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Research Article

Formulation And Evaluation of Anti-Acne Emulgel of Morus Rubra (Mulberry)And Antibacterial Test Against *Staphylococcus Aureus* and *Propionibacterium Acne* Bacteria

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ABSTRACT

Bacterial infections are a global public health concern due to their impact on the morbidity and mortality of various populations. Skin as a route of drug administration is useful for bacterial infection treatment which is a common problem in dermatology. Emulgel is a recently developed drug delivery system which allows for controlled emulsion and gel release for topical applications. Stability can be enhanced by incorporating an emulsion into a gel. In the current investigation, Emulgel containing extract of mulberry was formulated for the management of bacterial skin infections. The extracting procedures were performed with the emulsifying agent's tween 20 and span 20 to create the required emulsions. Emulgel was made by mixing emulsions with Carbopol 934.The preparation was done and evaluation parameters like pH, physical appearance, stability, spreadability, in vitro drug released study as well as antibacterial properties were checked. The formulation provided results that were acceptable for the tested parameters. The result showed formulation has Better spreadability, stability, and antibacterial properties than the other formulation. From those parameters, it was found that emulgel with leaves extract of Morus rubra (Mulberry) has the potential to treat skin bacterial infections while offering cosmetic advantages.

INTRODUCTION

Humans live in harmony with microorganisms; however, infections may arise when the immune system is weakened or when the levels of pathogens become excessively high. An infectious disease manifests when these agents provoke a response from the body, leading to observable clinical signs and symptoms. Various organisms, such as bacteria, viruses, parasites, fungi, prions, worms, and helminths, are responsible for infectious diseases. While bacterial infections were historically the most feared, advancements in control measures have shifted the focus to fungi, which now represent the most significant threat. Humans coexist peacefully with microorganisms; however, infections can occur when the immune

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system is compromised or when pathogen levels surge. An infectious disease occurs when these agents elicit a response from the body, resulting in noticeable clinical signs and symptoms. A range of organisms, including bacteria, viruses, parasites, fungi, prions, worms, and helminths, can cause infectious diseases. Although bacterial infections were once the primary concern, improvements in control strategies have redirected attention towards fungi, which now pose the greatest risk. Emulsion gels offer several benefits compared to conventional creams and ointments, such as enhanced application characteristics, quicker and more thorough drug release, and the convenience of application on hairy skin without leaving a greasy feel or residue. These formulations can incorporate both water-based and oil-based substances, allowing for the inclusion of hydrophobic or poorly soluble medications, like antibacterial agents, by choosing the suitable oily phase. The skin, recognized as the largest sensory organ of the body, maintains a pH level between 4.0 and 5.6. It is composed of four distinct layers: the non-viable epidermis, the viable epidermis, the viable dermis, and the subcutaneous connective tissue. Topical medications penetrate the skin through three primary mechanisms: transcellular, intracellular, and follicular. The transcellular route is the most direct and shortest, while the intercellular pathway is the most prevalent. The follicular route involves absorption through hair follicles and sweat glands. Topical formulations are specifically designed for delivery through the skin, with a significant advantage of bypassing first-pass metabolism. Morus rubra leaf extract possesses anti-inflammatory and antibacterial characteristics, making it advantageous for skin prone to acne. It aids in alleviating inflammation, fighting bacteria responsible for acne, and may also help diminish the visibility of acne scars. Morus rubra leaf extract is rich in various chemical compounds that are advantageous for treating acne. It includes antioxidants such as quercetin and kaempferol, along with anti-inflammatory agents like flavonoids and anthocyanins. Additionally, mulberry leaves provide essential minerals, including zinc, calcium, and iron, which are vital for overall well-being and may promote healthy skin. Furthermore, these leaves are an excellent source of vitamins A, C, and E, all of which play a crucial role in maintaining skin health and can aid in alleviating acne. [1][2]

MATERIALS AND METHODS

Materials

Morus rubra leaves were gathered from the vicinity of Loni Khud village. Carbopol 934, Span 20, Tween 20, Propylparaben, Propylene glycol, Ethanol, and Triethanolamine were sourced from the chemical store at Pravara Rural College of Pharmacy in Loni. All materials and reagents utilized in this study are of analytical grade.

METHODS

Preparation of mulberry leaf extract

To prepare Morus rubra leaf extract, dried leaves are first cleaned thoroughly. For aqueous extraction, fresh leaves are chopped, blended with distilled water in a 1:10 ratio, and heated at 60–80°C for 30–60 minutes before cooling and filtering. The extract can be stored as is or concentrated by evaporation. For solvent extraction, dried leaves are ground into a fine powder and soaked in ethanol (1:10 ratio) for 24–48 hours. The mixture is then filtered, and the solvent is evaporated to obtain a concentrated extract. The final extract is stored in an airtight container for future use.[3]

Preparation of Emulgel

The oil phase of the emulsion was created by dissolving Span 20 in light liquid paraffin, while the aqueous phase was formed by dissolving Tween 20 in purified water with continuous stirring. Methyl paraben and propyl paraben were dissolved in propylene glycol, and Isotretinoin was

dissolved in ethanol; both solutions were then incorporated into the aqueous phase. The oil and aqueous phases were separately heated to a temperature range of 70-80 °C. Subsequently, the oil phase was gradually added to the aqueous phase while stirring continuously until the mixture cooled to room temperature. The gel was formulated using Carbopol 934 as the gelling agent, to which a sufficient amount of distilled water was added while stirring at a moderate speed with a mechanical shaker. The pH was adjusted to between 6 and 6.5 using triethyl amine (TEA). Finally, the emulsion was incorporated into the gel formulation with continuous stirring, resulting in the preparation of the emulgel.[4][5]

Table 1. Formulation of emulgel

Ingredients	Category	Quantity taken
Morus rubra leaf extract	Anti-Bacterial	2gm
Carbopol	Gelling agent, emulsifier	1gm
Span 20	Co-surfactant	1.25ml
Light liquid paraffin	Softening agent	2.25ml
Tween 20	Non-ionic detergent	2.5ml
Propylparaben	Preservative	0.25gm
Propylene glycol	Emollient	2.5ml
Ethanol	Antiseptic, astringent	5ml
Triethanolamine	Buffer, pH indicator	Q.S.
water	Vehicle	Q.S.

EVALUATION OF EMULGEL

1)Physical Assessment of Emulgel Formulation:

The emulgel formulations were subjected to a visual inspection to evaluate their colour, Odor, appearance, homogeneity, phase separation, consistency (through texture analysis), and drying time.[6]

2)pH Measurement of Emulgel Formulation:

To assess the pH of the emulgel, 1 gram of the formulation was precisely weighed and dissolved in 100 ml of distilled water. The pH was then measured using a digital pH meter, which had been calibrated prior to use with a standard buffer solution. Measurements were conducted in triplicate, and averages were computed. The pH values of all emulgel formulations fell within the range of 5.8 to 6.8, which is consistent with the normal skin pH and is unlikely to cause any skin irritation.[7]

3)Study of Spreadability in Emulgel Formulation:

Spreadability refers to the degree to which an emulgel can easily cover a surface upon application to the skin or the affected area. The bioavailability of the emulgel is also influenced by its spreadability. To assess the spreadability of the formulation, 0.5 grams of emulgel was placed within a circle of 1 cm in diameter on a 4 cm glass plate, which was then covered with a second glass plate. A weight of 20 grams was applied to the upper plate for 5 minutes. The time taken for the diameter to increase due to the spreading of the emulgel was calculated using the following formula:

$$S = ML / T$$

Where S represents spreadability, M is the weight on the upper plate, L is the length of the glass slide, and T is the time taken.[8]

4)Determination of Drug Content in the Formulated Emulgel:

A sample of the emulgel formulation containing 0.5 mg of Morus rubra leaf extract was combined with 10 ml of dichloromethane. The mixture was gently agitated with a glass rod until the emulgel was fully dispersed. The resulting solution was transferred to a volumetric flask and diluted to 100 ml with 5 ml of 0.1 M HCl and 96% ethanol. The solution was then filtered using Whatman filter paper. The absorbance of the solution was measured at approximately 360 nm using an ethanolic hydrochloric solution in the reference cell, and the drug content was calculated based on the absorbance. This experiment was repeated five times, and the average drug content was determined.[9][10]

5)Evaluation of Emulgel Stickiness:

The stickiness of the emulgel was assessed by applying a small amount of the mulberry emulgel and observing whether stickiness was present or absent.[11]

6)Assessment of Odor in Emulgel Formulation:

The odour of the emulgel formulation was evaluated by having 5-6 volunteers assess the scent. The results were categorized as acceptable, unacceptable, or alcoholic.[12]

7)Viscosity study of emulgel formulation:

The viscosity of the emulgel formulation was measured undiluted using a Rheometer (Brookfield viscometer). The recorded values for both the sample and water were documented. The formulation was placed in a beaker and allowed to equilibrate at room temperature. Subsequently, the spindle was lowered and rotated at speeds of 2, 5, 10, and 12 rpm. The viscosity, expressed in centipoises, was observed and recorded. The Brookfield factor finder was utilized as follows:

Dial reading x factor = Viscosity in centipoises (CP. S).[13]

8)In vitro drug release study using a Franz diffusion cell:

The study was conducted to assess drug release through semi permeable membrane utilizing a Franz diffusion cell. The formulation was applied to a dialysis membrane, which was positioned between the receptor and donor compartments of the cell. The donor compartment was left open at the top, allowing exposure to the atmosphere. For the in vitro diffusion process, a dissolution medium consisting of a mixture of phosphate buffer at pH 5.8 and ethanol (in a 65:35 v/v ratio) was employed. Prior to use, the dialysis membrane was soaked in phosphate buffer for 24 hours. The temperature was maintained at 37°C using a water circulating jacket. The setup was placed on a magnetic stirrer for a duration of 6 hours, with continuous stirring of the solution facilitated by a magnetic bead. A corresponding blank setup was prepared with fresh dissolution medium. At specified time intervals of 15 minutes, 30 minutes, 1 hour, 2 hours, and up to 6 hours, 2 ml of the phosphate buffer solution was withdrawn using a syringe and replaced with an equal volume of fresh phosphate buffer solution. The samples were subsequently analysed using a spectrophotometer at a wavelength of 370 nm, and the percentage of drug release was calculated.[14][15]

9)Anti-bacterial activity

The Zone of Inhibition test assesses the antibacterial efficacy of various products. Extracts from Morus rubra leaves demonstrate significant antibacterial properties. This test involves the application of a bacterial culture onto a nutrient agar-based petri dish. A pure bacterial culture is evenly spread across Mueller-Hinton agar plates using a swab. The sample of the product being tested is then placed on the agar plate with sterile forceps. The petri dish is incubated for 18 to 24

hours at a temperature of 36°C, along with other optimal conditions that facilitate bacterial growth. After the incubation period, a clear area, known as the zone of inhibition, appears around the antibacterial product sample, indicating its antimicrobial effectiveness against *Staphylococcus aureus* and *Propionibacterium acne* bacteria. This zone is measured and analysed; a larger zone of inhibition signifies stronger antibacterial activity of the treated products, and vice versa. The product sample is placed on nutrient agar, and observations are made after a few days.[16][17]

RESULT AND DISCUSSION

1)Physical Assessment of Emulgel Formulation:

The formulation exhibited a white colour. Its consistency ranged from good to excellent, and no phase separation was observed. The results are detailed in the table below.

Table 2. Physical appearance of the formulation

Sr. No	Parameters	Result
1.	Colour	White
2.	Consistency	Excellent
3.	Phase separation	No phase separation

2)pH Measurement of Emulgel Formulation:

The pH measurement of the emulgel formulation derived from Morus rubra leaves extract was recorded at 6.1, which falls within the typical pH range for skin. This formulation does not cause any irritation to the skin.

3)Study of Spreadability in Emulgel Formulation:

The spreadability of the emulgel was assessed to determine how easily it can be applied to the skin. The evaluation revealed that the emulgel formulation exhibited a spreadability of 22.31 gm cm/sec.

4)Determination of Drug Content in the Formulated Emulgel:

The percentage of drug content in the prepared emulgel was evaluated using a UV spectrophotometer. The absorbance readings obtained were utilized to calculate the percentage of drug content. The drug content across the emulgel formulations was consistent, and found to be 96.23%.

5)Evaluation of Emulgel Stickiness:

Emulgel of Morus rubra leaves extract was free from Stickiness after application and it was freely spread on the skin.

6)Assessment of Odor in Emulgel Formulation:

The scent of the emulgel formulation was assessed by conducting evaluations with 5 to 6 volunteers. Based on their feedback, the odour was found to be acceptable.

7)Viscosity study of emulgel formulation:

The viscosity of the emulgel formulations was assessed using a Brookfield Viscometer, revealing a viscosity measurement of 1498 centipoise (CP. S).

8)In vitro drug release study using a Franz diffusion cell:

The anti-acne emulgel of Morus rubra leaf extract's percentage release pattern is displayed in the in vitro drug release investigation. 92.76% was found to be the formulation's greatest medication release percentage.

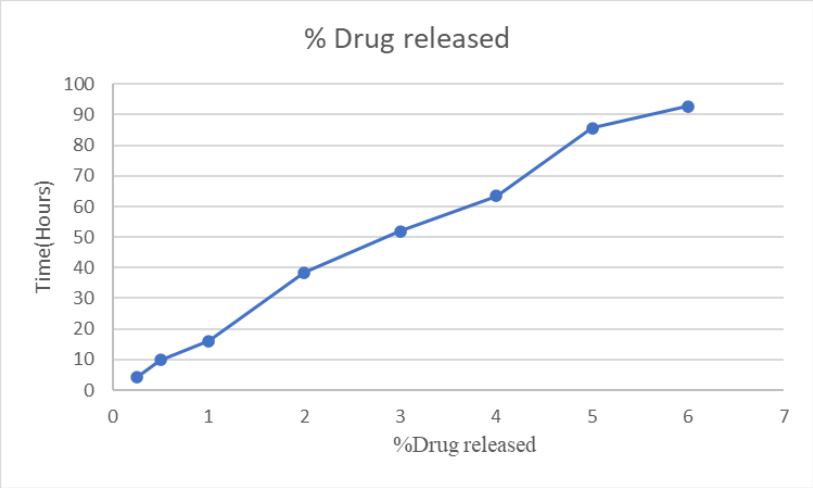

Figure 1. Drug released profile

9)Anti-bacterial activity:

Table 3. Antibacterial activity of emulgel formulation.

Sr. No.	Parameter	Result
1.	Zone of inhibition	6, 5.6, 6.1

Figure 2. Zone of inhibition of emulgel against bacteria.

CONCLUSION

The current study effectively formulated an herbal emulgel utilizing the antibacterial properties of Morus rubra. Various emulgel formulations containing Morus rubra were successfully created, and several physical parameters were assessed, including pH, stability, spreadability, drug content, viscosity, drug release, and antibacterial activity. The observed antibacterial effects are likely attributed to the chemical compounds found in the extract of Mulberry leaves. Therefore, the topical emulgel derived from Morus rubra extract presents a promising alternative for a topical drug delivery system in the treatment of acne.

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