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Original Article

OPTIMIZATION AND CHARACTERIZATION OF ESSENTIAL OILS FORMULATION FOR ENHANCED STABILITY AND DRUG DELIVERY SYSTEM OF MEFLOQUINE

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ABSTRACT

Objective: This work aims to choose suitable essential oil formulations to improve the bioavailability and long-term aqueous stability of mefloquine in drug delivery systems.

Methods: Oil phases of pomegranate oil, black cumin seed oil, and garlic oil. To choose the proper oil and surfactant for creating pseudo-ternary phase diagrams, cremophore EL, tween®20 and tween®80 (surfactants), and brij 35 (co-surfactants) were used in a variety of concentrations and combinations (Smix). Mefloquine was estimated to be soluble in a variety of oils, surfactants, and co-surfactants. Drug solubility, drug release research, thermodynamic stability, mean hydrodynamic size and zeta potential.

Results: Garlic with smix of cremophore EL and brij 35, Pomegranate with Tween 20, and Black cumin seed oil with Tween 80 showed the highest solubilization and emulsification capabilities and were further investigated using ternary phase diagrams. When combined with the co-surfactants under investigation, cremophore EL demonstrated a greater self-emulsification zone than tween® 80 and tween 20. Garlic oil, cremophore EL, and brij 35 nanoemulsion showed smaller size, greater zeta potential, less emulsification time, high transmittance, and better drug solubility than microemulsion formulations on especially those made with tween®20 and tween 80. Mefloquine loaded garlic oil nanoemulsion showed considerably low release in body fluid (32.48%) and a good release in intestinal fluid (82.78%) by 12 h in a drug release study.

Conclusion: Garlic oil as the oil phase and a mixture of cremophore EL and brij 35 as the surfactant phase are ideal surfactants and co-surfactant for mefloquine loaded garlic oil nanoemulsion with greater drug release in release kinetics investigation.

Keywords: Essential oil formulation, Microemulsion, Nanoemulsion, Garlic oil nanoemulsion, Mefloquine delivery system, Release kinetics, Oral bioavilability

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INTRODUCTION

There is a lack of amount proportionality, significant between-and within-subject variability, poor oral bioavailability, and, poor water solubility in a lot of prospective drugs and many established therapeutic compounds [1]. The most significant challenge is finding ways to use oral therapies that have appropriate bioavailability while using poorly water-soluble drugs. It is the pharmaceutical industry's most popular drug administration method due to its patient-friendliness, practicality, economy, and lack of invasiveness [2]. Several formulation approaches, including the use of emulsions, nanoparticles, solid dispersions, permeation enhancers, and lipid-based formulations, have been used to increase the oral bioavailability of purely water-soluble drugs [3]. Lipid-based formulations have recently received much attention to boost the oral bioavailability of lipophilic and weakly water-soluble drugs. Emulsifying formulations, emulsions, and liposomes are some examples of these carriers, including oils and surfactant dispersions [4]. When introduced into the aqueous phase while being gently stirred, nano-emulsifying drug delivery systems have the novel property of forming fine oil-in-water (o/w) nano emulsions in the nanometric range (10-100 nm) [5]. They are mixes of oil, surfactant, co-surfactant, and drugs that are isotropic and thermodynamically stable [6]. Natural products also outperform chemical ones in efficacy, bioavailability, and application [7]. Wild edible plants include vitamins, minerals, hormone precursors, protein, and energy, therefore many have been explored recently [8]. Drug release and absorption are made easier by the nano-sized droplets' large interfacial surface area [9] the addition of an oily phase to the formulation influences the absorption of drugs in addition to solubilization, increasing bioavailability [10]. Mefloquine is a malaria medication that has FDA approval [11]. One of the widely accepted explanations for the anti-malarial properties of mefloquine is that it interacts with heme to prevent the synthesis of b-hematin, which causes a toxic build-up of heme by-products (ferriprotoporphyrin IX) in the parasite's feeding vacuole [6, 12]. Despite its significant medicinal interest, mefloquine demonstrated low oral bioavailability (39%), owing to its high lipophilicity (log P = 5.04) and poor water solubility, both of which are key hurdles in creating formulations for clinical efficacy [13]. To address this issue, several attempts have been undertaken to improve mefloquine solubility and thus bioavailability [14]. Despite their high efficacy, numerous drugs have significant limitations due to their poor water solubility, limiting their absorption ability [15]. Many nanotechnologies have effectively treated low-water-soluble drugs [6]. Emulsions are the ideal delivery method for pharmaceuticals in biopharmaceutics Classification System (BCS) classes II and IV because they provide a chance to improve the *in vitro* and *in vivo* performance of drugs with low water solubility [16]. The surfactant and co-surfactant systems, which are crucial to an emulsion's performance, must be carefully designed [17]. The current work uses various characterization and optimization properties to describe the effect of various oils, surfactants, and cosurfactants on the phase behavior and characteristics of both microemulsion and nanoemulsion [18]. This study aided in the selection of oils (fig. 1) for the preparation of a highly stable formulation containing mefloquine, as well as the drug's solubility and drug release in different and oral administrations in the selected oil.

MATERIALS AND METHODS

Materials

Garlic Oil, Black cumin seed oil, Pomegranate oil, and Mefloquine Hydrochloride were bought from Sigma Aldrich, India, and Cremaphor EL from HI Media, India, Tween 80, Tween 20 Brij35 from SRL, India, and ultra-pure water from Cascada™ bio water system, Pall Corporation, USA with resistivity 18.2 MΩ cm was used for the preparation of all solutions. The chemicals required for release studies are monobasic phosphate, NaOH, $CaCl_2$, KCl, $MgSO_4$, $NaCl_2$, $NaHPO_4$, saccharose, hepes, and nitrocellulose membrane all

purchased from SRL and HI Media, India. All the above chemicals are of analytical grade.

Fig. 1: Visual appearance of different oils

Methodology

Oil and Surfactant initial screening for their emulsification ability

The chosen different oil phases such as Black cumin, Garlic, and Pomegranate oil (fig. 1) were combined with 250 µl of each chosen different surfactant. The mixes were slowly heated between (25 °C and 40 °C) to homogenize the ingredients. The resultant fine emulsion was created by adding the ratio (1:1:48-1:9:40) of oil with surfactant and Milli-Q in a stoppered volumetric beaker in a magnetic stirrer between 100-130 RPM. The number of flask inversions essential to produce a homogenous emulsion was used to determine how easy an emulsion was to produce [19]. The produced emulsions were left to remain overnight and their turbidity was measured spectrophotometrically The Date and Nagarsenker method [20] was to test the emulsification optical density (OD) of various at 600 nm using a UV spectrophotometer (JASCO V-630) with Milli-Q water used as a blank. Additionally, turbidity and phase separation in the emulsions were visually checked.

Construction of pseudo ternary diagram

By constructing a pseudo-ternary phase diagram, the ideal concentrations or ranges of oil, surfactant, and co-surfactant required to cause emulsification are identified [21]. To prevent the globules from coalescing, surfactant, and co-surfactant are selectively adsorbed at the interface. This lowers tension and forms a mechanical barrier. The objective was to choose the best surfactant for each oil. In short, Milli-Q is added in an increasing manner to a predetermined concentration of oil and surfactant while being stirred at 130-200 rpm (SpinIt 4010, Tarsons Products Pvt. Ltd., India) [22]. Using tabulated visual observations of different types, such as transparent or clear, clouded, or muddy, pseudo-ternary phase diagrams have been produced for the clear emulsions. Pseudo-ternary phase diagrams of mixed surfactant and co-surfactant (Smix), oil, and water, each representing a side of the triangle without drug inclusion, were plotted [1]. Using the aqueous titration technique, pseudo-ternary phase diagrams were created. To each weight ratio of oil, surfactant, and Smix, slow titration with an aqueous phase was performed. Observations for phase clarity were made while titrating the oil-surfactant, Smix ratio, and the total amount of water consumed was recorded [23]. Aqueous titration was used to construct pseudo-ternary phase diagrams. It was a slow titration with an aqueous phase to each weight ratio of oil and Smix. While titrating the oil-Smix ratio, observations for phase clarity were made, and the total volume of water used was noted.

Formulation process

Each oil was mixed with different surfactants (Tween 80, Tween 20, Cremophore EL, Brij 35) and Milli-Q at 300 rpm for an emulsion volume of 5 ml, resulting in a set of emulsions with ratios ranging from 1:1:48 to 1:9:40 (oil: surfactant: water). After the preliminary screening of different surfactants for different oil, garlic oil emulsions were created using cremophore EL as the surfactant and brij 35 as a co-surfactant according to the HLB value 14, whereas black cumin seed oil and pomegranate oil emulsions were created using Tween 80 and Tween 20. The set of emulsions is called a spontaneous/microemulsion. For the purpose of converting these emulsions into nanoemulsions, they were subjected to a 20-minute, 40%-intensity sonication process using an ultrasonicator (Sonics, Vibra cell, USA) [24].

Mefloquine standard graph

The maximum concentration of mefloquine (10µg/ml) was determined using a spectrophotometer (U2910, Hitachi, Japan) with methanol as the solvent, blank, and reference. This drug's absorption spectra (200 to 800 nm) in the UV and visible regions were used to calculate the concentration. The standard graphs were created using the absorbance of each concentration at 284 nm (max). In 15 ml volumetric flasks, a stock solution of mefloquine was generated at a concentration of 2 µg/ml. It was then diluted to 1 to 10µg/ml in each of the oils [25].

$$\text{LOD} = 3.3 \times \text{Standard deviation of the regression line} / \text{Slope(S) from regression line.}$$

$$\text{LOQ} = 10 \times \text{Standard deviation of the regression line} / \text{Slope(S) from regression line.}$$

Mefloquine solubility in different oils

It was established that mefloquine is soluble in a variety of oils (Pomegranate oil, Black cumin seed oil, Garlic oil), surfactants (including Tween® 20, Tween® 80, Cremophore EL, Brij 35) 2 ml of each oil were placed in screw-capped glass vials with an excess amount of mefloquine added, and the mixture was constantly stirred for 2 min with a magnetic stirrer (REMI 1 MLH) [16]. The mixes were then agitated (100 rpm) in an orbital shaker (REMI-BL) for 72 h at 25 0.5 °C, then allowed to stabilize for 24 h. The REMI C-24 BL was used to extract the samples and centrifuge them for 30 min at 5000 rpm [26]. The supernatant solution was filtered using a millipore membrane filter (0.45 m), and it was then properly diluted using a 50:50 methanol-to-water system. The amount of mefloquine was determined spectrophotometrically by utilizing a UV-Visible spectrophotometer with a methanol-water system (50:50) as a blank (JASCO V-630) at 268 nm. Three duplicates of the experiment were conducted.

Characterization of emulsions

The set of microemulsions and nanoemulsions was characterized by the following methods.

Thermodynamic stability

These tests aimed at screening the stability and phase integrity of different emulsions under various centrifugal force and temperature change conditions [27]. In this investigation, three distinct tests were done, such as the Centrifugation study, Heating, and cooling Freeze-thaw cycle.

Centrifugation study

Phase separation, creaming, cracking, and drug precipitation were all looked for after formulations (REMI C-24 BL) were centrifuged at 10,000 rpm for 30 min. The heating-cooling cycle was applied to the formulations that showed no signs of instability.

Heating and cooling cycle

With storage at each temperature for at least 48 h, three heating and cooling cycles were performed. The formulations used for the freeze-thaw stress test had to function well at these temperatures without showing any creaming, cracking, coalescence, phase separation, or phase inversion.

Freeze-thaw cycle

Three freeze-thaw cycles, each lasting at least 48 h, were used, with storage at each temperature ranging from-20 °C to+25 °C. After that,

phase separation on the formulations was visually examined. Only formulations with the ability to resist phase separation were chosen for the dispersibility research.

Mean hydrodynamic size and zeta potential

Using a nanoparticle analyzer (SZ-100, Horiba, Japan) and a diode-pumped frequency-doubled laser operating at 532 nm (10mW) at 25 °C with detectors at 90° and 173° for backscatter detection, dynamic light scattering (DLS) was used to determine the hydrodynamic diameter of the oil droplets [17, 28]. It also looked at how poly-disperse the droplets were. To reduce the impacts of repeated scattering, all of the formulations were diluted with water by a factor of 1:4 prior to readings. At a constant temperature of 25 °C, the laser doppler method in the nano-size analyzer was used to determine the electrophoretic mobility of the droplets. Using the provided software, the potential was estimated from the doppler frequency shifts of scattered laser light, which were used to assess the droplet mobility in this case. A capillary channel enclosed in an electric field was used to carry the particles through.

Physiochemical characterization

Only the optimized emulsions' physicochemical properties were identified. A pH meter (Mark VI, Systronics, Ahmedabad, India) was used to calculate the apparent pH. With water serving as the control and reference, the absorbance at 600 nm of the emulsions was measured using a UV-Visible spectrophotometer (U2910, Hitachi, Japan).

Study of emulsions stability

To check for kinetic stability, the finalized drug-loaded and drug-unloaded nanoemulsions were centrifuged at 1500 g for 30 min [7] chose the least surfactant-concentrated clear emulsions without phase separation as the optimized emulsions and employed them in subsequent testing. The product's long-term stability was assessed for a period of 5 mo and during that time, the droplet size and PDI concentration were determined.

Drug release study

Simulated intestinal fluid (SIF) and simulated body fluid (SBF) made in accordance with the US Pharmacopoeia as previously described were used to investigate the kinetics of Mefloquine release [29, 30]. The kinetics of drug release was calculated using the franz diffusion equipment. A cellulose nitrate dialysis membrane separated the 500 µl donor chamber from the 5 ml receiver compartment in the device [6]. The recipient chamber was spinning at 50 rpm and held at 25 °C. Samples were taken from the recipient compartment every 15 min for the first hour, then every hour for the next 12 h, in order to maintain the overall volume of the release medium. The mefloquine concentration was assessed by measuring the absorbance at 284 nm following an appropriate dilution. The control was mefloquine suspended in methanol at the same concentration [31, 32].

Statistical analysis

Using GraphPad Prism v6.01 (GraphPad Software Inc., CA), the statistical analysis was carried out.

RESULTS AND DISCUSSION

Oil and surfactant initial screening for their emulsification ability

A number of factors, such as the lipid-surfactant affinity, the hydrophilic-lipophilic balance (HLB) value of the surfactant, and the viscoelasticity of the emulsion base, affect the emulsification process and efficiency [33]. Emulsion droplet size and spontaneity of emulsification are influenced by the surfactant's HLB value. O/W nanoemulsions can be formed using surfactants with HLB>10 [34]. Since they are less toxic [35], less affected by variations in pH and ionic strength, and often have lower critical micelle concentrations than their ionic counterparts [36, 37], non-ionic surfactants are frequently taken into consideration for use in pharmaceutical and nanoemulsion formulations. Most often, oral intake is permitted [38]. Utilizing garlic, black cumin seed, and pomegranate oil as the oily phases, the effectiveness of the chosen surfactant's emulsification was evaluated [39–41]. Using various oily phases, the emulsification effectiveness of the chosen surfactants was evaluated. According to reports, well-formulated substances dissolve quickly with gentle stirring [42].

Table 1 lists the optimization of different formulation values and the number of inversions for various combinations and their characteristics (visuality). The findings suggested that tween® 20 (HLB 16.7), tween® 80 (HLB 15.0), cremophore EL (HLB 16.0), and co-surfactant brij 35(HLB 16.9), among between all the surfactant smix of cremophore and brij 35 exhibit the highest percentage highest emulsification efficiency in garlic oil, tween 80 in black-cumin oil and tween 20 in pomegranate oil. The studied surfactants' HLB values, which were different between cremophore EL, tween® 20, tween® 80, and co-surfactant brij 35 due to differences in their structure and chain length, could be the cause of the observed variation in their ability to emulsify [44]. The production of a more stable microemulsion and nanoemulsion in fig. 2 with different oil and surfactant; when exposed to water is correlated with higher HLB surfactants [45]. Smix with garlic oil demonstrated the highest emulsification efficiency without phase separation in both microemulsion and nanoemulsion because they produced transparent nanoemulsions in a shorter amount of time. However, when compared to microemulsion, garlic nanoemulsion showed a more-in-less ratio with less smix. In fig. 2 garlic oil was chosen as the oil phase with no phase-separation, the stability and less size capacity from different screens for further research, cremephore EL, a surfactant, and brij 35, a co-surfactant, both of which have high solubilities and emulsification were used.

Table 1: Composition of different formulations (Microemulsion and nanoemulsion) by using garlic, black cumin seed and pomegranate oil with the combination of Tween 80, Tween 20, Cremophore EL and Brij 35, all values presented as mean±standard deviation, n=3

S. No.	Formulation-code	Oil	Surfactant and Co-surfactant relative (%)			MiliQ	Characteristics (Micro-emulsion)	Characteristics (Nano-emulsion)
	Different oils (Garlic, Black cumin, Pomegranate)	Relative (%)	Tween 80	Tween 20	Cremophore ELand Brij 35	Relative (%)	Visual-clarity	Visual clarity
1.	GE-1	2.0	-	-	2.0	96.0	Milky	Turbid
2.	GE-2	2.0	-	-	4.0	94.0	Turbid	Clear
3.	GE-3	2.0	-	-	6.0	92.0	Turbid	Clear
4.	GE-4	2.0	-	-	8.0	90.0	Clear	Clear
5.	GE-5	2.0	-	-	10.0	88.0	Clear	Clear
6.	BE-1	2.0	2.0	-	-	96.0	Milky	Turbid
7.	BE-2	2.0	4.0	-	-	94.0	Milky	Turbid
8.	BE-3	2.0	6.0	-	-	92.0	Milky	Clear
9.	BE-4	2.0	8.0	-	-	90.0	Turbid	Clear
10.	BE-5	2.0	10.0	-	-	88.0	Clear	Clear
11.	PE-1	2.0	-	2.0	-	96.0	Milky	Turbid
12.	PE-2	2.0	-	4.0	-	94.0	Milky	Turbid
13.	PE-3	2.0	-	6.0	-	92.0	Turbid	Clear
14.	PE-4	2.0	-	8.0	-	90.0	Turbid	Clear
15	PE-5	2.0	-	10.0	-	88.0	Turbid	Clear

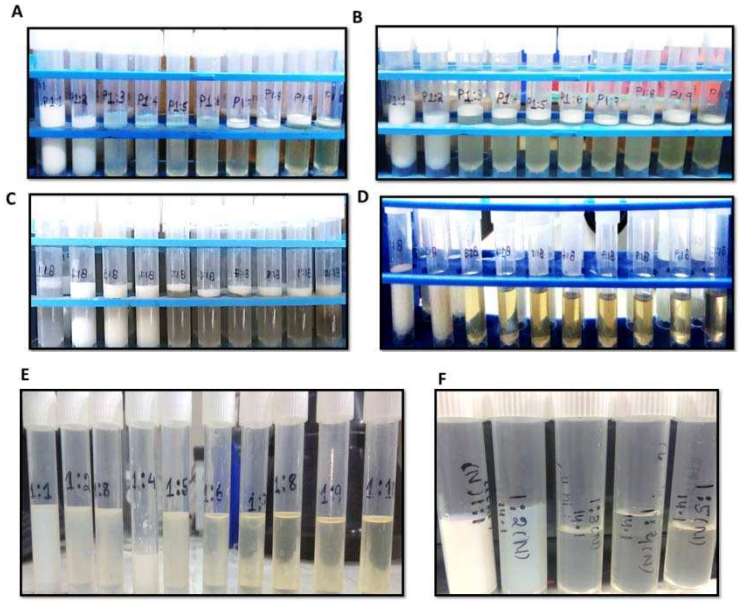

Fig. 2: Visual appearance of formulated micro and nanoemulsion (A)Pomegranate oil microemulsion (B) Pomegranate oil nanoemulsion (C) Black cumin seed oil microemulsion (D) Black cumin seed nanoemulsion (E) Garlic oil micro-emulsion (F) Garlic oil nanoemulsion

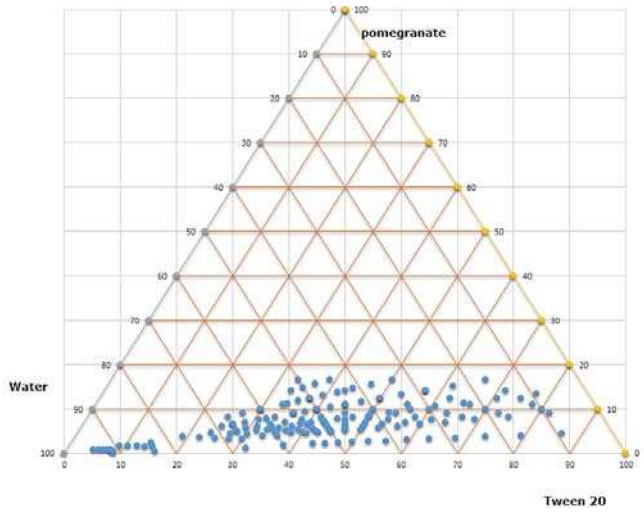

Fig. 3(A): Ternary phase diagram construction of nanoemulsions: oil (Pomegranate), Surfactant (Tween 80), and, Mili-Q

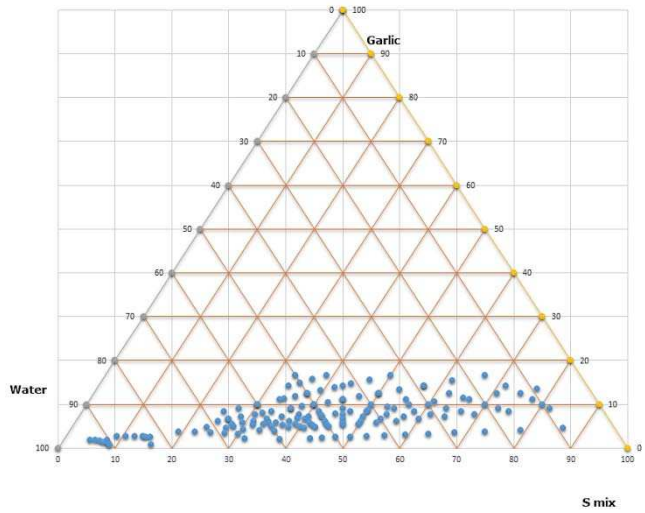

Fig. 3(B): Ternary phase diagram construction of nanoemulsion: Oil (Garlic), S mix (Cremophore EL and Brij 35), Mili-Q

Construction of pseudo ternary diagram

Ternary phase diagrams were created without mefloquine in order to locate the emulsifying zone and select the right ratio of oil, surfactant, and co-surfactant for the creation of formulations [46, 47]. These phase diagrams are crucial for understanding the phase behaviour of the produced emulsions [48]. It was made very evident that using brij 35 as a co-surfactant with chromophore EL in a garlic oil emulsion formed an isotropic emulsion area that was clear, whereas tween 80 with black cumin and pomegranate with tween 20 formed a clear emulsion fig. 3, (A), (B) and (C). Due to their higher HLB and hence more hydrophilic character cremophore EL and brij35 demonstrated more emulsification with the garlic oil region when compared to tween® 8,0 and tween 20. A good oil-water emulsion must have a higher HLB value because an oil-surfactant mixture with a higher hydrophilicity property would emulsify more quickly when it comes into contact with water [36, 49].

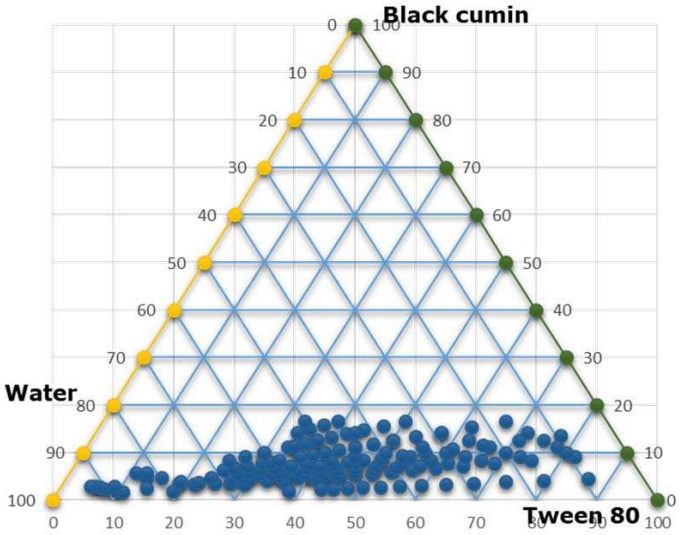

Fig. 3(C): Ternary phase diagram construction of nanoemulsion: Oil (Black cumin seed oil), Surfactant (Tween 80), and Mili-Q

According to the present findings, increasing the surfactant proportion (Smix) resulted in a more advantageous formation of both emulsions fig. 3(A, B, C). The free energy of emulsion formation depends on how much the surfactant reduces the surface tension of the oil-water interface and the change in dispersion entropy [50, 51]. In order to lower the surface tension between the oil phase and the aqueous phase, the surfactant builds a coating around the oil globule so that the polar head faces the aqueous phase and the non-polar tail draws oil out [34, 52]. The spontaneity of the self-emulsification process was also improved by increasing the surfactant concentration. The region where emulsions develop is reduced as co-surfactants are added because they aid surfactant in reducing interfacial tension rather than having much of an impact on it directly in fig. 3(A, B, C).

Solubility of drug

Considering that many formulations precipitate before undergoing in situ solubilization, the drug's solubility in excipients is a key factor in determining formulation stability [46]. Combinations of oils, surfactants, and co-surfactants are essentially what make up formulations [8]. At room temperature, this mixture should be a transparent, isotropic, monophasic liquid. With the aim to include the therapeutic dose in the least amount of combination possible, and should have strong solubilizing capacity [27, 31]. After phase separation in different oil formulations was confirmed, the ratio of 1:5 and beyond for spontaneous emulsion and 1:3 and beyond for nanoemulsion in three oils showed good stability [53]. Garlic oil, however, demonstrated superior stability when compared to the other two oils. Among those studied oil systems, pomegranate has the lowest solubility while garlic has the highest solubility as table 2 displays the solubility of mefloquine in various oils with LOD, LOQ values.

Due to its higher solubilization (R^2) value capability compared to the other two oils among the several vehicles examined in fig. 4(A, B, C) garlic oil was chosen as the oil phase. Previous studies have shown that medium-chain monoglycerides, such as garlic have a strong ability to dissolve hydrophobic lipophilic medicines and enhance the water permeability and self-dispersibility of lipid formulations when hydrated [54]. Additionally, because garlic is trapped in high HLB surfactant, which facilitates the emulsification process when diluted with an aqueous medium, garlic is expected to improve the interfacial fluidity of surfactant boundaries in the micelles.

Characterization of emulsions

Kinetic stability which represents the thermodynamic stability of the two systems, is the primary distinction between emulsions and nanoemulsions [2]. The formulation is in situ solubilized to formulate a nanoemulsion system; as a result, it must be stable enough to avoid precipitation, creaming, or cracking [45]. However, in numerous cases, prolonged storage may result in the medication precipitating from the nanoemulsion; at this point, little crystals may start to form and eventually grow into massive crystalline materials that will precipitate out at the vessel's bottom [18, 41]. In order to remove the metastable components, centrifugation, a heating and cooling cycle, and a freeze-thawing cycle were used to test the stability of the formulation [56]. The optimized both microemulsion and nanoemulsion formulations thermodynamic stability test of different oil formulations mentioned in (table 3).

Table 2: LOD, LOQ, and mefloquine solubility in different oils, all values presented as mean±standard deviation, n=3

Oils	LOD(µg/ml mean±SD)	LOQ (µg/ml mean±SD)	Mefloquine solubility (µg/ml mean±SD)
Garlic oil	0.96±0.013	2.13±0.055	14.64±2.1
Black cumin seed oil	0.77±0.041	1.05±0.021	9.64±1.6
Pomegranate oil	0.47±0.012	0.98±0.22	6.35±1.23

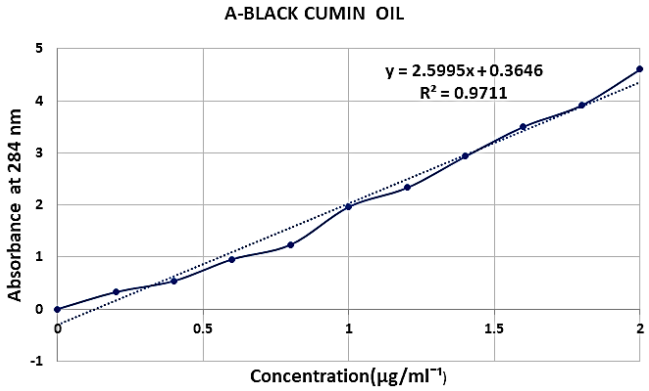

Fig. 4(A): Standard graphs for mefloquine in black cumin oil from 1 to 10µg/ml, all values presented as mean, n=3

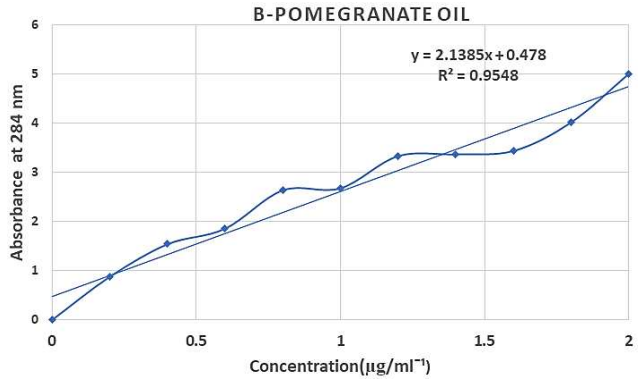

Fig. 4(B): Standard graphs for mefloquine in pomegranate oil from 1 to 10µg/ml all values presented as mean, n=3

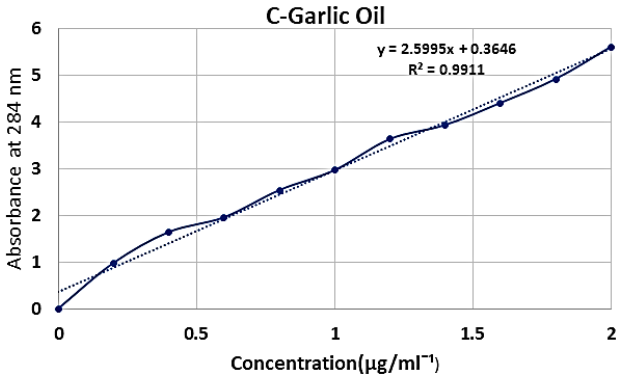

Fig. 4(C): Standard graphs for mefloquine in garlic oil from 1 to 10 µg/ml, all values presented as mean, n=3

Table 3: Evaluation of thermodynamic stability of different oil formulations

Formulation code	Microemulsion			Nanoemulsion		
Oils	Centrifugation	Freeze-thaw	Heating-cooling	Centrifugation	Freeze thaw	Heating cooling
GE-1	Fail	Fail	Fail	Fail	Fail	Fail
GE-2	Fail	Fail	Fail	Passed	Passed	Passed
GE-3	Fail	Fail	Fail	Passed	Passed	Passed
GE-4	Passed	Passed	Passed	Passed	Passed	Passed
GE-5	Passed	Passed	Passed	Passed	Passed	Passed
BE-1	Fail	Fail	Fail	Fail	Fail	Fail
BE-2	Fail	Fail	Fail	Fail	Fail	Fail
BE-3	Fail	Fail	Fail	Passed	Passed	Passed
BE-4	Passed	Passed	Passed	Passed	Passed	Passed
BE-5	Passed	Passed	Passed	Passed	Passed	Passed
PE-1	Fail	Fail	Fail	Fail	Fail	Fail
PE-2	Fail	Fail	Fail	Fail	Fail	Fail
PE-3	Fail	Fail	Fail	Passed	Passed	Passed
PE-4	Passed	Passed	Passed	Passed	Passed	Passed
PE-5	Passed	Passed	Passed	Passed	Passed	Passed

In the ratios of 1:1, 1:2, 1:3,1:4 and 1:5, the spontaneous emulsion of three oils-garlic oil, black cumin seed oil, and pomegranate oil-was unstable, showed phase separation, and was milky and turbid. This may have been caused by the internal phase coagulation, which caused phase separation [57]. While in two ratios of 1:1,1:2, in nano-emulsion revealed unstable, phase separation as well as milky and turbid. A steady and clear emulsion in a nanoemulsion is produced from a ratio of 1:3 onward. In the trials on thermostability (table 4), both formulations of three oils displayed fewer changes [16]. But nanoemulsion in three oils with different ratios has stable result than microemulsion. To enhance the biological activity, stability, safety, and bioavailability of numerous water-insoluble bioactive compounds nanoemulsion can further proceed. In contrast to, nanoemulsion which are intrinsically unstable and is only kinetically stable, microemulsions are inherently stable emulsions and are thermodynamically stable [30, 44].

Nanoemulsions are being employed more frequently because they produce stable emulsions with significantly lower surfactant concentrations [19]. The other characteristics of both emulsions mean hydrodynamic size, PDI value, Zeta Potential, turbidity, and pH method, are listed in table 4. Garlic in both emulsions showed a smaller size (1-20 nm) as compared to other oils, as shown in table 4 mention of the mean hydrodynamic value. According to earlier studies on the size of oil-in-water emulsions, sizes between 1 to 20 nm are consistently chosen for research on drug release and delivery [22] Droplet size is one of a nanoemulsion's most important properties for stability testing and a critical step in the process of enhancing a drug's bioavailability [59]. A larger interfacial surface area for medication absorption results from a smaller droplet size, which may speed up absorption and improve bioavailability [56]. Consequently, the effective drug release may be controlled by the nanoemulsion's droplet size [60]. Due to a higher surfactant fraction compared to co-surfactant, the droplet size reduced as the oil content fell. The stability of the oil droplets brought on by the surfactant molecules' interpretation at the oil-water interface probably explains this [16]. Smaller droplet sizes may result from the interfacial film condensing and stabilising after the addition of the surfactant, while the film may stretch after the addition of the co-surfactant [27].

Table 4: Physiochemical characteristics of optimized microemulsion and nanoemulsion

Formulation code	Microemulsion					Nanoemulsion				
Oils	Droplet size (nm)	PDI	Zeta potential	Turbidity	pH	Droplet size (nm)	PDI	Zeta Potential	Turbidity	pH
GE-3	31.5	0.163±0.02	-1.89±0.0	0.108±0.0	6.21±0.18	19.5	0.214±0.14	-2.15±0.0	0.049±0.0	6.03±0.12
GE-4	29.3	0.207±0.003	-1.23±0.0	0.105±0.0	6.24±0.20	15.9	0.258±0.06	-2.98±0.0	0.038±0.0	6.14±0.2
GE-5	22.9	0.387±0.014	-1.96±0.0	0.129±0.0	6.28±0.14	14.3	0.395±0.01	-3.15±0.0	0.025±0.0	6.19±0.0
BE-3	51.5	0.398±0.002	-0.893±0.0	0.138±0.0	5.02±0.25	29.7	0.387±0.18	-1.20±0.0	0.191±0.0	5.04±0.0
BE-4	49.9	0.254±0.028	-0.982±0.0	0.025±0.0	5.14±0.74	24.3	0.275±0.04	-1.63±0.0	0.172±0.0	5.09±0.0
BE-5	38.3	0.158±0.006	-1.256±0.0	0.451±0.0	5.37±0.21	21.5	0.189±0.01	-1.89±0.0	0.155±0.0	5.48±0.0
PE-3	48.5	0.269±0.005	-0.789±0.0	0.392±0.0	3.14±0.00	26.2	0.296±0.13	-0.98±0.0	0.198±0.0	3.56±0.0
PE-4	40.5	0.198±0.015	-0.963±0.0	0.275±0.0	3.45±0.10	24.2	0.243±0.0	-1.56±0.0	0.185±0.0	3.96±0.0
PE-5	39.9	0.231±0.004	-1.149±0.0	0.221±0.0	3.57±0.01	22.1	0.266±0.0	-1.69±0.0	0.171±0.0	3.99±0.0

All values presented as mean±standard deviation, n=3

As a result, the relative proportion of surfactant to co-surfactant has varying impacts on the droplet size. Zeta potential, pH, and turbidity values for both microemulsions and nanoemulsions are listed in table 4. Garlic has a higher zeta potential value than the other two oils, which explains the surface charge and indicates longer-term emulsion stability. The type of surfactants used to create nano-emulsion systems has a significant impact on the zeta potential, which is used to assess stability. When the surfactant in question is either anionic or cationic, it becomes more prominent [47] additionally, it was found that the pH of the garlic oil nanoemulsion was 5.36 0.14. Garlic oil nanoemulsion and microemulsion have turbidities of 0.0010.004 and 0.0920.00, respectively.

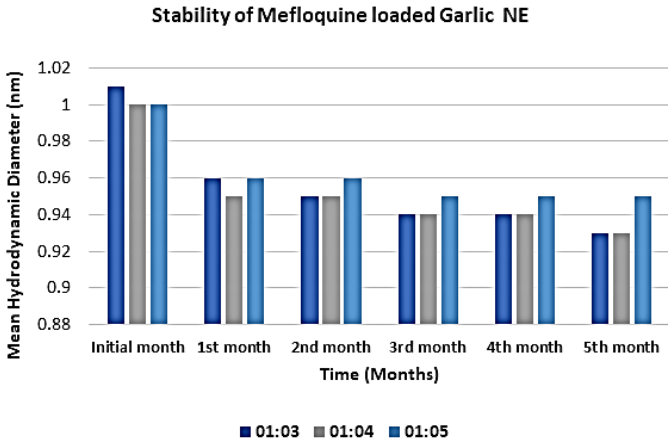

Fig. 5: Long-term stability analysis of mefloquine-loaded garlic nanoemulsion, all values presented as mean, n=3

Mefloquine is encapsulated in nanoemulsion improving the emulsion's stability, shelf life, and oral bioavailability. Mefloquine's prolonged stability in the garlic nanoemulsion for up to 5 mo shows how protective the emulsion's oil is for long-term storage capacity [61]. Emphasizing improving the solubility, characterization, and stability of the garlic-loaded mefloquine nanoemulsion, which was selected for the drug release profile with various simulated fluids to test its oral bioavailability [62].

Drug release kinetics of mefloquine loaded garlic nanoemulsion

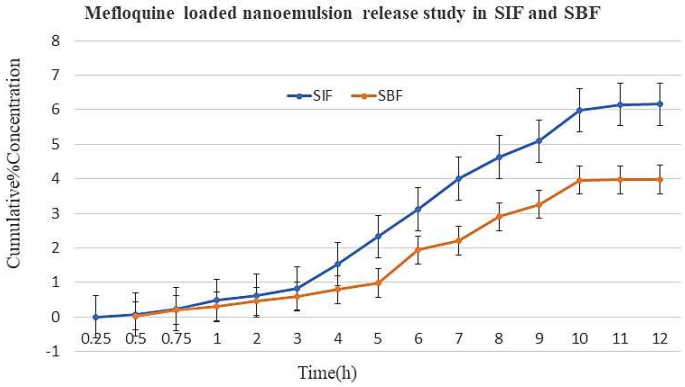

Fig. 6: Drug release study of optimized mefloquine-loaded garlic oil nanoemulsion in two simulated intestinal and body fluid, all values presented as mean±standard deviation, n=3

Simulated body fluid, showed 10-20% after 3 h, while simulated intestinal fluid showed 5-10% after 3 h (fig. 4A and B). Interestingly mefloquine loaded garlic nanoemulsion remained in simulated intestinal fluid for a period of up to 12 h until 60-70% of saturation was reached 12 h until they reached saturation (60-70%). In contrast, simulated body fluid Showed 150-20% release at an early time point of 8h of sustained release. R^2 value (coefficient determination) indicative of the drug release in the simulated intestinal fluid was observed to be 0.989 for mefloquine in garlic oil respectively. The 35-40% release of mefloquine from garlic oil nanoemulsion occurs for 3 h, and there is an extended release until 12 h. This is important for absorption as mefloquine levels can be maintained as mefloquine is rapidly broken down by the body [62], and garlic oil also contains polyunsaturated fatty acids and is one of the best oils for human benefits [42].

CONCLUSION

Garlic oil, cremophore EL, and brij 35 were found to be the best-suited oils, surfactants, and co-surfactants, respectively, according to the thermodynamic stability studies, ternary phase diagrams, and characterization. An acceptable working range for the concentration of the surfactant and co-surfactant was established with the aid of the research. Garlic oil nanoemulsion containing mefloquine are exceptionally biocompatible, have low toxicity, and can assist improve mefloquine's poor oral bioavailability and stability. One of the best oils available for its antioxidant and anticancer properties is garlic oil, which can also be used to deliver mefloquine orally. Mefloquine nanoemulsion formulation and *in vitro* release are the mere topics covered in this study.

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AUTHORS CONTRIBUTIONS

Priyadarshini Mohapatra: Investigation, Methodology, Visualization, Formal analysis, Writing-Original Draft.

N. Chandrasekaran: Conceptualization, Methodology, Supervision, Project administration, Writing-Review and editing.

CONFLICT OF INTERESTS

The authors involved reveal no sources of conflict of interest.

REFERENCES

1. Li B, Tan T, Chu W, Zhang Y, Ye Y, Wang S. Co-delivery of paclitaxel (PTX) and docosahexaenoic acid (DHA) by targeting lipid nanoemulsions for cancer therapy. Drug Deliv. 2022;29(1):75-88. doi: 10.1080/10717544.2021.2018523, PMID 34964421.
2. Begum A, Uma Rani G. Formulation and evaluation of floating drug delivery system of repaglinide. Vol. 16; 2023. doi: 10.22159/ajpcr.2023v16i6.47271.
3. Zhao P, Tang X, Huang Y. Teaching new tricks to old dogs: a review of drug repositioning of disulfiram for cancer nanomedicine. View. 2021;2(4):20200127. doi: 10.1002/VIW.20200127.
4. Ahmad MZ, Ahmad J, Aslam M, Khan MA, Alasmary MY, Abdel Wahab BA. Repurposed drug against Covid-19: nanomedicine as an approach for finding new hope in old medicines. Nano Express. 2021;2(2). doi: 10.1088/2632-959X/abffed.
5. Prasher P, Sharma M. Nanotechnology-based self-sterilizing surfaces and their potential in combating Covid-19. Nanomedicine (Lond). 2021;16(14):1183-6. doi: 10.2217/nnm-2021-0079, PMID 33973804.
6. Sarvan VH. Types and application of pharmaceutical nanotechnology: a review. Int J Curr Pharm Res. 2023 May 15;14-8.
7. Das S, Biswas P, Dutta C, Biswas DS. Fabrication and release kinetics of piperazine citrate tablets using natural gum. Vol. 16; 2023. doi: 10.22159/ajpcr.2023v16i6.47323.
8. Seal T, Pillai B, Chaudhuri K. Effect of cooking methods on total phenolics and antioxidant activity of selected wild edible plants. Int J Pharm Pharm Sci. 2023 Jul 1:20-6.
9. Capek I. Preparation of metal nanoparticles in water-in-oil (w/o) microemulsions. Adv Colloid Interface Sci. 2004;110(1-2):49-74. doi: 10.1016/j.cis.2004.02.003, PMID 15142823.
10. Guo Y, Wu Z, Shen S, Guo R, Wang J, Wang W. Nanomedicines reveal how PBOV1 promotes hepatocellular carcinoma for effective gene therapy. Nat Commun. 2018;9(1):3430. doi: 10.1038/s41467-018-05764-7, PMID 30143633.
11. Li YH, Yang SL, Zhang GF, Wu JC, Gong LL, Ming Zhong. Mefloquine targets β-catenin pathway and thus can play a role in the treatment of liver cancer. Microb Pathog. 2018;118:357-60. doi: 10.1016/j.micpath.2018.03.042, PMID 29578061.
12. Kucharski DJ, Jaszczak MK, Boraty PJ. A review of modifications of quinoline antimalarials; 2022.
13. Weinke T, Trautmann M, Held T, Weber G, Eichenlaub D, Fleischer K. Neuropsychiatric side effects after the use of mefloquine. Am J Trop Med Hyg. 1991;45(1):86-91. doi: 10.4269/ajtmh.1991.45.86, PMID 1867351.
14. Lundstrom Stadelmann B, Rufener R, IJP HA. Drugs and drug resistance drug repurposing applied: activity of the antimalarial me floquine against Echinococcus multilocularis (Jun); 2020.
15. Xu X, Wang J, Han K, Li S, Xu F, Yang Y. Antimalarial drug mefloquine inhibits nuclear factor kappa B signaling and induces apoptosis in colorectal cancer cells. Cancer Sci. 2018;109(4):1220-9. doi: 10.1111/cas.13540, PMID 29453896.
16. Çalıs S, Ozturk Atar K, Arslan FB, Eroglu H, Çapan Y. Nanopharmaceuticals as drug-delivery systems. Nanocarriers Drug Deliv. 2019:133-54.

17. Ashaolu TJ. Nanoemulsions for health, food, and cosmetics: a review. Environ Chem Lett. 2021;19(4):3381-95. doi: 10.1007/s10311-021-01216-9, PMID 33746662.
18. Mcclements DJ, Jafari SM. General aspects of nanoemulsions and their formulation. Elsevier Inc. 2018. p. 3-20. doi: 10.1016/B978-0-12-811838-2.00001-1.
19. Fardous J, Omoso Y, Joshi A, Yoshida K, Patwary MKA, Ono F. Development and characterization of gel-in-water nanoemulsion as a novel drug delivery system. Mater Sci Eng C Mater Biol Appl. 2021;124:112076. doi: 10.1016/j.msec.2021.112076. PMID 33947568.
20. Alhajamee M, Marai K, Al Abbas SMN, Homayouni Tabrizi M. Co-encapsulation of curcumin and tamoxifen in lipid-chitosan hybrid nanoparticles for cancer therapy. Mater Technol. 2022;37(9):1183-94. doi: 10.1080/10667857.2021.1926811.
21. Abrar M, Ayub Y, Nazir R, Irshad M, Hussain N, Saleem Y. Garlic and ginger essential oil-based neomycin nanoemulsions as effective and accelerated treatment for skin wounds' healing and inflammation: *in vivo* and *in vitro* studies. Saudi Pharm J. 2022 Dec;30(12):1700-9. doi: 10.1016/j.jsps.2022.09.015.
22. Ganta S, Singh A, Rawal Y, Cacaccio J, Patel NR, Kulkarni P. Formulation development of a novel targeted theranostic nanoemulsion of docetaxel to overcome multidrug resistance in ovarian cancer. Drug Deliv. 2016;23(3):968-80. doi: 10.3109/10717544.2014.923068, PMID 24901206.
23. Izadiyan Z, Basri M, Fard Masoumi HR, Abedi Karjiban R, Salim N, Shameli K. Modeling and optimization of nanoemulsion containing sorafenib for cancer treatment by response surface methodology. Chem Cent J. 2017;11(1):21. doi: 10.1186/s13065-017-0248-6, PMID 28293282.
24. Yang W, Veroniaina H, Qi X, Chen P, Li F, Ke PC. Soft and condensed nanoparticles and nanoformulations for cancer drug delivery and repurpose. Adv Ther (Weinh). 2020;3(1):1900102.
25. Noor A. A data-driven medical decision framework for associating adverse drug events with drug-drug interaction mechanisms. J Healthc Eng. 2022;2022:1-7. doi: 10.1155/2022/9132477.
26. Mathew C, Lal N, SL, Tra, Varkey J. Antioxidant, anticancer and molecular docking studiesof novel 5-benzylidene substituted rhodanine derivatives. Int J Pharm Pharm Sci. 2023 Jul 1:7-19.
27. Sanchez Lopez E, Guerra M, Dias Ferreira J, Lopez Machado A, Ettcheto M, Cano A. Current applications of nanoemulsions in cancer therapeutics. Nanomaterials (Basel). 2019;9(6). doi: 10.3390/nano9060821, PMID 31159219.
28. Gorain B, Choudhury H, Nair AB, Dubey SK, Kesharwani P. Theranostic application of nanoemulsions in chemotherapy. Drug Discov Today. 2020;25(7):1174-88. doi: 10.1016/j.drudis.2020.04.013, PMID 32344042.
29. Sugumar S, Clarke SK, Nirmala MJ, Tyagi BK, Mukherjee A, Chandrasekaran N. Nanoemulsion of eucalyptus oil and its larvicidal activity against culex quinquefasciatus. Bull Entomol Res. 2014;104(3):393-402. doi: 10.1017/S0007485313000710, PMID 24401169.
30. Mishra P, Jerobin J, Thomas J, Mukherjee A, Chandrasekaran N. Study on antimicrobial potential of neem oil nanoemulsion against Pseudomonas aeruginosa infection in Labeo rohita. Biotechnology and Applied Biochemistry. 2014;61(5):611-9.
31. Sugumar S, Ghosh V, Nirmala MJ, Mukherjee A, Chandrasekaran N. Ultrasonic emulsification of eucalyptus oil nanoemulsion: antibacterial activity against Staphylococcus aureus and wound healing activity in wistar rats. Ultrason Sonochem. 2014;21(3):1044-9. doi: 10.1016/j.ultsonch.2013.10.021, PMID 24262758.
32. Anjali CH, Sharma Y, Mukherjee A, Chandrasekaran N. Neem oil (Azadirachta indica) nanoemulsion-a potent larvicidal agent against culex quinquefasciatus. Pest Manag Sci. 2012;68(2):158-63. doi: 10.1002/ps.2233, PMID 21726037.
33. Ghosh V, Mukherjee A, Chandrasekaran N. Formulation and characterization of plant essential oil based nanoemulsion: evaluation of its larvicidal activity against Aedes aegypti formulation and characterization of plant essential oil based nanoemulsion: evaluation of its larvicidal activity aga. Asian Journal of Chemistry. 2013;25 Suppl:S321-S323.
34. Anwer MK, Jamil S, Ibnouf EO, Shakeel F. Enhanced antibacterial effects of clove essential oil by nanoemulsion. J Oleo Sci. 2014;63(4):347-54. doi: 10.5650/jos.ess13213, PMID 24599109.
35. Date AA, Dixit R, Nagarsenker M. Self-nanoemulsifying drug delivery systems: formulation insights, applications and advances. Review. 2010;5:1595-616.
36. Journal A, Issue S. Formulation and characterization of plant essential oil based nanoemulsion: evaluation of its larvicidal activity against Aedes aegypti. Asian Journal of Chemistry. 2013;25:18-20.
37. Ghosh V, Saranya S, Mukherjee A, Chandrasekaran N. Cinnamon oil nanoemulsion formulation by ultrasonic emulsification: investigation of its bactericidal activity. J Nanosci Nanotechnol. 2013;13(1):114-22. doi: 10.1166/jnn.2013.6701, PMID 23646705.
38. Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: an advanced mode of drug delivery system. 3 Biotech. 2015;5(2):123-7. doi: 10.1007/s13205-014-0214-0, PMID 28324579.
39. Nicastro HL, Ross SA, Milner JA. Garlic and onions: their cancer prevention properties. Cancer Prev Res (Phila). 2015;8(3):181-9. doi: 10.1158/1940-6207.CAPR-14-0172, PMID 25586902.
40. Sharma P, McClees SF, Afaq F. Pomegranate for prevention and treatment of cancer: an update. Molecules. 2017;22(1):1-18. doi: 10.3390/molecules22010177, PMID 28125044.
41. Farshi P, Tabibiazar M, Ghorbani M, Hamishehkar H. Evaluation of antioxidant activity and cytotoxicity of cumin seed oil nanoemulsion stabilized by sodium caseinate-guar gum. Tabriz University of Medical Sciences. 2017;23(4):293–300. doi: 10.15171/PS.2017.43.
42. Ragavan G, Muralidaran Y, Sridharan B, Nachiappa Ganesh R, Viswanathan P. Evaluation of garlic oil in nano-emulsified form: optimization and its efficacy in high-fat diet induced dyslipidemia in wistar rats. Food Chem Toxicol. 2017;105:203-13. doi: 10.1016/j.fct.2017.04.019, PMID 28428086.
43. Alkhatib MH, Binsiddiq BM, Backer WS. *in vivo* evaluation of the anticancer activity of a water-in-garlic oil nanoemulsion loaded with docetaxel. Int J Pharm Sci Res. 2017;8(12):5373-9.
44. Plaza Oliver M, Santander Ortega MJ, Lozano MV. Current approaches in lipid-based nanocarriers for oral drug delivery. Drug Deliv Transl Res. 2021;11(2):471-97. doi: 10.1007/s13346-021-00908-7, PMID 33528830.
45. Ganta S, Talekar M, Singh A, Coleman TP, Amiji MM. Nanoemulsions in translational research-opportunities and challenges in targeted cancer therapy. AAPS PharmSciTech. 2014;15(3):694-708. doi: 10.1208/s12249-014-0088-9, PMID 24510526.
46. Nabila N, Suada NK, Denis D, Yohan B, Adi AC, Veterini AS. Antiviral action of curcumin encapsulated in nanoemulsion against four serotypes of dengue virus. Pharm Nanotechnol. 2020;8(1):54-62. doi: 10.2174/2211738507666191210163408, PMID 31858909.
47. Periasamy VS, Athinarayanan J, Alshatwi AA. Anticancer activity of an ultrasonic nanoemulsion formulation of Nigella sativa L. essential oil on human breast cancer cells. Ultrason Sonochem. 2016;31:449-55. doi: 10.1016/j.ultsonch.2016.01.035, PMID 26964971.
48. Alkhatib MH, Binsiddiq BM, Backer WS. *In vivo* evaluation of the anticancer activity of a water-in-garlic oil nanoemulsion loaded with docetaxel. Int J Pharm Sci Res. 2017;8(12):5373-9.
49. Press D. Eucalyptus oil nanoemulsion-impregnated chitosan film: antibacterial effects against a clinical pathogen, Staphylococcus aureus, *in vitro*; 2015. p. 67-75.
50. El-readi MZ. Review article cancer nanomedicine: a new era of successful targeted therapy. Vol. 2019; 2019.
51. Ghosh V, Mukherjee A, Chandrasekaran N. Ultrasonic emulsification of food-grade nanoemulsion formulation and evaluation of its bactericidal activity. Ultrason Sonochem. 2013;20(1):338-44. doi: 10.1016/j.ultsonch.2012.08.010, PMID 22954686.
52. Jerobin J, Sureshkumar RS, Anjali CH, Mukherjee A, Chandrasekaran N. Biodegradable polymer based encapsulation of neem oil nanoemulsion for controlled release of Aza-A. Carbohydr Polym. 2012;90(4):1750-6. doi: 10.1016/j.carbpol.2012.07.064, PMID 22944443.

53. Ye H, He X, Feng X. Developing neobavaisoflavone nanoemulsion suppresses lung cancer progression by regulating tumor microenvironment. Biomed Pharmacother. 2020;129(May):110369. doi: 10.1016/j.biopha.2020.110369, PMID 32563983.
54. Satyal P, Craft JD, Dosoky NS, Setzer WN. The chemical compositions of the volatile oils of garlic (Allium sativum) and wild garlic (allium vineale). Foods. 2017;6(8):1-10. doi: 10.3390/foods6080063, PMID 28783070.
55. Dziri S, Casabianca H, Hanchi B, Hosni K. Composition of garlic essential oil (Allium sativum L.) as influenced by drying method. J Essent Oil Res. 2014;26(2):91-6.
56. Liu Y, Miyoshi H, Nakamura M. Nanomedicine for drug delivery and imaging: a promising avenue for cancer therapy and diagnosis using targeted functional nanoparticles. Int J Cancer. 2007;120(12):2527-37. doi: 10.1002/ijc.22709, PMID 17390371.
57. Ghosh V, Mukherjee A, Chandrasekaran N. Mustard oil microemulsion formulation and evaluation of bactericidal activity. Int J Pharm Pharm Sci. 2012;4(4):497-500.
58. Zheng Y, Xie Q, Wang H, Hu Y, Ren B, Li X. Recent advances in plant polysaccharide-mediated Nano drug delivery systems. Int J Biol Macromol. 2020;165(B):2668-83. doi: 10.1016/j.ijbiomac.2020.10.173, PMID 33115646.
59. Mondal A, Banerjee S, Bose S, Mazumder S, Haber RA, Farzaei MH. Garlic constituents for cancer prevention and therapy: from phytochemistry to novel formulations. Pharmacol Res. 2022;175:105837. doi: 10.1016/j.phrs.2021.105837. PMID 34450316.
60. Cao Y, Liu J, Ma H, Bai J, Qi C. Novel nano drug delivery systems for hepatic tumor. SPIE Proceedings. Eighth International Conference on Photonics and Imaging in Biology and Medicine (PIBM 2009). 2009;7519:751912. doi: 10.1117/12.844928.
61. Liu Y, Chen S, Xue R, Zhao J, Di M. Mefloquine effectively targets gastric cancer cells through phosphatase-dependent inhibition of PI3K/Akt/mTOR signaling pathway. Biochem Biophys Res Commun. 2016;470(2):350-5. doi: 10.1016/j.bbrc.2016.01.046. PMID 26780727.
62. Yan KH, Lin YW, Hsiao CH, Wen YC, Lin KH, Liu CC. Mefloquine induces cell death in prostate cancer cells and provides a potential novel treatment strategy *in vivo*. Oncol Lett. 2013;5(5):1567-71. doi: 10.3892/ol.2013.1259, PMID 23759954.