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in-vitro Evaluation of Curcurmin Nanoemulsion for its Antioxidant and Anti -Microbial Activity on Face Pathogens

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Abstract

Curcumin is an inherent component present in the spice turmeric. Renowned for its vibrant yellow hue, this subject has been thoroughly examined for its possible advantages to one's well-being. Curcumin demonstrates potent anti-inflammatory and antioxidant characteristics, which could aid in the treatment of several health disorders. Although curcumin has advantages, it has limited bioavailability. Hence, a nanoemulsion formulation of curcumin was synthesised and subsequently assessed for its potential in conducting antioxidant and antibacterial investigations. In the current research work accelerated stability tests assessed the changes in the quality of medication over time. From month 1 to month 4, the CUNL (curcumin nanoemulsion) observed an upward trend in zeta potential, particle size, polydispersity index, and viscosity, while the pH decreased. The research further revealed that encapsulation significantly decreased its scavenging activity, especially at low doses (< parts per million). However, the scavenging activity of both samples was determined to be comparable when the concentration ranged from 18 to 23 parts per million (ppm). In addition to this, CUNL was found to be highly efficient against Staphylococcus aureus (ATCC-6538), Streptococcus pyogenes (SF370), Staphylococcus epidermidis (ATCC 12228), Corynebacterium minutissimum (DSM 7174), and Cutibacterium acnes NCTC 737. The highest level of inhibition was seen for Cutibacterium acnes (17.2 ± 0.1), followed by Staphylococcus epidermidis (18.6 ± 0.2), Corynebacterium minutissimum (12.4 ± 0.1), Staphylococcus aureus (11.6 ± 0.2), and Streptococcus pyogenes (10.7 ± 0.2) as determined by CUNL. The results clearly showed the effectiveness of the curcumin nanoemulsion in killing microorganisms.

Keywords: Antimicrobial, Curcumin, Face Sanitation, Microbes, Nanoemulsion

INTRODUCTION

Self-soothing is a cognitive process that the brain activates to alleviate feelings of boredom and worry (Grimmer, 2022). Touching one's face satisfies the demand for self-soothing. However, this could result in self-inoculation, which is a type of contact transmission (Dreisoerner et al., 2021). In this process, an individual's contaminated hands come into contact with other areas of their own body, introducing unclean material to those sites (Mok, 2020). Contaminated hands touching the face has been identified as a potential means of spreading respiratory tract diseases (Przekwas, and Chen 2020). Health care workers (HCW) do not consistently adhere to hand hygiene protocols, either after coming into touch with an infected individual or their contaminated surroundings, resulting in self-inoculation (Alkan et al., 2021). Hence, increasing awareness that touching one's face can potentially lead to self-inoculation may prompt healthcare workers (HCWs) to recognise this message, thereby decreasing colonisation and the transfer of infections (Michael and Nguyen, 2022). Several hand sanitisers, such as Dettol, Lifebuoy, Savalon, Godrej Protekt, are widely advertised, however there is limited information about face sanitisers. Face sanitisers are a limited number of pharmacological medications that can provide protection against self-inoculation (Rivers et al., 2021). Facitize, a product manufactured by West coast Pharmaceuticals in Gujarat, India, is a marketed sanitiser that is 100% natural and organic. It contains a moisturiser and offers immediate protection against infections. Additionally, this product is devoid of alcohol and can be used safely by both males and females. The product consists of natural components such as tea tree oil, rosemary oil, turmeric oil, liquorice oil, and eucalyptus oil. Moreover, the absence of sulphates and parabens effectively prevents skin irritation. However, due to its gel-based nature (composed of cellulose, water, and alcohol), it has a limited length of effectiveness and can only provide protection for a maximum of 6 hours. As a result, it needs to be reapplied frequently. Face Guard X Sanitising Face Spray, manufactured by Adamson Analyticals USA, is another type of facial sanitiser that comes in a spray form. It consists of aloe vera gel, eucalyptus oil, and isopropyl alcohol. This spray was produced amidst the COVID outbreak in the year 2020. However, it has not yet received approval from the US Food and Drug Administration (FDA). Consequently, it is currently unavailable in the market. Furthermore, its period of effectiveness was limited to a maximum of 5 hours. The Dermaglo - Face Hand and Body Mist, developed by Dermaglove in Georgia, USA, is sold in Georgia as a head-to-toe sanitiser due to its approval by the USA FDA. However, the primary component of this product is ethyl alcohol, namely at

a concentration of 70%. It has the ability to cleanse, moisturise, and invigorate your entire body, but only for a limited period of time.

The world is currently experiencing the occurrence of contagious diseases, including H1N1 Swine Flu, Bird Flu, Ebola virus, Hantaviruses, and Covid 19 (Asif et al., 2020). Sanitisers are becoming an essential resource for prevention against communicable illnesses. Hand hygiene products are readily available in the market. These sanitisers have a warning to refrain from using them on the face. Research findings indicate that humans tend to touch their face frequently, averaging 23 times per hour (Zhang et al., 2020). This behaviour has consequences for the importance of maintaining proper hand sanitation. Currently, there is limited study conducted both in India and internationally on curcumin nanoemulsion face sanitiser. Nanoparticles provide increased stability and outstanding biocompatibility (Kang et al., 2020). Furthermore, these materials can be easily adjusted to enhance their antibacterial capabilities by surface area modifications. The primary objective of our work is to develop a new method for creating a nanoemulsion formulation that can offer long-lasting protection against infections. This formulation aims to address the drawbacks associated with alcohol-based formulations, such as their side effects. In addition, nanoemulsions that contain bioactive components have the ability to hinder the drug efflux mechanism of both gram positive ang gram negative bacteria.

Considering the above details, we have prepared, optimised and characterized curcumin nanoemulsion (CUNL) using GMS and Croduret 40, in our previous study. In the current research work we will be evaluating its in-vitro potential.

METHODOLOGY

Materials

Pune-based Bioprex Labs supplied the curcumin. Glyceryl monostearate (GMS), Croduret 40, Polyethylene glycol 400 (PEG 400), dimethyl sulfoxide (DMSO), triethanolamine (TEA), and potassium dihydrogen phosphate were purchased from Sigma-Aldrich. Hi-Media, India, supplied glycerine, propylene glycol, methylparaben, and propylparaben. Aayuraj Herbal (India) bought Sodium Polyacryloyldimethyl Taurate. New Delhi-based Shivchem Enterprises supplied analytical-grade methanol and acetonitrile. The Om Sterling Global University, Hisar Department of Biotechnology supplied deionised and double-distilled water.

Analytical equipments

The study used a magnetic stirrer (Macro Scientific Works, Delhi), cooling centrifuge (Remi C30 Plus, Mumbai), single punch IR hydraulic press KP, 795 (Kimaya Engineers, Thane, India), bath sonicator (Power sonic 410, Cyberlab), probe sonicator (Bandelin Sonoplus), and fourier transform infrared spectrophotometer.

Preparation, optimization, and characterization of Curcumin nanoemulsion using GMS and Croduret 40

In previous research paper we have made CUNL (Curcumin nanoemulsion) using ultrasonication. For variable optimisation, design expert software suggested 0.57 mg/ml GMS and 0.25% Croduret 40. 65.9% confined 187-nm nanoparticles. A stable CUNL with -25.4 mV zeta potential was produced. PDI was 0.55 (heterogeneous). The pH was found to be 6.03 and viscosity to be 10.87. CUNL released 40.2% and 75% curcumin after 3 and 24 hours. TEM revealed 87 nm nanoemulsion globules. The FTIR of CUNL matched pure curcumin. The primary functional group is the O-H bending at 1528.24 cm−1. Additional bands at 2965-2855 cm−1 occur from C-H bond elongation oscillation. CUNL spectrum bands are derived from functional groups O-C=O (2953.03 cm−1), O-H (2852.72 cm−1), and C-H (2922.16). Further research shows CUNL is a dependable, uniform nanoemulsion with non-Newtonian flow.

Test for Accelerated Stability of CUNL

In compliance with the standards of the International Conference on Harmonisation (ICH), accelerated stability studies were conducted on an optimised curcumin nanoemulsion (F3). Changes in the nanoemulsion's appearance, content, pH, sterility, phase separation, viscosity, particle size, polydispersity index, and zeta potential were monitored as part of the physical stability test (Melo et al., 2020). For a duration of 12 weeks, nanoemulsions were kept at room temperature (25 ± 2 ◦C) and were monitored at weeks 0, 4, 8, and 12.

Antioxidant assay -DPPH Radical Scavenging Activity

The DPPH assay was used to assess the antioxidant capacity of Curcumin, GMS, Croduret 40 and CUNL. DPPH, a compound consisting of 1,1-diphenyl-2-picrylhydrazyl, was dissolved in methanol at a concentration of 3.9 mg per 100 ml. The Curcumin, GMS, Croduret 40 and CUNL were mixed with DPPH and incubated in the dark for 30 minutes to measure the inhibition of DPPH (Santos et al., 2024). The absorbance was then measured at 517 nm using a UV spectrophotometer, and this process was repeated three times. The negative control in this study consisted of blank GMS and Croduret 40 NPs, while the positive control was pure

Curcumin. The % inhibition of DPPH by pure Curcumin and CULNs was determined using the following formula: (Wołosiak et al., 2021)

$$\% \text{ Radical scavenging activity} = \left(\frac{Ac - As)}{Ac}\right) \times 100$$

As- Absorbance of the sample, and Ac - Absorbance of the control

***in-vitro* Antimicrobial activity**

Evenly distributed the microorganism suspension onto the MHA (Mueller Hinton Agar) medium [Staphylococcus aureus (ATCC-6538), Streptococcus pyogenes (SF370), Staphylococcus epidermidis (ATCC 12228), Corynebacterium minutissimum (DSM 7174) and Cutibacterium acnes NCTC 737 [VPI 0389]]. Obtained a filter paper disc of 6 mm in diameter and deposited 10 µL of the nanoemulsion onto it. Next, carefully positioned the disc onto the agar plate. The nanoemulsion was allowed to disperse uniformly in the agar through natural diffusion for a period of 20 minutes at room temperature. The plate was placed in an incubator that was set to a temperature of 37°C for a period of 24 hours. Following the incubation time, the diameter of the zone of inhibition was measured in millimetres. The size of the zone of inhibition is used as a metric to determine the effectiveness of the nanoemulsion against the bacterium. A broader zone of inhibition indicated a greater degree of antibacterial efficacy. (Benkova et al., 2020)

Statistical analysis

The measurements will be performed thrice to determine the standard deviation (SD). The comparison of the means will be conducted using a one-way analysis of variance (ANOVA) and Duncan's test, with a significance level set at $p < 0.05$

RESULTS

Accelerated stability test

Accelerated stability tests evaluate the alterations in the quality of medication as time passes, which are affected by factors such as temperature, humidity, and light. Drug goods must undergo thorough physical and chemical analysis starting from the early stages of research and consistently throughout their planned storage duration. The results of the study, as shown in Table 1, suggest that there were no statistically significant changes noticed in any of the test parameters after 120 days.

During the period from month 1 to month 4, the CUNL experienced an increase in zeta potential, particle size, polydispersity index, and viscosity, while the pH fell (Coelho et al.,

2024). Increasing the temperature will enhance the kinetic energy of the dispersed phase, resulting in an expansion of the globule diameter. An increase in globule diameter leads to a commensurate increase in particle size, polydispersity index, and emulsion viscosity. Nevertheless, the statistical analysis revealed that there were no substantial changes ($P > 0.05$) in the particle size, polydispersity index, viscosity, and curcumin content seen over a period of four months. The remarkable stability can be attributed to the steric stabilising effect of the GMS (Subramanian et al., 2024). As a result, this event obstructs the flocculation and coalescence process.

Antioxidant activity

The DPPH technique is commonly employed to determine the antioxidant activity of encapsulated compounds. The 1,1-diphenyl-2-picrylhydrazyl is a chemically stable free radical with unpaired electrons spread out across the entire molecule, resulting in a deep violet colour. An absorption band is observed at a wavelength of around 517 nm. The violet colour vanished with the homogeneous mixing of a DPPH solution with a molecule possessing the ability to donate a hydrogen atom (oxidising property). Curcumin is a widely recognised chemical with antioxidant properties. The Curcumin solution was subjected to incubation with DPPH, a hydrogen atom donor, resulting in a noticeable colour change from violet to pale yellow. Therefore, the absorption band was diminished. The scavenging capacity of nano-encapsulated curcumin and free curcumin exhibited a dose-dependent relationship, ranging from 1 to 100ppm, as illustrated in the Figure below. It was discovered that encapsulation reduced its scavenging activity, particularly at low concentrations (< parts per million). Nevertheless, the scavenging activity of both samples was found to be similar when the concentration was between 18-23 ppm.

***in-vitro* Antimicrobial activity on face pathogens**

The overuse of antimicrobial drugs by people has led to the development of drug resistance in human illnesses, which presents a great challenge to researchers trying to create safe and effective treatments for infectious diseases (Aradhana et al., 2022). Numerous research groups have investigated the antibacterial and radical-scavenging properties of plant extracts of Curcumin, Thymus pulegioides, Stachys schtschegleevii, Azadirachta indica and many more in great detail, demonstrating their potent efficacy in eliminating radicals and fighting germs

without causing any negative side effects. Research has suggested that curcumin have antibacterial properties that can fight against kinds of bacterial infections.

Corynebacterium minutissimum can cause erythrasma, which is a common skin condition. Erythrasma presents as progressively enlarging patches of brown or pink, moderately scaly skin, commonly found in regions where the skin creases (Sun et al., 2020). The reference is from Fischer et al. (2017). Other common places for this condition include the interdigital spaces, the proximal medial thighs, the scrotum or vulva, the axillae, and the gluteal cleft (Murphy-Chutorian et al., 2013). At times, there can be no symptoms save for the existence of a rash, however in other instances, the affected areas may undergo itching. The effectiveness of the medication may take two to four weeks to become apparent. Usually, topical creams and solutions are first used, and if these initial treatments are not successful, oral antibiotics are next prescribed. Sometimes, a combination of oral and topical treatments is necessary. Currently, there is no existing data about the anti-microbial activities of Curcumin against Corynebacterium minutissimum. This is the inaugural form of research of its kind. Curcumin, when present at a concentration of 0.86 μg/mL in a myristic acid microemulsion, was able to block 50% of bacterial growth. This inhibitory effect was found to be 12 times more potent compared to curcumin dissolved in DMSO. The combination of myristic acid and curcumin in the microemulsion carrier shown a synergistic effect in reducing the growth of S. epidermidis.

Previous research have indicated that curcumin-loaded microemulsions have the potential to be used as a treatment for illnesses associated with S. epidermidis and acne vulgaris (Peralta et al., 2022)

In a laboratory setting, the study investigated the antibacterial effects of several fractions obtained from the rhizome of Curcuma longa on both standard strain and clinical isolates of Staphylococcus aureus. The clinical isolates displayed heightened sensitivity to different fractions in comparison to the reference strain of S. aureus. (ISAH, 2021)

Streptococcus pyogenes, a gram-positive bacterium that is pathogenic in the oral cavity, is responsible for causing severe human illnesses including rheumatic fever, sepsis, invasion of soft tissues, and a state similar to toxic shock. The objective of this study was to assess the inhibitory impact of Curcumin on the growth of Strept.pyogenes in comparison to Ciprofloxacin, using a well diffusion method (Akram et al., 2020). The study discovered that curcumin inhibited the growth of Strept. pyogenes with zones of 10.2mm, whereas Ciprofloxacin exhibited zones ranging from 15.52mm to 13.4mm. Curcumin's antibacterial

effects against Streptococcus pyogenes growth indicate its potential to reduce dental biofilms and prevent caries formation. ((Shoba, 2020)

Prior research has shown that the combination of probiotic LAB and Curcuma longa extract has a synergistic effect in fighting against C. acnes bacteria. This suggests that this synbiotic combination could be used in the development of cosmetics or pharmaceuticals that specifically target C. acnes (Szabo et al., 2022). The citation "Kim et al., 2020" refers to a study conducted by Kim and colleagues in the year 2020.

The CUNL demonstrated significant effectiveness against Staphylococcus aureus (ATCC-6538), Streptococcus pyogenes (SF370), Staphylococcus epidermidis (ATCC 12228), Corynebacterium minutissimum (DSM 7174), and Cutibacterium acnes NCTC 737 in the current research study. The greatest level of inhibition was seen for Cutibacterium acnes (17.2 ± 0.1), followed by Staphylococcus epidermidis (18.6 ± 0.2), Corynebacterium minutissimum (12.4 ± 0.1), Staphylococcus aureus (11.6 ± 0.2), and Streptococcus pyogenes (10.7 ± 0.2) as assessed by CUNL (Table 2). The results indicated that CUNL was highly effective in giving face cleansing (Chen et al., 2024).

CONCLUSION

Turmeric contains curcumin. Famous for its bright yellow colour, it has been extensively studied for its health benefits. Curcumin has significant anti-inflammatory and antioxidant properties that may help treat numerous diseases. Curcumin has benefits but low absorption. Thus, a curcumin nanoemulsion was synthesised and tested for antioxidant and antibacterial properties. Accelerated stability tests examined medicine quality over time in this study. From month 1 to month 4, CUNL (curcumin nanoemulsion) zeta potential, particle size, polydispersity index, and viscosity increased while pH declined. The study found that encapsulation greatly reduced scavenging activity, particularly at low dosages (< parts per million). However, both samples had equivalent scavenging activity at 18–23 ppm. Additionally, CUNL was highly effective against Staphylococcus aureus (ATCC-6538), Streptococcus pyogenes (SF370), S. epidermidis (ATCC 12228), C. minutissimum (DSM 7174), and C. acnes NCTC 737. CUNL showed that Cutibacterium acnes had the maximum inhibition (17.2 ± 0.1), followed by Staphylococcus epidermidis (18.6 ± 0.2), Corynebacterium minutissimum (12.4 ± 0.1), Staphylococcus aureus (11.6 ± 0.2), and Streptococcus pyogenes

(10.7 ± 0.2). The results proved curcumin nanoemulsion kills bacteria that cause face infections. Hence, this nanoformulations could serve as beneficial face sanitizer in future.

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Table 1: Various parameters studied for CUNL for a period of four months

Time (months)	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)	Viscosity	pH	Curcumin content
0	187±71.66	0.55± 0.01	-25.4±3.22	10.87± 0.12	6.03±1.87	98.13± 0.19
1	190± 20.12	0.57± 0.05	-25.0±3.02	10.98± 0.02	6.00±0.05	98.02± 0.11
2	191± 10.55	0.59± 0.002	-24.1±2.04	11.04± 0.003	5.98±0.32	98.00± 0.20
3	192± 20.12	0.60± 0.021	-21.87±0.022	11.99± 0.14	5.63±1.12	97.43± 0.16
4	195± 53.02	0.63± 0.003	-.20.11±1.22	12.51± 0.02	5.53±0.21	97.03± 0.08

Table 2: Growth inhibition zone diameter (mm) of Curcumin, CUNL and Ampicillin

	Inhibition Zone (mm)		
Tested Microbe	**Curcumin (300 µg/ml)**	**CUNL (10µg)**	**Ampicillin (10µg)**
Staphylococcus aureus (ATCC-6538)	7.8 ± 0.4	11.6 ± 0.2	19.2 ± 0.1
Streptococcus pyogenes (SF370)	7.5 ± 0.1	10.7 ± 0.2	20.5.2 ± 0.1
Staphylococcus epidermidis (ATCC 12228)	9.4 ± 0.2	15.6 ± 0.4	23.1 ± 0.1
Corynebacterium minutissimum (DSM 7174)	9.0 ± 0.4	12.4 ± 0.1	19.9 ± 0.1
Cutibacterium acnes NCTC 737	9.2 ± 0.1	17.2 ± 0.1	22.3 ± 0.1

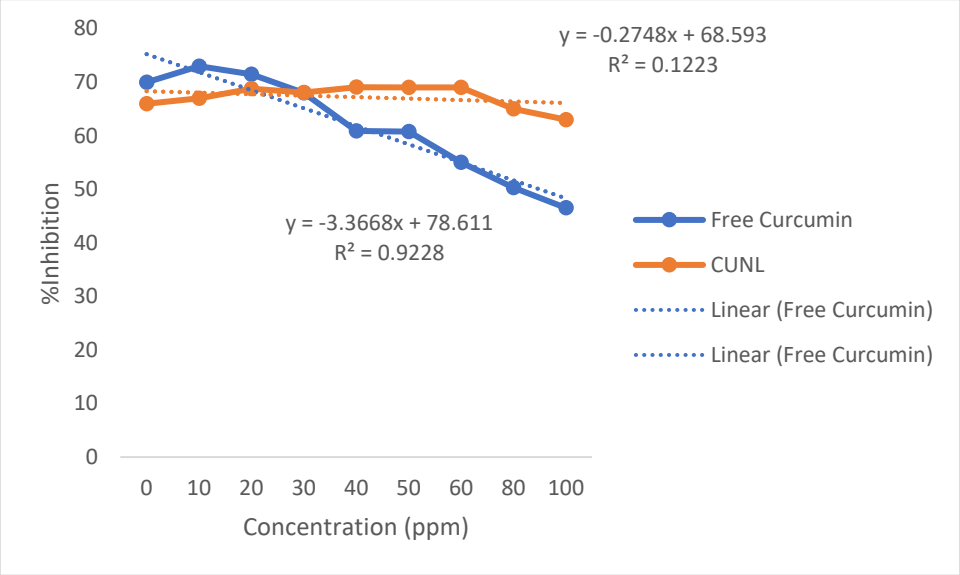

Figure 1: *in-vitro* antioxidant activity exhibited by curcumin and CUNL

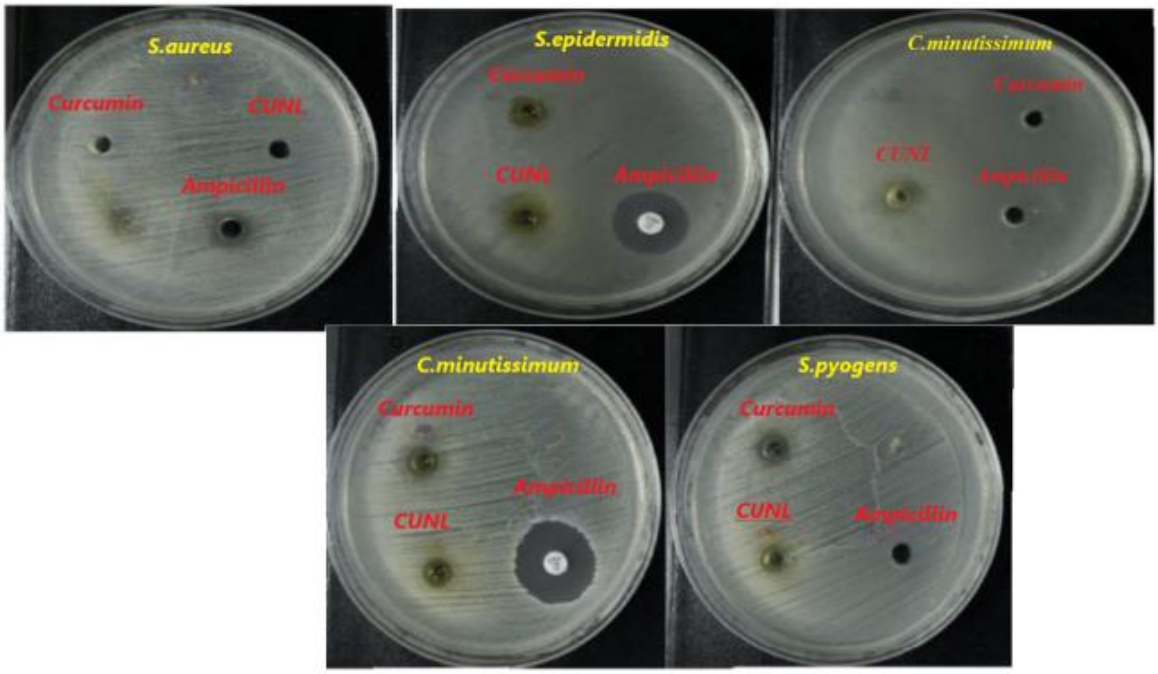

Figure 2: *in-vitro* antimicrobial activity exhibited by curcumin, CUNL, and ampicillin on various face pathogens