present. IR studies revealed that there was no drug Excipients interaction. During our physicochemical evaluation studies all the formulation were found to have good, pH and Viscosity, diffusion and stability studies. pH determination shows that all the formulation have near neutral pH and does not changed during the study period. Hence, there was no possibility of any kind of skin irritation. The drug content estimation showed uniform drug content in the all prepared formulations. In-vitro drug release studies showed that, the formulation F9 showed optimum drug release 97.17±2.000 in 3 hours. The tested organisms, particularly gram positive, gram-negative organisms and Fungi Candida albicans had shown high resistance towards different Antibiotics whereas they were found to be inhibited by Neem oil even at lower concentration. Thus neem oil is effective against drug resistant organisms.

Compliance with ethical standards

Acknowledgments

All the authors have contributed equally.

Disclosure of conflict of interest

No conflict of interest.

References


