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Research Article

Design and Evaluation of SMEDDS for Combined Oral Delivery of Curcumin and Thymoquinone and Its Anti-Microbial Assessments

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ABSTRACT

Curcumin and thymoquinone are well-known phytochemicals. However, these natural bio-actives poor water solubility and restricted penetration impede their effective delivery and potential therapeutic result. This study was carried out to design and characterize a novel solid self-micro emulsifying drug delivery system of class IV drugs by adsorption approach employing Syloid®244 FP as the solid carrier for solubility increase of Curcumin and Thymoquinone. The optimized liquid SMEDDS for Curcumin and Thymoquinone contains 30:15:45 oil Oleic acid, surfactant Tween20, and co-surfactant PEG 400. It demonstrated good self-emulsification performance and a clear appearance. Characterization investigations revealed that solidification using 50% (w/w) Syloid®244 FP in the liquid formulation results in a free-flowing powder with no agglomeration. The liquid and solid SMEDDS formulations created a fine oil-in-water microemulsion with mean globule sizes of 153±7.4 nm respectively. As a phosphate buffer pH 6.8 dissolution environment, the drug synthesized as solid SMEDDS was swiftly and thoroughly dissolved (90%) within 120 minutes, but crude curcumin powder was much less soluble. The Curcumin-Thymoquinone loaded SMEDDS demonstrated strong antibacterial activity against *Staphylococcus aureus*, antifungal activity against *Candida albicans*, and moderate antibacterial activity against *Escherichia coli*in antimicrobial assays. Curcumin-Thymoquinone loaded-SMEDDS formulation in liquid & solid dosage forms were successfully developed with an increased drug loading and dissolution rate, which could be the potential combined delivery system for various anti-bacterial and anti-fungal treatments.

INTRODUCTION

Curcumin (Curcuma longa) is a yellow substance found in the ginger family's turmeric spice (*Zingiberaceae*). Curcumin (1,7-Bis(4-hydroxy-3-methoxy phenyl)-1-6- heptadiene-3,5-dione), a low molecular weight hydrophobic polyphenol that is functionalized with hydroxyl and methoxyl groups, can exist in a variety of tautomeric forms. Turmeric is used to treat bacterial infections and keep people's blood sugar levels in a healthy range. Curcumin also exhibits potent *in vitro* antiproliferative properties against a variety of cancers. It also enhances the anticancer properties of some popular chemotherapy drugs, including doxorubicin, cisplatin, and paclitaxel.

Several studies have shown that Curcumin has antimicrobial activity against many foodborne pathogens and spoilage microbes, including *Escherichia coli, Yersinia enterocolitica, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Aspergillus niger, Penicillium notatum*, and *Saccharomyces cerevisiae*. The Oxford cup method was used to show that it had a broad-spectrum inhibitory effect on all species tested. The proinflammatory cytokine interleukin-1ß, which is elevated in the brains of APPsw transgenic mice (the model animals for Alzheimer's disease), as well as oxidized proteins and other inflammatory indicators, were also drastically decreased by Curcumin, providing evidence that it may prevent Alzheimer's disease [1-3].

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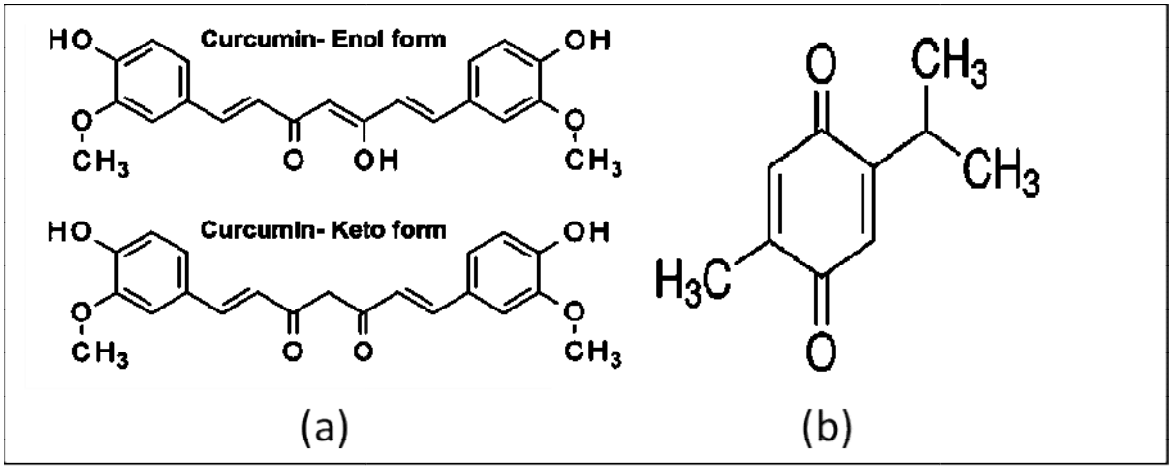

Figure 1: Chemical Structure of (a) Curcumin in enol and keto form and (b) Thymoquinone

Table 1: Saturation solubility profile of Thymoquinone and Curcumin in different excipients (oils, surfactants, and co-surfactants)

Vehicles		Solubility of Curcumin (mg/ml)	Solubility of Thymoquinone (mg/ml)
Oils	Oleic acid	26.12 ± 0.39	320.29 ± 0.40
	Castor oil	4.65 ± 0.40	20.23 ± 0.78
	Soyabean oil	8.45 ± 0.22	16.55 ± 0.49
	Corn oil	11.55 ± 0.19	25.86 ± 0.37
Surfactant	Tween 20	25 ± 0.44	109.43 ± 0.18
	Tween 80	13.017 ± 0.29	89.70 ± 0.28
	Span 20	6.559 ± 0.19	24.66 ± 0.45
	Span 80	13.981 ± 0.23	29.58 ± 0.43
Co-surfactant	PEG 400	110.52 ± 0.54	90.14 ± 0.19
	Ethyl Alcohol	1.779 ± 0.35	9.60 ± 0.14
	Isopropyl Myristate	0.956 ± 0.21	6.6 ± 0.28

Thymoquinone is a phytochemical that can be found in the plant *Nigella sativa* Linn (*Ranunculaceae*). It is an herbaceous plant that has been used to treat a variety of ailments for more than 2000 years in indigenous systems of traditional medicine. Thymoquinone (2-isopropyl-5-methylbenzo-1,4-quinone), the main phytochemical in black seed oil, has been shown to prevent heart, liver, and kidney damage in animal studies. It also has potential anticancer activities. The plant species *Nigella sativa* has a wide range of pharmacological effects that have been scientifically proven, including hypotensive, uricosuric, choleretic, anti-nociceptive, antidiabetic, anti-histaminic, anti-oxidant, anti-inflammatory, antibacterial, anti-tumor, and immunomodulatory activity. Additionally, it has been shown in animal models to have analgesic, anticonvulsant, antiangiogenic, and antiepileptic effects. Thymoquinone has several modes of action that support its anticancer effects, including those that are antiproliferative, induce apoptosis, stop the cell cycle, produce reactive oxygen species, and inhibit metastasis and angiogenesis. The anticancer properties of Thymoquinone have also been investigated in cancer xenograft mouse models for lung, pancreatic, prostate, and colon cancers [2-4].

Curcumin and thymoquinone combination treatment may enhance therapeutic effects while lowering toxicity. The current experiment will show that the combination of curcumin and thymoquinone is more potent at treating patients than either medicine alone, at low achievable doses along with improved therapeutic characteristics. The model drug Curcumin and Thymoquinone's concern for stability in the dosage form represented the biggest threat to formulation development. It was found that these compounds are unstable in liquid lipid systems, have poor solubility in water, and are sensitive to sunlight, which made them difficult to develop for clinical use. Poorly

water-soluble drugs can increase their oral bioavailability using various varieties of methods. Because it offers a high degree of patient compliance, the oral route has been the primary method of drug delivery for the chronic treatment of many disorders. The high lipophilicity of the drugs itself, however, makes oral administration of 50% of the drug molecules difficult. Nearly 40% of new drug candidates, including curcumin and thymoquinone, have limited solubility in water, which makes it difficult to establish the ideal oral solid dosage form in terms of formulation design and bioavailability of new pharmaceutical products. Numerous methods have been employed to alleviate these issues; including changing the solubility of the drugs or keeping it dissolved during the gastrointestinal transit time. Surfactants, micronization, salt creation, pH changes, nano-size delivery, solid dispersions, and permeation enhancers are a few examples of these techniques. Lipid solutions, emulsions, and emulsion pre-concentrates have received much attention since they can be created as physically stable formulations suited for encapsulating of attention since they can be created as physically stable formulations suited for encapsulating such poorly soluble medicines. Emulsion systems have unique complications, such as manufacturing issues and stability issues related to their commercial production. One formulation method that may provide a suitable solution to such issues is self-micro emulsification systems [5].

Due to their advantages over other systems, lipid-based micro drug delivery systems are an appealing strategy. Self-micro emulsifying drug delivery systems (SMEDDS) are an appealing method to increase the solubility and oral bioavailability of poorly soluble drugs among the different lipid-based drug delivery systems. Due to its strong solubilization capacity and small droplet size, SMEDDS, an isotropic mixture of oil and surfactants, creates an emulsion with slight agitation in the gastrointestinal fluid and is an anticipated technique for improving the water solubility of poorly water-soluble pharmaceuticals. An isotropic mixture of synthetic or natural oil, a solid or liquid surfactant, a co-surfactant, and co-solvents is referred to as SMEDDS. SMEDDS, on the other hand, refers to one or more hydrophilic solvents and co-solvents/surfactants that, with gentle agitation followed by dilution in aqueous media, such as gastrointestinal fluid, have the extraordinary ability to create fine oil-in-water (O/W) microemulsion. SMEDDS is a thermodynamically stable and optically transparent system. The size of the droplet is less than 100 nm. It has been demonstrated that this system's smaller droplet size improves medication absorption. These SMEDDS characteristics enable improved gastrointestinal membrane permeability and bioavailability of manufactured pharmaceuticals [6]. The ability of SMEDDS to promote transcellular absorption by increasing membrane fluidity, permitting paracellular transport by opening tight junctions, avoiding the hepatic first-pass effect, lowering cytochrome P-450 metabolism, and safeguarding the drug from enzymatic degradation may be the reason for the enhancement of drug absorption [7, 8].

The most recent conversion process, which allows for the development of liquid lipid formulation into solid dosage form, is one of the most crucial features of lipid-based SMEDDS.

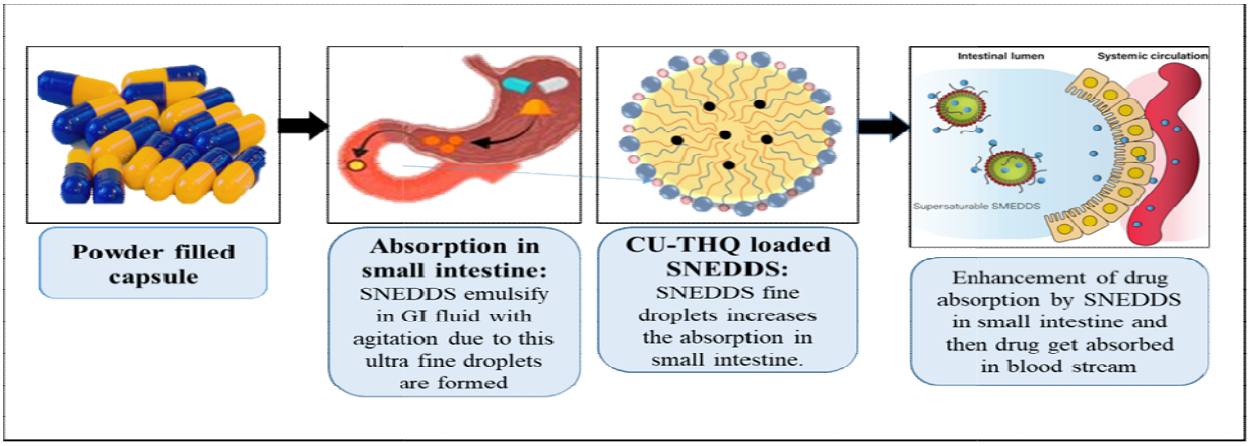

Figure 2: Mechanism of action of self-nano emulsifying drug delivery system on oral administration of the drug

The conversion of liquid lipid formulations using an adsorbent method, solid lipid nanoparticles technique, and fluid bed coating techniques is gaining greater attention for this methodology. The adsorbent approach for solidification is the quickest to design, easiest to use, and most affordable of all solidification procedures. Silica-based compounds, which have a high capacity to adsorb lipids (oils) and generate a free-flowing powder, are the main types of materials that are suitable for adsorption on surfaces. This free-flowing powder can either be enclosed in hard gelatin capsules or compacted straight into tablets using a one-step procedure [2].

The adsorption approach was taken into account in this research to improve the solubility and bioavailability of Curcumin and Thymoquinone dosage forms while maintaining cheap manufacturing costs ease of process control, high stability and repeatability, and higher patient compliance. To increase stability, reduce the impact of *in vitro* dissolution time, and transport the drug in solubilized form (avoiding precipitation) before entering the systemic circulation for absorption, the current studies sought to load the maximum amount of Curcumin and Thymoquinone into the most appropriate liquid SMEDDS, which was later converted into a solid dosage form.

MATERIALS AND METHODS

Materials

Curcumin (CURCUMIN, 99.5% pure) and Thymoquinone (purity >99.8%) were obtained from Yucca Enterprises, Mumbai, India. Oleic acid, ethyl oleate, castor oil, tween 80, tween 20, tween 40, PEG 200, PEG 400, and PEG 800 were purchased from Research-Lab Fine Chem Industries, Mumbai, India. Vivapur MCC sphere 100, Emcocel 50M (JRS Pharma, Germany), Syloid®244FP (Grace, Germany), HPLC grade solvents (Merk Pharmaceuticals), and 0.45µ membrane filter, Curcumin and Thymoquinone capsules (label claim 10.5 mg and 8 mg) were used for analysis.

Solubility Studies

The solubility of drug molecules in various components is one of the most crucial factors to consider when choosing the right components for the development of SMEDDS. The solubility of Curcumin and Thymoquinone in various excipients/components, such as oils, surfactants, and co-surfactants, was therefore determined by adding an excess amount of each drug to 1 mL of each component in a stoppered glass. To achieve equilibrium, the glass vials were vortexed for 5 min and then transferred to biological shaking for continuous shaking at 37±1°C for 48 h. To get rid of the undissolved medicines, the mixtures were centrifuged at 6000 rpm for 10 minutes. Various vehicles' supernatants were divided into aliquots, which were then properly diluted with methanol. These dilutions were then assayed analytically by UV spectrophotometry (UV-1800 Shimadzu, SDDVCOP Research Centre, Mumbai, India) at λmax 425 nm and 253 nm for Curcumin and Thymoquinone using the method described and validated by Sharma et al [9-11].

Construction of Pseudo-Ternary Phase Diagram

Pseudo-ternary phase diagrams are used to pinpoint the location of the microemulsion. It is easy to find out how various surfactant/co-surfactant weight ratios influence the size of an observed stable microemulsion area. To identify the "Region of Microemulsion," the phase diagram was created using the "Aqueous Titration Method." To create a microemulsion, different amounts of water, chosen oils, surfactants, and co-surfactants were combined.

Based on the solubility data of Curcumin and Thymoquinone in different components, Oleic acid, Tween 20, and PEG 400 were selected as oil, surfactant, and co-surfactant for the development of SMEDDS. However, de-ionized water was selected as the aqueous phase due to its frequent use in SMEDDS/microemulsion preparation. The weight ratios of surfactant to the co-surfactant combination (Smix) were taken as 1:1, 1:2, 1:3, 2:1, and 3:1. In a water titration, mixtures of the oil phase with the surfactant and co-surfactant were formed into several vials in the following ratios, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. The vials were filled with a modest amount of filtered water in 0.1 mL increments. The mixture in the vials was vortexed for 2–3 minutes after each addition and then left to equilibrate for 5 minutes. The mixes were visually inspected for phase separation and transparency upon equilibration. The endpoint of the titration was defined as the point at which the mixture turned turbid or displayed evidence of phase separation. This was left out overnight to see any turbidity changes. Using the free CHEMIX School 10.00 demo edition software, it was determined how much-purified water was needed to turn a clear microemulsion turbid, and the percentages of the oil phase, Smix, and

aqueous phase were displayed. The steady and transparent emulsion zone was located [12, 13].

Formulation of L-SMEDDS

Based on the idea that the concentration of the oil phase should be such that it can dissolve 150 mg of Curcumin and 70 mg of Thymoquinone for 10ml of SMEDDS readily, various formulations were chosen from the SMEDDS zones of each phase diagram. Different amounts of oleic acid that solubilized 150 mg of Curcumin and 70 mg of Thymoquinone were carefully selected from the generated pseudo-ternary phase diagrams to construct several SMEDDSs. The aqueous titration method was used to create appropriate SMEDDS. The pseudo-ternary phase diagrams were used to determine the composition of SMEDDS. Curcumin-Thymoquinone was dissolved in Oleic acid and sonicated for 20-30 minutes. Water and Smix (surfactant and co-surfactant) were also prepared, and they were stirred together using a magnetic stirrer until well mixed. Add the oil phase to a Smix and water mixture, and agitate it for at least 20 to 30 minutes at 30 to 40°C to create a clear, homogeneous emulsion [13].

Evaluation of L-SMEDDS

Thermodynamic Stability Studies

The selected formulation was subjected to a thermodynamic stability study to access its physical stability.

- Centrifugation Tests: The selected nanoemulsion were subjected to centrifugation at 5500rpm for 30 minutes and checked for any separation. The formulation that did not show any phase separation was taken for the heating and cooling cycle.
- Heating and Cooling Cycle Test: Six cycles were studied, between refrigerator temperature (5°C+3°C) and 45°C for 48 hours each, and formulations were examined for stability at these temperatures. The formulation which was found stable was subjected to a freeze-thaw cycle test.
- Freeze-Thaw Cycle Test: Formulation was kept in a chest freezer at -20°C for 24 hours. After 24 hours the formulation was removed and kept at room temperature. The physically stable nanoemulsion returned to its original form within 2-3 minutes and was selected. Such three cycles were repeated and observed for any phase separation.

Appearance and Dilutability

Lipid-based compositions' self-emulsifying effectiveness can be evaluated just visually. Against a black background, the microemulsion's appearance, including transparency, phase separation, and clarity, was assessed visually. A prepared, optimized batch of the microemulsion was visually examined for phase separation and clarity after being diluted 1:100 with distilled water [3].

Analysis of Globule Size

The transmission electron microscope (TEM) was used to study microemulsions. Studies using TEM were done to figure out how the interior oil droplets were shaped. A drop of the improved SMEDDS was put into a copper grid after being appropriately diluted with clean water and given 30 seconds to dry. A drop of phosphotungstic acid was then added to the grid for 10 seconds while it was still upside down. The excess phosphotungstic acid was removed by absorbing on filter paper, and the grid was examined using the point-to-point resolution TEM technique at 100 kV.

Zeta Potential and PDI

The optimized microemulsion polydispersity index and zeta potential were calculated using a Dynamic Light Scattering (DLS) method.

Dispersibility Test

The stability of SMEDDS and the characteristics of gastric fluid must both be described in terms of emulsification time. The self-emulsification capabilities of the preparations were assessed visually. After a light shake, prepared SMEDDS spontaneously distributed in an aqueous medium. The emulsification time of the drug-loaded L-SMEDDS formulations was evaluated by USP dissolving apparatus II. The sample was prepared by diluting 1 mL of L-SMEDDS 1:100 with 100 mL of double-distilled water, 0.1N HCl, and 6.8 pH phosphate buffer in separate glass beakers. The mixers were gently stirred for 1 minute at a temperature of 37°C. The microemulsion production time for each sample was immediately seen and recorded.

SMEDDS emulsions are divided into grades A, B, C, D, and E based on their ability to produce an emulsion in a specific amount of time and the presence of microemulsion.

Grade A: Rapidly forming (within 1 min) microemulsion, having a clear or bluish appearance.

Grade B: Rapidly forming, slightly less clear emulsion, having a bluish-white appearance.

Grade C: Fine milky emulsion that is formed within 2 min.

Grade D: Dull, greyish-white emulsion having a slightly oily appearance that is slow to emulsify (longer than 2 min).

Grade E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface [14].

Solidification of L-SMEDDS

Curcumin and Thymoquinone S-SMEDDS were produced using the adsorption method. Using the adsorption method, an improved L-SMEDDS preparation was converted into S-SMEDDS. It was hypothesized that one approach to producing free-flowing powders from liquid-SMEDDS mixtures would be adsorption onto solid carriers. Higher surface area, good physical adsorption, high dissolving efficiency, good homogeneity, and reproducibility are all advantages of adsorption processes. Solid carriers included Aerosil 200, Vivapur MCC spherical 100, Emcocel 50M, and cross-linked polymethyl methacrylates like Syloid244 FP.

The necessary amount of adsorbent and Curcumin-Thymoquinone loaded L-SMEDDS were added to a glass mortar drop wise, and the combination was well mixed to produce a homogenous solid powder. A tiny amount of solid S-SMEDDS was placed in a 25°C desiccator for additional characterizations before being mixed with the manufactured S-SMEDDS (in powder form) and put directly into size "00" firm gelatin capsules [2].

Evaluation of Solid SMEDDS

Bulk Volume (Vb): Accurately weighed 5gm of S-SMEDDS was placed in a flat ground measuring cylinder with a volume of 50mL. Unsettled apparent volume was read to the nearest millilitre.

Tapped Volume (Vt): After measuring bulk volume 100 taps were given to the cylinder and the apparent volume was read to the nearest millilitre.

Bulk Density: Apparent Density before setting;

$$\varrho = \frac{M}{Vb}$$

Tapped Density: Apparent Density after setting;

$$\varrho = \frac{M}{Vt}$$

Flowability: A standard funnel was used to measure the powder's flow. The test sample was added without compacting into a dry funnel with an appropriately blocked bottom aperture. The time required for the complete sample to flow out of the funnel was measured after clearing the funnel's bottom entrance.

$$Hausner's\ ratio = \frac{(Tapped\ Density)}{(Bulk\ Density)}$$

Compressibility Index: The compressibility index was determined according to Carr's index;

$$\text{Carr's index} = \frac{(Tapped\ density - Bulk\ density)}{(Tapped\ density)} \times 100$$

The Angle of Repose: The angle of repose was determined by measuring the height of the cone of powder and the radius of the base. The end of a funnel was placed 2 cm above the flat base. The funnel was filled with the powder (up to 5gm), therefore after releasing the powder out of the funnel, the top of the resulting cone reached the end of the funnel[1].

$$\theta = tan^{-1}\frac{height}{radius}$$

***In-Vitro* Dissolution Study**

In this study, we conducted an *in-vitro* dissolving test on pharmaceuticals loaded with L-SMEDDS, S-SMEDDS, and pure drugs (Curcumin and Thymoquinone) using a USP II dissolve - test apparatus (Paddle type).

According to reports, the drugs take around two hours to pass through the stomach (where the pH is quite acidic) before traveling to the intestine, where the pH quickly rises. To determine how pH varies from the stomach to the intestine, we did *in-vitro* research in several media, by transferring the formulation to an intestinal phosphate buffer with a pH of 6.8. The paddle speed was maintained at 100 RPM, while the bath apparatus's temperature was maintained between 36.5°C and 37.5°C. At regular intervals of 5, 10, 15, 30, 45, 60, 90, and 120 minutes, 5 mL samples were taken. To

maintain equilibrium, 5 mL of fresh sample was added to the medium volume. Withdrawn samples were filtered, diluted, and then tested with a UV spectrophotometer for the presence of Curcumin and Thymoquinone at wavelengths of 425 and 253 nm [6].

In-Vitro Antimicrobial Activity of Curcumin and Thymoquinone

The standard Curcumin and Thymoquinone were studied for antimicrobial activity using *Escherichia coli* (ATCC No. 25922) as a gram +Ve bacteria, *Staphylococcus aureus (*ATCC No. 6538) as a gram -ve bacteria and *Candida albicans* (ATCC No.10231) as a fungus. Potato Dextrose Agar is used as a medium for fungal growth, whereas nutrient agar is used as a medium for bacterial growth. Using DMSO (70%) as a solvent, the chosen formulation was tested using various ratios of Curcumin and Thymoquinone concentrations. To assess antibacterial activity, zone of inhibitions tests using the agar well diffusion method or cup plate method was performed for various concentration ratios [3].

RESULTS

Solubility Study

It is preferred to solubilize lipophilic drugs like Curcumin and Thymoquinone in o/w microemulsion. Greater solubility in the oil phase aids in reducing the formulation's volume to deliver the drug's therapeutic dose in an encapsulated form[6]. Based on the above study, it was concluded that the solubility in the oils, surfactants, and co-surfactants like Span 40, Span 80, Tween 20, Tween 80, Oleic acid, Castor Oil, Soyabean oil, Corn oil, PEG 200, PEG 400 was found to be soluble. The chosen oils' various physicochemical characteristics were examined and determined to be advantageous for the microemulsion drug delivery systems. Oleic acid was chosen as the oil phase because it had the maximum solubility (mg/mL) of thymoquinone and curcumin among the different experimental oils. This is because oleic acid has been shown in prior studies to have a permeation-enhancing property. Curcumin and Thymoquinone had the highest solubility in PEG 400, according to the solubility results; hence it was selected as a co-surfactant. Likely, Curcumin's capacity to create a hydrogen bond with the polyethylene oxide (PEO) groups accounts for its remarkable solubility in PEG 400. Tween 20 and Tween 80 outperformed the others in terms of the solubility of Curcumin and Thymoquinone. Both of these ingredients which are commonly used in pharmaceutical preparations are non-ionic and GRAS (generally recognized as safe) excipients. Tween 20 is the most hydrophilic surfactant among the Tweens and it showed increased solubilization capacity [11].

Construction of Pseudo Ternary Phase Diagram

The construction of the pseudo-ternary phase served to both locate the microemulsion zone and maximize the concentration of the chosen vehicles, such as oleic acid, Tween 20, and PEG 400. The area of the monophasic region for the SMEDDS formulation was provided by optimized excipient concentration ratios determined by phase diagram experiments. To ensure proper aqueous dilution without damaging the microemulsion, this area must be identified. The phase diagrams for five separate oil-surfactant-water systems are shown in Fig. 3(A to E).

Different regions of transparent, microemulsions, and coarse emulsions might be found in these phase diagrams. The mixture of Tween 20: PEG 400 (Smix) (1:3 w/w), Oleic acid, and distilled water with varied Km (such as 1>2>3) made up the pseudo-ternary phase diagrams of O/W microemulsion. However, it was also shown that raising the PEG 400 concentration facilitated fast emulsification in addition to helping to reduce viscosity. Therefore, several selected compositions among all three pseudo-ternary graphs were tested for thermodynamics, dispersibility, and drug loading to take advantage of the combined benefits of speedy emulsification, low viscosity, and high drug loading of Curcumin and Thymoquinone.

Development of L-SMEDDS

Oils were a crucial component of the system to solubilize significant amounts of Curcumin and Thymoquinone; it may also facilitate transport via the intestinal lymphatic system (depending on lipophilicity) and so increase the absorption of drugs from the gastrointestinal tract. Long and medium-chain triglyceride oils either from natural sources or minimally modified with varied degrees of saturation have been widely used in the development of SMEDDS systems. The surfactant is a crucial excipient for stabilizing SMEDDS and allowing for the uptake of large amounts of medicinal molecules due to increased solvent capacity. Table 2 shows the excipients utilized to design SMEDDS in this study and how they were mixed to represent

distinct formulation systems. Table 2 demonstrates that thirteen formulations with varying amounts of oils, surfactants, and/or co-solvents were created. The first three formulations (F1-F3) each included 5% Oleic acid, while formulations F4-F5 contained 15% Oleic acid. The F3 and F11 formulations had a high proportion of surfactant and co-solvent (50-60%), resulting in a clean and transparent microemulsion system; it was also shown that raising the PEG 400 concentration facilitated fast emulsification in addition to helping to reduce viscosity.

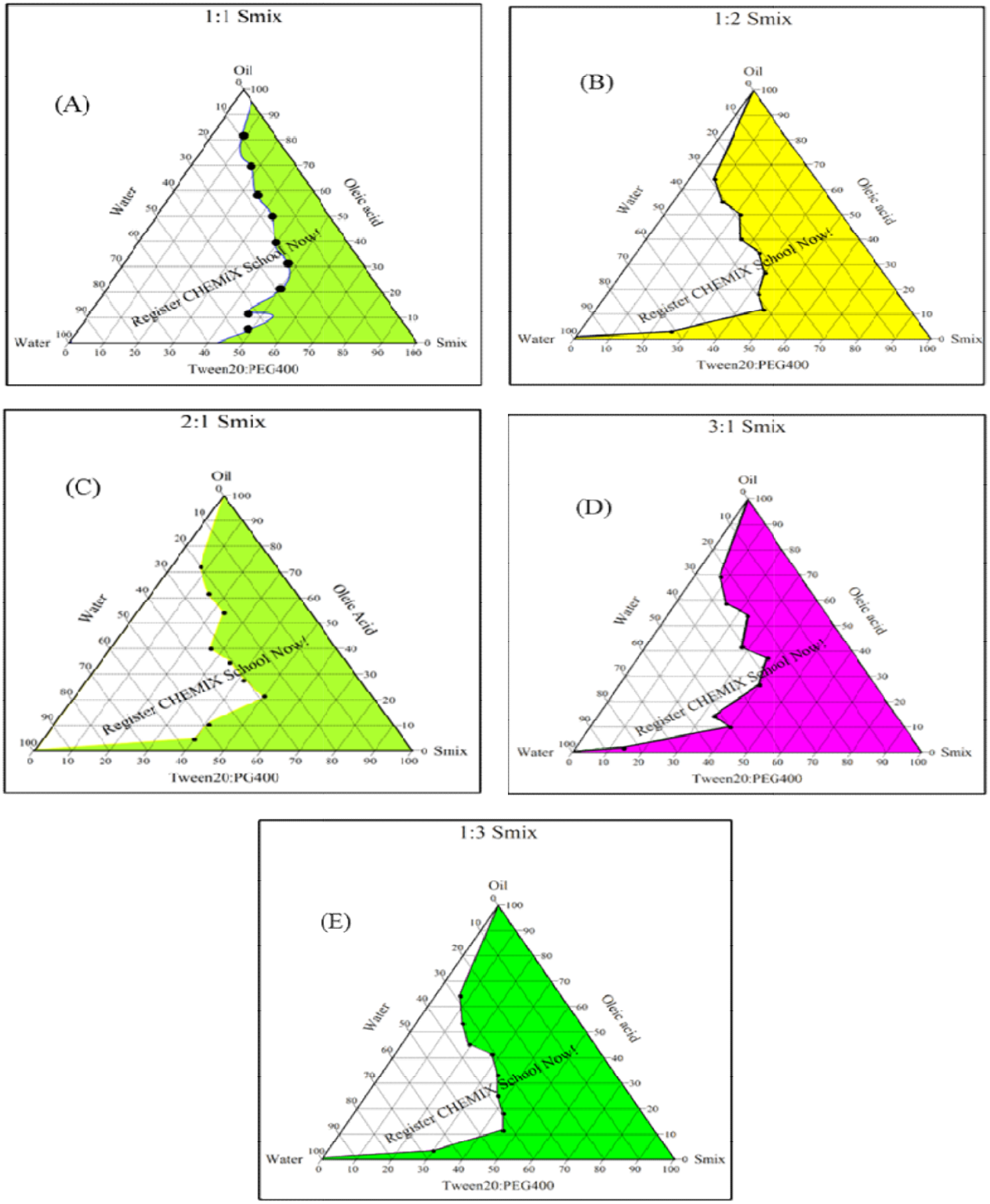

Figure 3: Pseudo-Ternary Phase Diagram (3A-3E)

Evaluation of L-SMEDDS

Appearance and Dilutability

Visual assessment was used in the study to help determine the self-emulsification properties of the formulation labelled SMEDDS. The following factors for visual assessments were taken into account during the optimization of SMEDDS: miscibility of the oil/surfactant mixture, homogeneity, and appearance upon aqueous dilution (ratio maintained, formulation: water 1:100). Table 3 displayed the efficiency assessment results, which revealed that two formulations, primarily F3-F11, were transparent following aqueous dilution. As a result of their ''transparent" looks, these formulations were classified as SMEDDS and subjected to additional experimental testing.

Table 2: Composition of selected microemulsion formulation with Observations after 24 hours.

Batch No.	Oil (Oleic acid)	Smix (Tween20: PEG400)	Water	Observation after 24 hrs.
F1	5	30	65	Hazy
F2	5	40	55	Hazy
F3	5	50	45	Clear
F4	15	45	40	Phase Separation
F5	15	55	30	Phase separation
F6	25	40	35	Hazy
F7	25	45	30	Hazy
F8	25	50	25	Hazy
F9	30	40	30	Almost Clear
F10	30	50	20	Almost Clear
F11	30	60	10	Clear
F12	35	40	25	Phase Separation
F13	45	20	35	Phase Separation

Table 3: Observations of Optimised Formulations for Appearance, Dilutability, and Thermodynamic stability screening

Batch No.	Oil (Oleic acid)	Tween 20	PEG 400	Water	Appearance	Thermodynamic Stability Screening		
						Centrifugation	Heating Cooling Cycle	Freeze-Thaw Cycle
F3	5	12.5	37.5	45	Clear	Clear and Stable	Opaque and Stable	Phase Separation
F11	30	15	45	10	Clear	Clear and Stable	Clear and Stable	Clear and Stable

Thermodynamic Stability Studies

The chosen formulations were subjected to a variety of thermodynamic stability experiments, including heating and cooling, centrifugation, and the freeze-thaw cycle. Some formulations were turbid during physical stability testing, and some formulations experienced phase separation, as shown in Table 3.

Particles Size

The emulsion droplet size is an important aspect of self-emulsification performance since it influences the rate and degree of drug release as well as absorption. Transmission electron microscopy (TEM) was used to assess the mean globule size of microemulsions. TEM was used to determine the morphology of microemulsions. Fig. 4 revealed that the oral formulation is spherical, and the particle size obtained was the 100 nm.

Zeta Potential and Polydispersity Index (PDI)

The physical stability of microemulsions is governed by the PDI, which should be as low as feasible for the long-term stability of microemulsions. A PDI of 0.1-0.25 implies a very narrow size range, whereas a PDI larger than 0.5 indicates a very broad distribution. F3 has a polydispersity index (PID) of 0.191 and F11 has a PID of 0.137. As a result, a highly homogeneous Curcumin and Thymoquinone microemulsion with a limited particle size distribution was created.

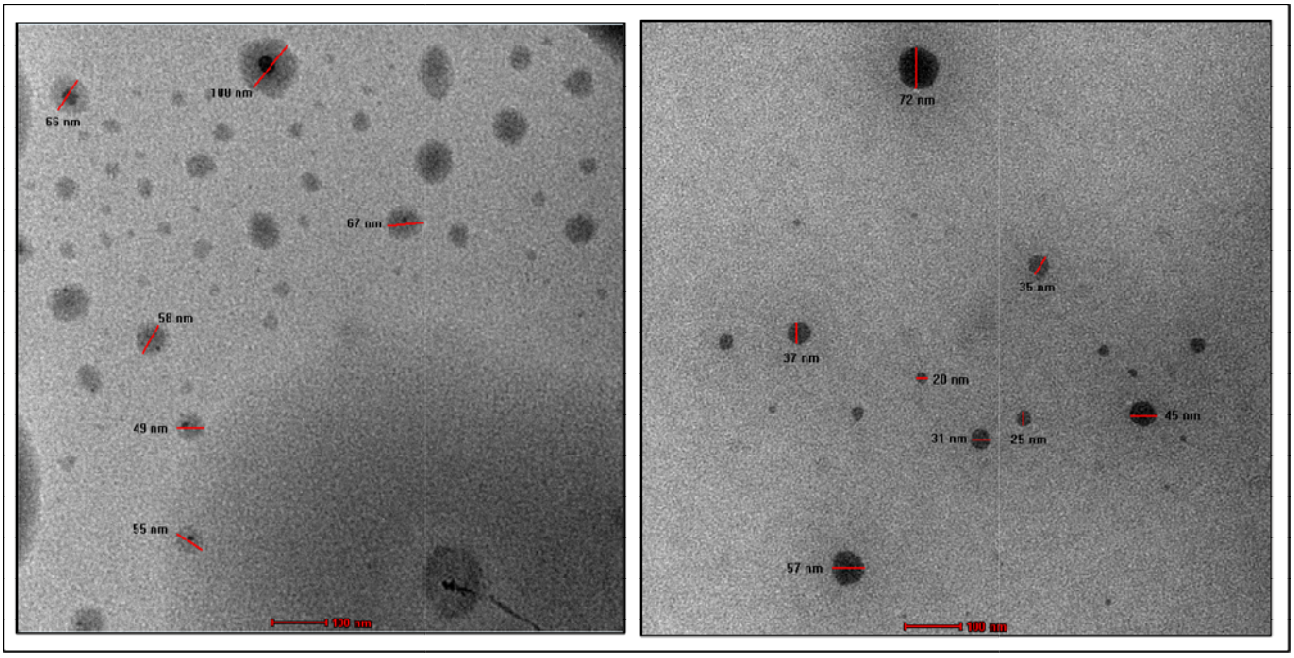

Figure 4: TEM micrographs of Curcumin and Thymoquinone loaded SMEDDS(Images at different magnifications of F11 SMEDDS respectively).

Table 4: Physical characterization studies of optimized L-SMEDDS formulations.

Batch No.	Particles Size	Polydispersity Index (PDI)	Zeta Potential
F3	100 nm	0.191	-19.88
F11	100 nm	0.137	-23.15

Curcumin and Thymoquinone-loaded SMEDDS had absolute zeta potential values of F3: -19.88 and F11: -23.15 mV, respectively. The presence of lipid excipients in the formulation could explain the negative surface charge value. The greater surface charge indicates that the SMEDDS has been stabilized for a long time.

Dispersibility Test

F11 passed the thermodynamic stress tests out of all thirteen formulations chosen from pseudo-ternary diagrams. As a result, dispersibility testing was performed on the F11 formulation. Because SMEDDS is a thermodynamically stable system, it easily forms globules following dispersion in a medium with no phase separation. When SMEDDS formulation is diluted, possible that SMEDDS will separate into various phases, resulting in the precipitation of poorly soluble drugs [15]. When the F11 formulation was disseminated in the aqueous phase, it spontaneously emulsified, resulting in an exceedingly transparent emulsion with a yellowish tint colour (Grade A).

Solidification of L-SMEDDS

According to the findings of the tests, solidification with 50% (w/w) adsorbent in the liquid formulation produces free-flowing powder with no agglomeration. The silica material Syloid®244 FP produced smoother granules than any other adsorbents and kept the drugs stable in an amorphous condition. *In vitro* dissolving tests revealed that F11 liquid SMEDDS formulations and solid SMEDDS made with Syloid®244 FP silica material exhibited outstanding dissolution efficiency and repeatability for Curcumin and Thymoquinone. However, as compared to other adsorbents for Curcumin and Thymoquinone, Syloid®244 FP silica material demonstrated a higher rate of dissolution (more than 65%).

Evaluation of Solid SMEDDS

Evaluation of solid SMEDDS of batch F11 is shown in Table 5, where bulk density is 0.1 gm/mL, tapped density shown is 0.11 gm/mL, Carr's index was 10.20-11.78, Hausner's ratio was 1.09-1.12, and angle of repose is 26.00-27.00°. The results of solid SMEDDS showed that they possess good flow properties and are ready for capsule filling.

Table5: Physical characterization studies of optimized S-SMEDDS formulations.

Physical characterization	Observations
Bulk Volume (Vb)	49 mL
Tapped Volume (Vt)	44 mL
Bulk Density	0.1020 gm/mL
Tapped Density	0.1136 gm/mL
Hausner's Ratio	1.11
Compressibility Index	10.20
Angle of Repose	26.85°

In-Vitro Dissolution Study

Curcumin and Thymoquinone are widely recognized to be poorly soluble in water and aqueous solutions with acidic pH. Pure Curcumin and Thymoquinone powder, liquid SMEDDS, and solid SMEDDS containing Curcumin and Thymoquinone were used in the *in vitro* dissolution tests. Curcumin and Thymoquinone from F11 were compared to simple Curcumin and Thymoquinone *in vitro* dissolution profiles. To simulate the intestinal situation, release tests were carried out using the USP II dissolving equipment in a pH 6.8 buffer. The release profiles of pure Curcumin and Thymoquinone powder, as shown in Fig. 5, exhibited very low release (less than 25%) even after 120 minutes in the intestinal medium (pH 6.8).

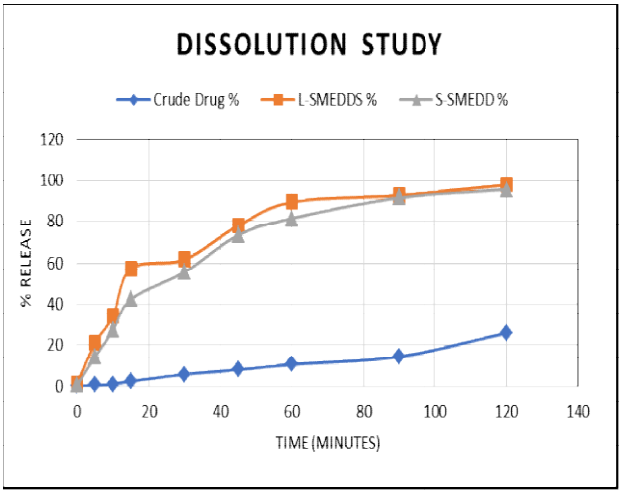

Figure 5: *In-vitro* Dissolution Profile of Solid and Liquid SMEDDS loaded with Curcumin and Thymoquinone & Crude Drug sample of Curcumin and Thymoquinone.

Curcumin and Thymoquinone dissolving from liquid SMEDDS had the best dissolution performance, with approximately 35% of both drugs released in the medium within 10 minutes. Liquid SMEDDS diffused quickly in the dissolution media after water dilution to form microdroplets. SMEDDS solidification produced different findings than SMEDDS liquid. The S-SMEDDS exhibits rapid, uniform dispersibility and the ability to preserve the drug solubilized without precipitation of Curcumin and Thymoquinone, demonstrating that the resulting microemulsion is stable even at pH 6.8.

In-Vitro Antimicrobial Activity of Curcumin and Thymoquinone

The resultant F11 formulation was tested for various combinations of Curcumin and Thymoquinone doses with DMSO (70%). According to this, a dose of 15mg/mL of Curcumin and 7mg/mL of Thymoquinone was chosen from the microbiological investigations, as shown in Table 6. F11 formulation had strong antimicrobial action against *Staphylococcus aureus* and antifungal activity against *Candida albicans* at this dose, as well as moderate antibacterial activity against *Escherichia coli*.

Table 6: Microbial Study of Curcumin and Thymoquinone with *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*

Concentration of Curcumin (mg/mL)	Concentration of Thymoquinone (mg/mL)	Zone of Inhibition (mm)		
		E. coli	*S. aureus*	*C. albicans*
20	1.5	10	14	13
15	7	12	17.5	15
18	3.5	11	15	13.9
Control		20	25	12

DISCUSSION

In this study, a novel liquid SMEDDS was formulated using Oleic Acid, Tween 20, and PEG 400 as an oil phase, a surfactant, and a co-surfactant in the ratios of 30:15:45, respectively, and then developed into a solid SMEDDS by adsorption using Syloid® FP 244 as the solid carrier and then encapsulated in hard gelatin capsule shells. This solid SMEDDS retained the liquid SMEDDS's self-emulsification capability and the dissolving rate in buffer pH 6.8 dissolution media was faster than the crude powder. Curcumin and Thymoquinone water solubility was increased. Given the limitations of liquid SMEDDS, a solid powder formulation should be a more viable option. Furthermore, the results indicate that the solid SMEDDS could be investigated and further evaluated for the oral delivery of lipophilic poorly soluble medicines where an oral mode of administration is preferred.

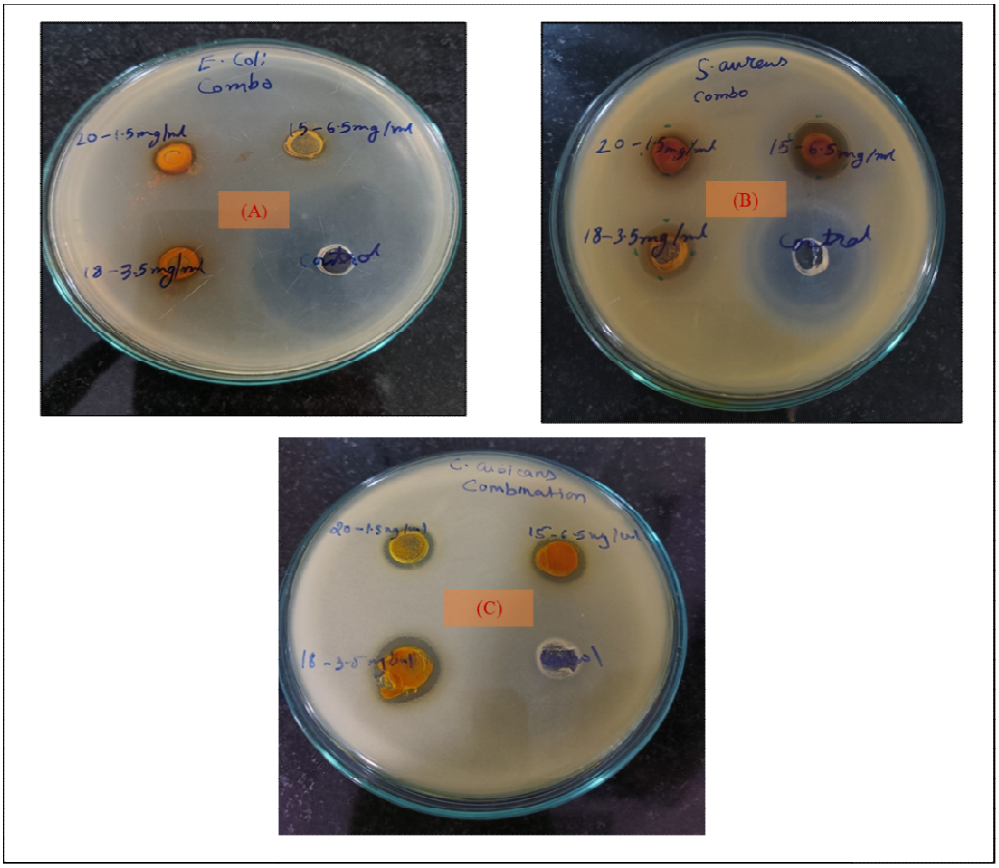

Figure 6: (A)*Escherichia coli* microbial study with Bactrim as a Control sample. (B)*Staphylococcus aureus* microbial study with Linezolid as a Control sample. (C)*Candida albicans* microbial study with Fluconazole as a Control sample.

This study shows that using a self-micro emulsifying drug delivery system improves the solubility and dissolution of very weakly water-soluble drugs like Curcumin and Thymoquinone. According to the antimicrobial experiments, the Curcumin-Thymoquinone loaded SMEDDS displayed excellent antimicrobial activity against *Staphylococcus aureus* and antifungal activity against *Candida albicans*, and moderate antibacterial activity against *Escherichia coli*.

CONCLUSION

SMEDDS was effectively created for the simultaneous oral drug delivery of BCS class IV drugs, Curcumin and Thymoquinone, using a well-known bioactive component, which is described in Table 1. Because they retain at least 90% of the curcumin and thymoquinone solubilized during dispersion and digestion in the GI tract, these excipients are excellent transporters. They are capable of producing translucent, homogeneous, and stable microdroplets. This could make medications pass through the intestinal barrier more easily and absorb more. Because of the enhanced surface activity and micron size of the system, medications can be delivered more stably through the GI boundary layer, enhancing drug absorption and causing an immediate effect.

We also examined the antibacterial and antifungal effects of curcumin and thymoquinone combination therapy, and it is hypothesized that this combination may be particularly beneficial when manufactured as a solid Self Micro Emulsifying Drug Delivery System (s-SMEDDS).

Despite the above-mentioned improvements and modifications, SMEDDSs still have a few difficulties that must be addressed before they can be considered commercially viable. Future studies should prioritize understanding the mechanisms of action of various SMEDDS formulations, pharmacokinetic research, particularly on human subjects, and cost-effectiveness. They should also concentrate on their business use.

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