

Evaluation of Anti-microbial potential of Nanoemulsion of Hippaestrum vittatum (Lily) flowers extract

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ABSTRACT

Fungal skin infections are one of the most prevalent dermatological issues nowadays. According to research, 40 million people have fungal infections. The present study was based on the evaluation of anti-microbial potential of nanoemulsion of Hippaestrum vittatum (Lily) flowers extract. The flowers of H. vittatum were collected from the Unnao region, Uttar Pradesh. These were identified and authenticated by a botanist. These were washed, dried under shade and sieved for making dust-free and kept at room temperature. The powder of Hippaestrum vittatum were weighed and extracted through maceration using ethyl alcohol. The flowers of Hippaestrum vittatum were soaked into a beaker containing ethyl alcohol solvent for 15 days with frequent stirrings. The beaker was mounted with aluminium foil. The anti-microbial potential was determined using disc diffusion method. In results, the H. vittatum flowers were extracted through cold maceration process using ethyl alcohol and percentage yield was found to be 11.29 %. In concluded that lily's flowers extract based nanoemulsion (F1-F6) showed a statistically significant anti-microbial potential which might be due to release of more phytochemical constituents in the flowers extract. F3 showed the highest zone of inhibition i.e., 18.4 mm, 14.6 mm, 23.6 mm, and 15.7 mm against S. aureus, B. subtilis, E. coli and S. pneumoniae, respectively. In suggests, the fellow researchers to determine the mode of action for its anti-microbial activity thus H. vittatum (lily) flowers extract-based nanoemulsion can be utilized in cure of numerous microbial infections...

Keywords Lily, Hippaestrum vittatum, nanoemulsion, anti-microbial, E. coli...

INTRODUCTION

Fungal skin infections are one of the most prevalent dermatological issues nowadays. According to research, 40 million people have fungal infections [1]. Fungal infections that appear superficially on the skin, hair, or nails are frequent and typically challenging to treat [2]. Dermatophytes are one of the most prevalent causes of tinea, which includes capitis, manuum, pedis, cruris, corporis, barbae, and onychomycosis. Pityriasis versicolor and candidal infections are two additional common superficial cutaneous fungal infections [3]. To treat these fungal infections, a variety of synthetic antifungal medications are utilized in clinical settings. These medications can be roughly categorized into four classes: azoles, allylamines, echinocandins, and polyenes [4]. Despite this, the path to modern treatment has not been without issues and challenges, most notably drug resistance. Azole resistance, however, is widely known [5].

Because of their higher surface-to-volume ratio, which promotes dispersibility in the emulsion, the active components are better suited for a variety of activities when they are present as nanoparticles [6]. Nanoemulsions can be categorized based on their morphology. The opposite scenario, where water is the continuous phase and oil is the dispersed phase, produces an oil-based or water-in-oil (W/O) emulsion [7]. The small droplet size of the nanoemulsion allows for the uniform deposition and penetration of active substances through the skin's surface. The contents of nanoemulsions permeate components more efficiently because of the large surface area and low surface tension of the entire emulsion system; only 3–10% of surfactants are needed during preparation [8].

The genus *Hippeastrum*, which contains several alkaloids, is gaining popularity as a remedy for neurological conditions and neurodegenerative diseases [9]. The tropical and subtropical parts of South America are home to its more than 70 species [10]. *Hippeastrum* plants come in a variety of hybrids, and most modern commercial hybrids are descended from *Hippeastrum vittatum* Herb [11]. *Hippeastrum vittatum* is native to Central & South America, Brazil, and Peru, and is currently the world's most often cultivated *Amaryllis*, which originated in South Africa in the Cape of Good Hope in 1633 and was introduced to Europe [12]. The ethanolic fresh flower extract from *Hippeastrum vittatum* revealed the distinctive alkaloids, tannins, glycosides, including Ismine, Lycorine, Crinine, Vittacarboline, Haemanthamine, Narciclasine, Galanthamine, Tazettine [13]. *Hippeastrum vittatum* possesses anticancer, antiviral, anti-inflammatory, and other pharmacological activities. Malignant tumours have emerged as one of the most serious hazards to human health and mortality [14][15]. The present work was centered on the evaluation of anti-microbial capability of nanoemulsion of *Hippeastrum vittatum* (Lily) flowers extract.

MATERIALS AND METHODS

Experimental requirements

Flowers of *Hippeastrum vittatum*, PF127 (Sigma Aldrich, India), ethanol, phosphate buffer and agar, Chlorhexidine acetate, microbial strains and distilled water.

Collection, Identification & Authentication of plant

The flowers of *Hippeastrum vittatum* were collected from the Unnao region, Uttar Pradesh. These were identified and authenticated by a botanist. These were washed, dried under shade and sieved for making dust-free and kept at room temperature.

Extraction of plant

The powder of *Hippeastrum vittatum* were weighed and extracted through maceration using ethyl alcohol. The flowers of *Hippeastrum vittatum* were soaked into a beaker containing ethyl alcohol solvent for 15 days with frequent stirrings. The beaker was mounted with aluminium foil. At last, it was filtered with whatman filter paper thus obtained the herbal extract in a homogenous manner. A rotating evaporator was used to dry the brownish, semisolid extract obtained under partial vacuum. The % yield of the extract was calculated [16].

Preparation of formulation (Spontaneous emulsification method)

The essential oil included dissolved *Hippeastrum vittatum*, Surfactant PF127 and preservative methyl paraben were dissolved in water to prepare the aqueous phase. In order to avoid heating the system, nanoemulsion was made by gradually integrating the oil phase into the aqueous phase using an ultrasonic processor. The temperature was kept as $15 \pm 8^\circ\text{C}$. The formulation was prepared and stored at 25°C / room temperature. In order to assess the stability of the dispersion, we changed the ratios of peppermint essential oil from 5% to 10% and the quantity of surfactant in the aqueous phase from 5% to 15% [17].

Table 1. Formulation composition (Oil phase)

Formulation	Oil phase	
	Herbal extract (%)	Peppermint oil (%)
F 1	5.0	35
F 2	5.0	40
F 3	5.0	35
F 4	5.0	35
F 5	5.0	35
F 6	5.0	40

Table 2. Formulation composition (Aqueous phase)

Formulation	PF127 (surfactant)			Methyl paraben (%)	Distilled water (%)
	5 %	10 %	15 %		
F 1	5.0	—	—	0.1	55
F 2	—	—	5.0	0.1	50
F 3	10.0	—	—	0.1	50
F 4	—	10.0	—	0.1	50
F 5	—	—	10.0	0.1	50
F 6	—	—	5.0	0.1	50

Evaluation of anti-microbial activity

All the materials required for the method were sterilized prior and were kept in the aseptic area in the Ultra-Violet laminar air flow. Bacterial suspensions (3 mL) were then poured in the petri-plates. As soon as nutrient agar attained 50° C temperature, 20 mL media was poured into the petri-plates containing bacterial suspension or fungal suspension and plates were rotated to mix the suspension with media. When the agar got solidified, bores were made in the plate with sterile borer of 8 mm diameter. In each plate, four bores were made. Out of which, one is meant for addition of standard, one for control and remaining two bores for addition of same concentrations of sample. 0.1ml of sample was added in each cylinder. The plates were kept to allow diffusion at room temperature for three hr and then incubated in the upright position in incubator at 37° C for about 21 hr for bacterial growth. The diameter of zone of inhibition was accurately measured for bacterial growth in each treated plate. The zone of inhibition of bacterial growth and fungal growth by the test solution was compared with the zone of inhibition by the standard at tested concentrations [18].

The followings microbial strains were used in the screening of anti-microbial activity:

Streptococcus aureus

Bacillus subtilis

E. coli

Streptococcus pneumoniae

RESULTS AND DISCUSSION

Percentage yield

The *H. vittatum* (lily) flowers were extracted through cold maceration process using ethanol and percentage yield was found to be 11.29 %.

Evaluation of anti-microbial potential

Hippaestrum vittatum (Lily) flowers extract based nanoemulsion (F1-F6) showed the significant anti-fungal potential through increasing the zone of inhibition against all the microbial strains used i.e., *S. aureus*, *B. subtilis*, *E. coli* and *S. pneumoniae*. F3 showed the highest zone of inhibition i.e., 18.4 mm, 14.6 mm, 23.6 mm, and 15.7 mm against *S. aureus*, *B. subtilis*, *E. coli* and *S. pneumoniae*, respectively.

Moreover, Chlorhexidine acetate exhibited the zone of inhibition as 19.8 mm, 16.4 mm, 25.7 mm and 16.9 mm, against *S. aureus*, *B. subtilis*, *E. coli* and *S. pneumoniae*, respectively.

Table 3. Anti-microbial potential of Nanoemulsion of *H. vittatum* (Lily) flowers extract

Formulation	Zone of inhibition (mm)			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. pneumoniae</i>
F1	17.3	13.7	23.2	15.5
F2	16.8	13.2	21.5	14.3
F3	18.4	14.6	23.6	15.7
F4	16.9	13.9	21.9	14.8
F5	17.6	14.2	22.3	14.1
F6	17.8	13.5	22.1	14.4
Chlorhexidine acetate	19.8	16.4	25.7	16.9

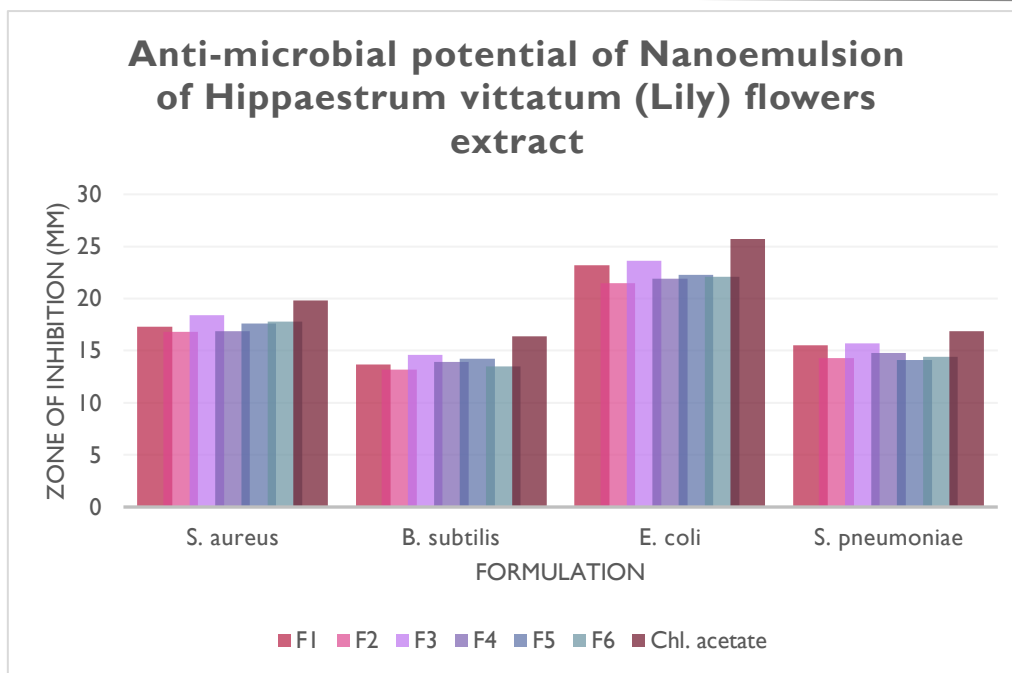


Fig. 1 Anti-microbial potential of Nanoemulsion of *H. vittatum* (Lily) flowers extract

Antimicrobial assessment showed significantly high ZOI for PHFX1 against selected bacteria. Gram-positive bacteria, such as *B. subtilis*, *S. pneumoniae*, *S. aureus*, and *S. mutans*, showed susceptibility toward PHFX1 with ZOI of 23.37 ± 1.20 , 20.43 ± 0.71 , 24.03 ± 0.60 , and 16.53 ± 0.71 mm compared to the standard CHD-A ZOI of 18.07 ± 0.91 , 16.13 ± 0.67 , 16.87 ± 1.20 , and 23.27 ± 1.90 mm, respectively ($p \leq 0.05$). Moreover, Gram-negative bacteria, such as *Escherichia coli* and *K. pneumoniae*, also showed susceptibility toward PHFX1 with ZOI of 26.53 ± 0.60 and 21.17 ± 0.61 mm, which were similar to the standard CHD-A ZOI of 26.53 ± 0.60 and 23.27 ± 1.60 mm, respectively. In addition, the ZOI values for PHFX1 were significant compared to free CCM and pure CEO ($p \leq 0.05$). PHFX1 inhibited the growth of *B. subtilis*, *S. pneumoniae*, *S. aureus*, *S. mutans*, and *Klebsiella pneumoniae* even at one-third of the concentration of formulation and was most active against *E. coli* where it was a potent inhibitor, inhibiting the growth at one-fourth of the concentration. Standard CHD-A on the other hand inhibited bacteria at 42% of the working concentration. [19].

The well-established antibacterial properties of neem, which contains azadirachtin, nimbin, and quercetin—compounds known to suppress acne-causing microbes—are supported by the notable inhibitory zones against *Propionibacterium acnes* and *Staphylococcus aureus* [20]. The solubility and permeability of lipophilic and poorly water-soluble actives are known to be improved by nanoemulsions. Because neem extract and vitamin E are hydrophobic, they both benefited from better solubilization and prolonged release in this formulation. Over the course of eight hours, the in vitro diffusion research verified a greater cumulative release from the nanoemulgel (82.5%) than from the conventional gel (51.8%). Maintaining constant medication levels at the application site, lowering application frequency, and enhancing patient compliance are all made possible by this extended release profile [21]. A herbal nanoemulsion-based topical gel containing *Azadirachta indica* (neem) and vitamin E was successfully developed and assessed in a prior study for the targeted treatment of acne and skin irritation. The nanoemulsion showed excellent stability, low PDI, and appropriate droplet size (142.6 ± 3.2 nm), all of which improved skin penetration and prolonged drug release. The incorporation of the nanoemulsion into a Carbopol 940 gel base yielded a nanoemulgel with favorable rheological and textural properties, suitable for dermal application. The formulation exhibited significant antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus*, and strong anti-inflammatory potential, comparable to standard synthetic drugs. Importantly, the nanoemulgel was non-irritant in skin safety tests, reinforcing its suitability for long-term topical use. These findings support the therapeutic potential of herbal nanoemulgel systems as a safe, effective, and patient-friendly alternative to conventional acne therapies. Future studies, particularly clinical trials and long-term stability assessments, are recommended to validate the commercial viability and broaden the therapeutic applications of this novel herbal formulation [22].

CONCLUSION

It is concluded that lily's flowers extract based nanoemulsion (F1-F6) showed a statistically significant anti-microbial potential which might be due to release of more phytochemical constituents in the flowers extract. F3 showed the highest zone of

inhibition i.e., 18.4 mm, 14.6 mm, 23.6 mm, and 15.7 mm against *S. aureus*, *B. subtilis*, *E. coli* and *S. pneumoniae*, respectively.

It suggests, the fellow researchers to determine the mode of action for its anti-microbial activity thus *H. vittatum* (lily) flowers extract-based nanoemulsion can be utilized in cure of numerous microbial infections.

CONFLICT OF INTEREST

Authors declare for none conflict of interest....

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