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Formulation and Evaluation of Natural Oil (Turmeric and Chenopodium Oil Nanoemulsion for Effective Treatment of Cancer

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ABSTRACT

Background: Turmeric oil and Chenopodium oil are natural oils with much evidence for their therapeutic actions including anticancer activity. Aim: Present studied was to explore the anticancer activity of nanoemulsion prepared by Turmeric and Chenopodium oil. Method: Tu-Ch oil nanoemulsion prepared using Tween 80 by ultrasonication. The anticancer activity of Nanoemulsion estimated by MTT cell viability assay against HeKa Toungue carcinoma cells. Results: To increase the stability of essential oils through Nanoemulsion. The optimized formulation of Nanoemulsion was studied at increased (oil: surfactant) ratio and evaluation by analysis of $_{P}$H, Viscosity, Zeta potential, Poly dispersity index, particle size distribution, and cell viability at different concentrations. The particle size of developed nanoemulsion (A2) was found to be 172.3 nm, viscosity of the nanoemulsion formulation was (28.55 ± 2.03mP) and Zeta potential was 0.240 ± 0.05, PDI value -28.0± 0.24 for Tu-Ch oil-loaded nanoemulsion. turmeric oil and chenopodium oils together in nanoscale showed a concentration-dependent cytotoxicity effect. Conclusion: This study proves that the Combination of Turmeric and Chenopodium Oil nanoemulsion is a permissible natural medicine that can overcome the drawbacks in cancer treatment.

INTRODUCTION

Oils are essential for a healthy diet. They frequently act as transmitters for fat-soluble vitamins including A, D, E, and K in addition to providing energy. Oils not only enhance food flavour but also provide essential fatty acids including linoleic, linolenic, and arachidonic acids. The organisation and content of the fatty acids present, affect the physical properties of triglycerides, which are the main constituents of oil (Mohammadi *et al.*, 2016; Rodriguez *et al.*, 2016). Considering Natural oils' nutritional and medicinal importance, there has been growing interest in technologies for modifying these oils in recent years. More and more research is being done on modification technologies to change the characteristics of oils and adapt them to particular applications. Researchers have developed different technologies to improve the quality and safety of food. Nanotechnology improves solubility, oral bioavailability, and heat stability (Hamad *et al.*, 2018). According to Pathania *et al.* (2018), nanoemulsions are actively stable emulsions in which surfactant molecules stabilise the aqueous and oily phase by lowering surface tension and occasionally by adding a co-surfactant. A nanoemulsion is an emulsion with nanoscale particles. To boost the bioavailability of pharmaceuticals, much research is being done on nanoemulsions as drug carriers. Viscous resistance has been successfully eliminated using high-energy ultrasound to produce strong shear and minimise droplet size. Translucent nanoemulsions have been successfully prepared on a laboratory scale using high-power ultrasonic. Due to the Nanoemulsion's small particle size, the medicine is retained for a more extended period and is more bioavailable, avoiding drug loss. The most alluring high-energy technique for creating nanoemulsions is ultrasonication. Due to its ability to produce tiny droplets with high efficiency, ultrasonic homogenization has proven to be both economical and practical minimum amount of energy consumed (Nirmala, Durai, Gopakumar, et al., 2020).

Nanoemulsions, which have a tiny size range of 20 nm to 200 nm, are essentially emulsions with a lipid phase distributed in a continuous aqueous phase and any oil. The droplet is surrounded by a thin boundary layer made up of emulsifying particles. They are considered profound, essential oil delivery vehicle systems with exclusive benefits. There are two advantages to having tiny droplets. First, the possibility of reinforcement boosts the stability and physicochemical properties of the material. On the other hand, by increasing the surface area of the unit, it increases the ability to promote biological activity-lipophilic substances. The bioavailability of encapsulated compounds is increased using nanoemulsion-based delivery methods, which also use low-dose active components. Numerous research have shown the antibacterial power of essential oils on nanoemulsions (Dhankhar et al., 2021). The combination of nanotechnology and recent years has seen significant advancement in medicine. Among the nanocarriers that enable the selective delivery of drugs to the tumor, nanoemulsions are highly valued for their extraordinary properties. Due to their variable rheology, high surface area per unit volume, and kinetic stability, many other advantageous characteristics, nanoemulsions are applied in various fields (Gupta *et al.*, 2016).

The related mortality of cancer has reduced due to improved cancer treatment. Cancer treatment is mainly based on the targeted therapy of different signalling pathways. Indeed, at some point, aggressive cancer patients have a terrible prognosis and develop resistance to chemotherapy medications. Preclinical and clinical studies and animal models used in recent cancer research have demonstrated the effectiveness in various natural compounds, primarily phytochemicals derived from plant extracts, in treating and chemo prevention of cancer (Faivre *et al.*, 2006).

Through various methods of action and structural changes, phytochemicals (bioactive compounds) prevent tumour development, angiogenesis, metastasis, and cell proliferation. They are extensively studied to discover how bioactive chemicals impact health. The molecular pathways of these natural substances control the suppression of cancer by cell cycle arrest, apoptosis, necrosis, and autophagy. Despite these benefits, the efficiency of these bioactive compounds might be impacted by variables such as stomach residence length and instability. Additionally, the suppression of apoptotic and anti-apoptotic protein expression levels during

cancer therapy has led to a rise in drug resistance. Therefore, these active substances need to overcome the cells' natural defence mechanism. The pharmaceutical industry has a significant problem in creating an effective cancer therapy because of the negative side effects of traditional medicines and the sharp rise in mortality to 8.8 million per year (Nirmala, Durai, Rao, *et al.*, 2020). Turmeric and Chenopodium oils are predominantly significant essential oils used in traditional medicine. In this study, nanoemulsions were formulated using these oils, and cytotoxicity was estimated using an MTT assay.

Materials and Methods

Turmeric oil was purchased from Kanta Enterprises, Noida, and Chenopodium oil from BO International. Delhi. The non-ionic surfactant Tween 80 and water were used to create a nanoemulsion of Chenopodium oil and turmeric oil. From Merck (Merck, India), PEG 400 and Tween 80 were received as a gracious gift sample from Gattefosse (Mumbai, India).

Formulation and Optimisation

Tween 80 and water were used to create a nanoemulsion out of chenopodium and turmeric oil. The non-ionic surfactant Tween 80's sufficient solubility with essential oils and ability to reduce droplet width by sticking to the droplet surface make the oil-in-water (O/W) emulsion system more stable. O/W nanoemulsions are made by combining the right proportions of water, Tween 80, and oil (turmeric and chenopodium oil) while stirring at 500 rpm for 10 minutes in a magnetic stirrer. The oil content was maintained at 6% (the same proportion of Chenopodium oil to turmeric oil). The necessary nanoemulsions are then made utilising a PCI (Probe sonicator-Advanced model) 20 kHz ultrasonicator with a 750 W input power processor to produce the emulsions. Each concentration had a different sonication time of 5, 10, and 15 minutes. By creating turbulence, the high-energy shock waves can tear the droplets apart. To check for any slight heat produced, the sample was put in a container with ice (Nirmala, Durai, Rao, *et al.*, 2020).

Characterisation of Nanoemulsion

Measurement of absorbances

The nanoemulsions absorbances at 600 nm were measured using a UV-visible spectrophotometer. The dynamic light scattering approach determined the droplet size and polydispersity index of nanoemulsions with different ratios. To decrease the impacts of various scattering effects, deionized water was used to dilute all samples to 10% before the experiment.

Viscosity, refractive index, conductivity, and pH

The Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, Middleboro, MA, USA) was used to measure the viscosity of the nanoemulsion. For each of the various nanoemulsion formulations, the refractive index was measured in triplicate at 25 °C using an Abbe's refractometer (Nirmal International, Delhi, India). The pH of the improved nanoemulsion was measured in triplicate at room temperature using a calibrated digital pH metre (Mettler Toledo MP 220, Greifensee, Switzerland). A digital thermal conductivity metre (1152, Emcee Electronics, Venice, FL, USA) was used to measure conductivity and monitor current flow (Baboota *et al.*, 2011).

Surface morphology by transmission electron microscopy

Using a Morgagni 268D transmission electron microscope (TEM) (FEI, Netherlands) that operates at 70 KV and has point-to-point resolution, the morphology and structure of the nanoemulsion were examined. The shape and size of the nanoemulsion droplets were revealed using a combination of diffraction modes and bright field imaging at escalating magnification. A drop of nanoemulsion containing 2% phosphotungstic acid was put on a carbon-coated grid and left for 30 seconds in order to conduct the TEM observations. On a slide, a cover slip was placed over the dried coated grid. Under the electron microscope, the slide was examined (Ali *et al.*, 2014).

Polydispersity Index and Zeta potential of Nanoemulsion

With clean water, the emulsion sample was diluted 100 times. To measure the PDI and particle size, the created Nanoemulsion was 100 times diluted and then injected into a disposable zeta cell (DT1060C) and the measurement chamber of a dynamic light scattering device. Following equilibration at 25°C for 2 minutes, samples were measured (Goh *et al.*, 2021). The high surface charge is the cause of the nanoemulsion's remarkable stability at high zeta values since stable particles are those with potentials between +30 mV and 30 mV. This increases the stability of the system,

encourages redistribution, and lessens the possibility of coagulation brought on by electrostatic repulsion between particles with the same electrical charge (Lee *et al.*, 2021). Using a Zetasizer Nano ZS, the zeta potential of the Tu-Ch oil nanoemulsions was calculated. A novel mixed-mode measuring methodology or light scattering phase analysis approach was used to estimate the zeta dimension. Extreme accuracy may be achieved in determining both the mean zeta potential and the distribution using this method (Azam *et al.*, 2023).

Anticancer activity:

Cell Culture

DMEM (Dulbecco's Modified Eagle Media) medium supplemented with 10% foetal bovine serum, two mM L-glutamine, 100 units/mL penicillin, and 100 g/mL streptomycin was used to grow HeKa 293 cell lines, while K562 cells were grown in the same medium. Cancer cell lines were grown at 37°C in incubators with 5% CO_2 and humidified air (Panyajai *et al.*, 2022).

Cell viability Measurement

10,000 HeKa293 cells were plated in 96-well plates and incubated for one day in RPMI 1640 medium at 37°C and 5% CO2. The next day, they received increasingly more treatments using the upgraded Tu-Ch oil nanoemulsion. A surfactant and water combination served as the adverse control. After a 48-hour treatment period, an MTT test was performed by adding MTT solution (0.5 mg/ml) to each well and incubation for around three hours. Dark blue Formazan crystals show that functional cells are present after reconstitution in DMSO. These crystals are not created by non-viable cells. At 570 nm, the absorbance was measured using a microplate reader. Cell viability was calculated as the percentage of formazan uptake (Yoon *et al.*, 2018). Results were expressed as the mean, standard deviation after every trial was carried out in triplicate throughout the three independent experiments. The cell viability was measured against varying concentrations, and data were analyzed through two-way ANOVA using SPSS software.

After that, the emulsions were prepared by making the necessary nanoemulsions using a PCI (Probe sonicator-Advanced model) 20 kHz ultrasonicator with a 750 W input power processor. Each concentration had a different sonication time of 5, 10, and 15 minutes. By

Results

Formulation and Optimisation

A nanoemulsion was made using Chenopodium and turmeric oil (1:1) water and Tween 80. The non-ionic surfactant Tween 80's sufficient solubility with essential oils and ability to minimise droplet width by sticking to the droplet surface make the oil-in-water (O/W) emulsion system more stable. The correct quantities of oil (Turmeric and Chenopodium oil), surfactant (Tween 80), and water are combined in a magnetic stirrer at a speed of 500 rpm for 10 minutes to create O/W nanoemulsions. In Table 1, the formulation is indicated. The oil content was maintained at 6% (the same proportion of Chenopodium oil to turmeric oil). creating turbulence, the high-energy shock waves can split the droplets apart. By putting the sample in a container with ice, the minimal heat produced was determined (Nirmala, Durai, Rao, *et al.*, 2020).

Table 1: Various formulations for optimizing Turmeric and Chenopodium oil nanoemulsion

Formulation code	Oil: Surfactant ratio	Turmeric + Chenopodium oil (Tu-Ch): Tween 80: Water(v/v)	Sonication time
F1-A1	1:1	6:6:88	5 mins
F2-B1	1:2	6:12:82	5 mins
F3-C1	1:3	6:18:76	5 mins
F1-A2	1:1	6:6:88	10 mins
F2-B2	1:2	6:12:82	10 mins
F3-C2	1:3	6:18:76	10 mins
F1-A3	1:1	6:6:88	15 mins
F2-B3	1:2	6:12:82	15 mins
F3-C3	1:3	6:18:76	15 mins

Characterisation of Nanoemulsion

The visual appearances of nanoemulsions after sonication time of 5, and 10, 15 mins were shown in Fig. 1.

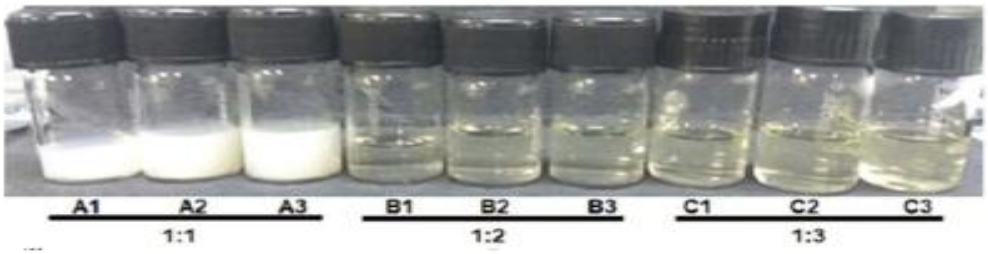

Fig. 1: Color changes of formulation from white to clear solutions after sonication time intervals

Measurement of absorbances

All Formulations underwent physicochemical evaluation with an ideal sonication time of 5 minutes. With increased surfactant content, a drop in absorbance is shown in Fig 2. With increased surfactant concentration, pH levels and Viscosity rise, as shown in (Fig. 4 & 5).

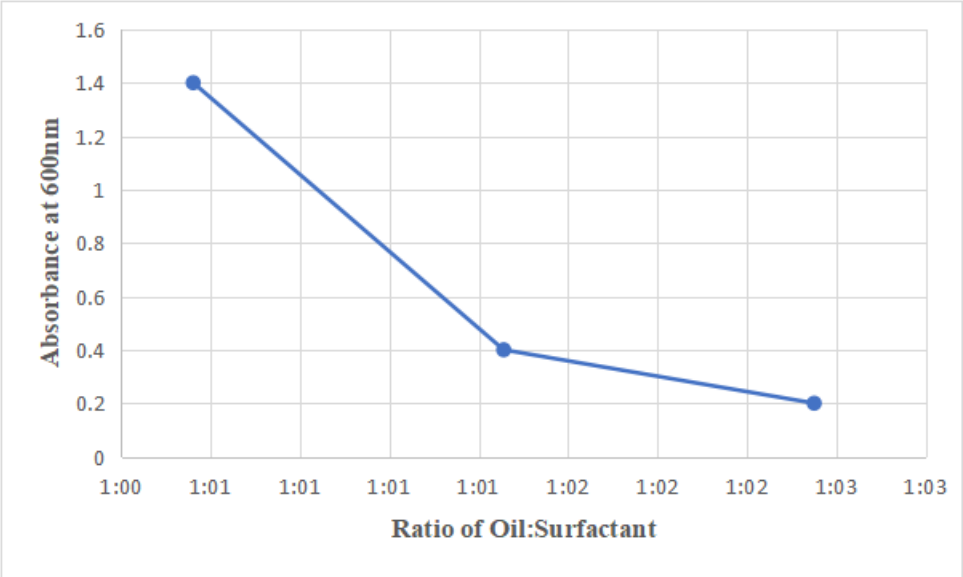

Fig. 2: Absorbance of Formulations with Oil: Surfactants at ratios of 1:1 1:2 and 1:3 measured at 600 nm.

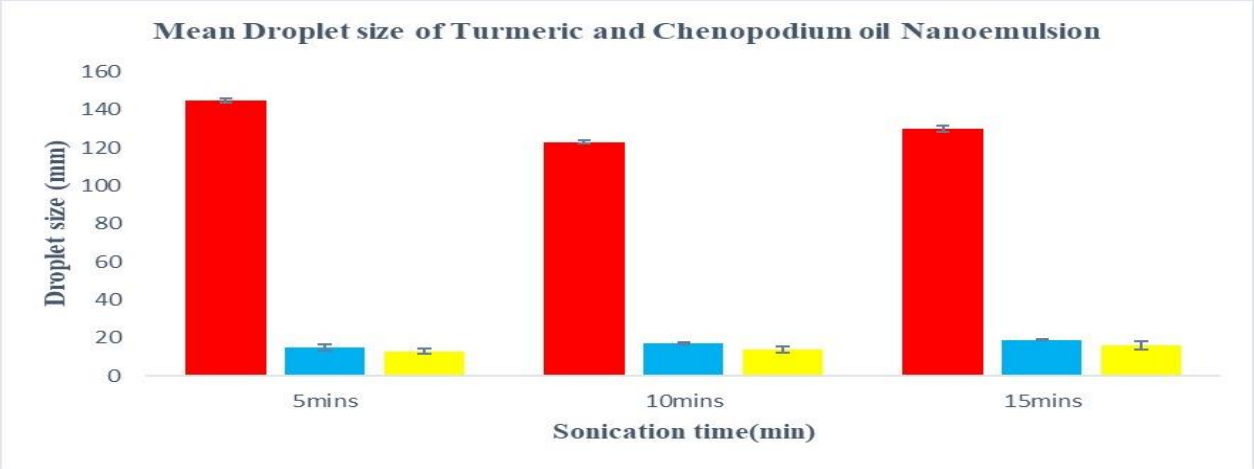

Fig. 3: Effect of Sonication time on varying formulations of Mean Droplet size of Turmeric and Chenopodium oil based nanoemulsion.

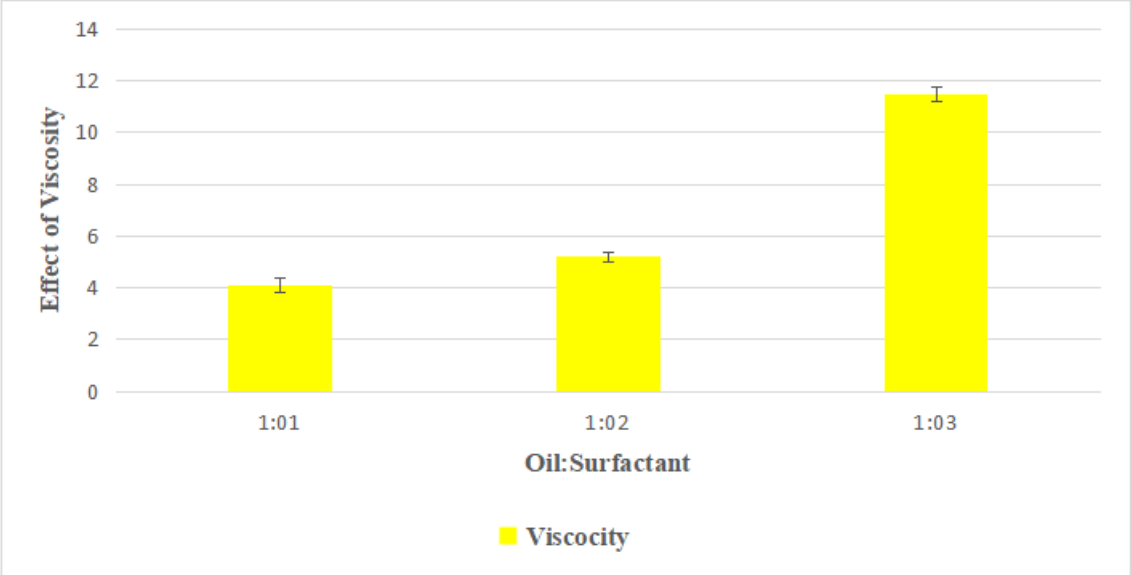

Fig. 4: Effect of Viscosity ($kgm^{-1}s^{-1}$) on Various Formulations with Oil: Surfactant ratio 1:1 to 1:3.

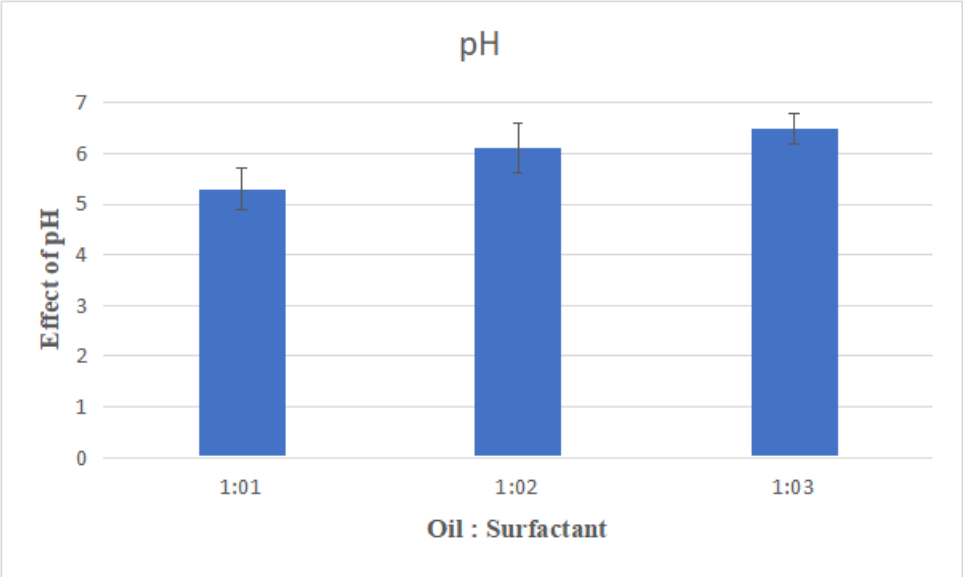

Fig. 5: Effect of pH on Various Formulations with Oil: Surfactant ratio 1:1 to 1:3.

Viscosity, refractive index, conductivity, and pH

As would be predicted for an o/w emulsion, the nanoemulsion (A2) formulation's viscosity was very low (28.55 ± 2.03mP). The low viscosity might be due to a shortage of oil and a low concentration of Tween 80, a fatty acid polyhydric alcohol ester with a high intrinsic viscosity. Refractive index, which indicates the formulation and represents the net value of an emulsion's component components, has an isotropic nature. Using an Abbes type refractometer (Nirmal International, New Delhi, India), the refractive index of the nanoemulsion was determined at a temperature of 25 ± 0.5 °C. It was discovered that the refractive index for the formulation A2 had a mean value of 1.409. It was discovered that nanoemulsion A2 has a specific conductivity of 10^{-4} s cm^{-1}. By using a pH metre (Accument AB 15, Fisher Scientific, USA) in triplicate at 25 ± 1°C, the apparent pH of the formulation was determined to be 6.4 (Table 2).

Polydispersity index (PDI) and Zeta potential:

Table 2. Optimized formulations of Tu-Ch oil nanoemulsions polydispersity index and zeta potential.

Formulation code	Poly Dispersity Index (PDI)	Zeta potential
F1-A1	0.32 ± 0.05	14.38±.14
F2-B1	0.34 ± 1.2	16.23±.21
F3-C1	0.28 ± 0.09	13.21±.16
F1-A2	0.240 ± 0.05	-28.0± 0.24
F2-B2	0.248 ± 0.53	15.17±.29
F3-C2	0.270 ± 0.117	17.57±.27
F1-A3	0.313 ± 0.98	14.46±.39
F2-B3	0.261 ± 0.45	15.22±.13
F3-C3	0.273 ± 0.32	14.35±.11

Zeta potential of was found to be -28.0± 0.24mv for Tu-Ch oil-loaded nanoemulsion (A2).

Particle size Distribution: Tu-Ch oil-loaded nanoemulsion (A2) showed a mean hydronamic diameter of less than 200nm, i.e 172.3 nm shown in Fig. 6.

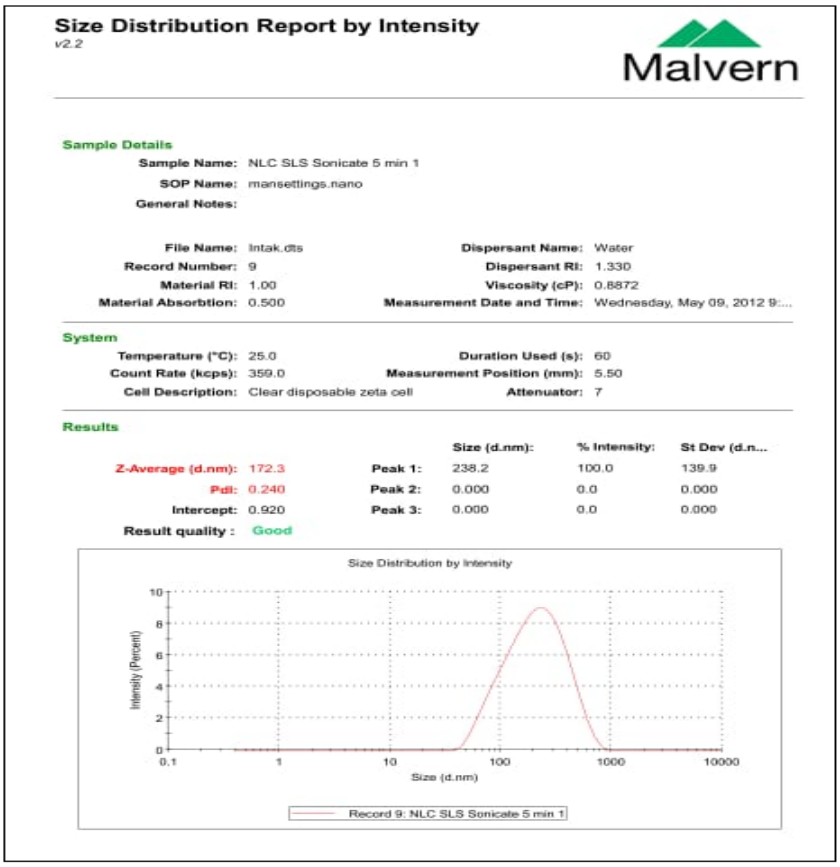

Fig. 6: Particle Size Distribution of nanoemulsion.

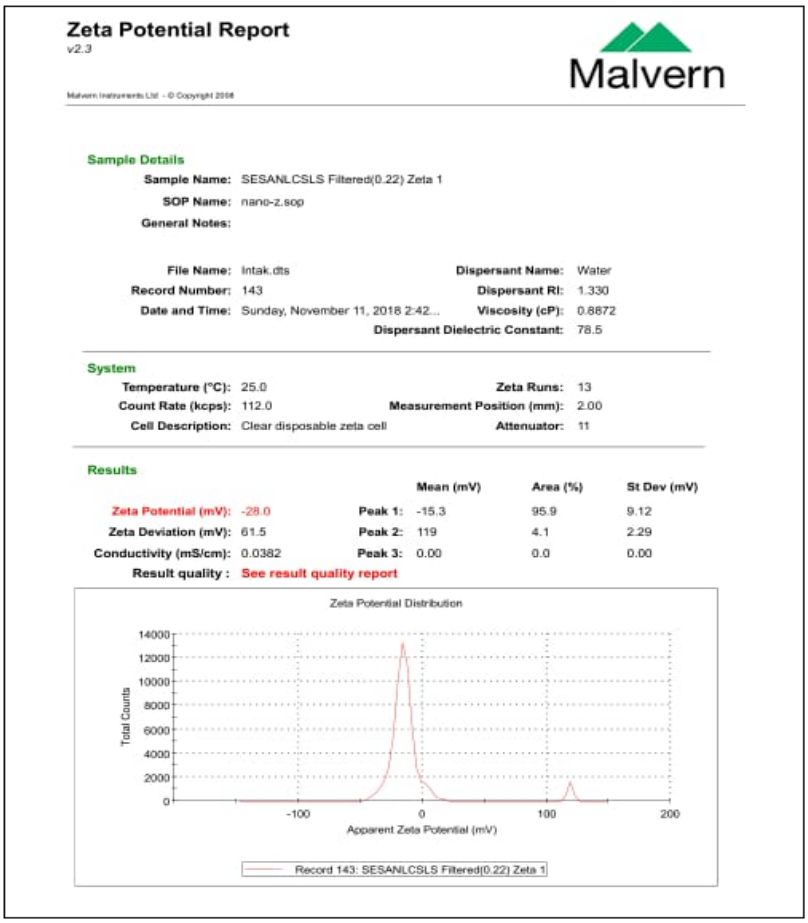

Fig 7: Zeta potential of optimized Tu-Ch oil-loaded emulsion

It is well known that the emulsifier employed, the kind of oil, and the manufacturing process all have an impact on the shape and size of nanoemulsion droplets (Lee *et al.*, 2021). Fig. 8 displays the TEM images from the surface of the Tu-Ch oil-loaded nanoemulsion used in this investigation. The Tu-Ch oil-loaded nanoemulsion had an agglomeration between the particles and a heterogeneous structure. This form is most likely a result of the sample being dried before TEM imaging.

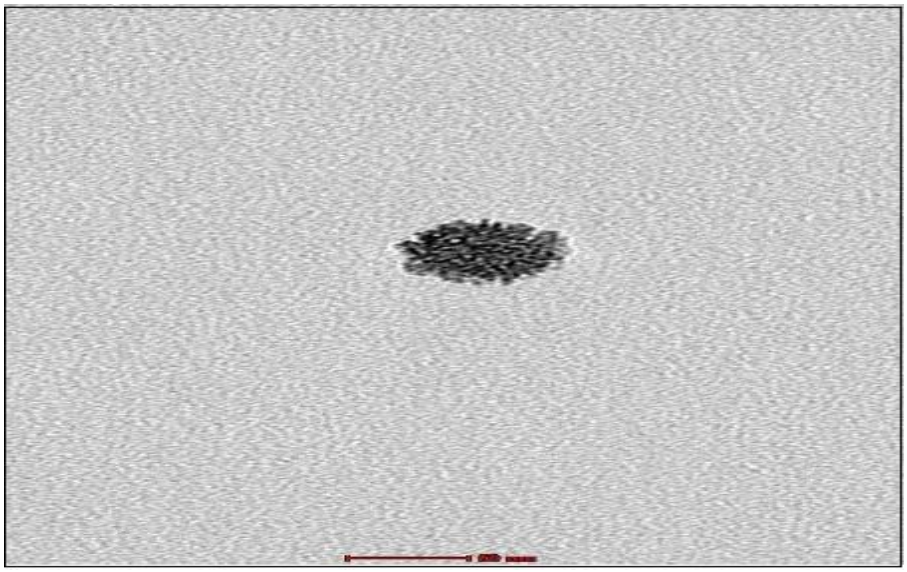

Fig 8: TEM image of Tu-Ch oil-loaded optimized Nanoemulsion.

Cytotoxicity of Tu-Ch loaded Nanoemulsion:

By measuring the amount of decreased MTT tetrazolium-linked formazan crystals produced by viable cells, cell viability was evaluated. The MTT (Related to Cell Metabolism and Cell Activity) methodology is a quantitative colorimetric method for assessing cytotoxicity, cell viability, and cell growth. HeKa 293 cell line, however, did not exhibit any cytotoxicity in response to the Nanoemulsion (Figure 9), indicating that the Nanoemulsion exclusively targets and suppresses tumour cell proliferation and expansion. With increasing exposure concentration, cell viability decreased.

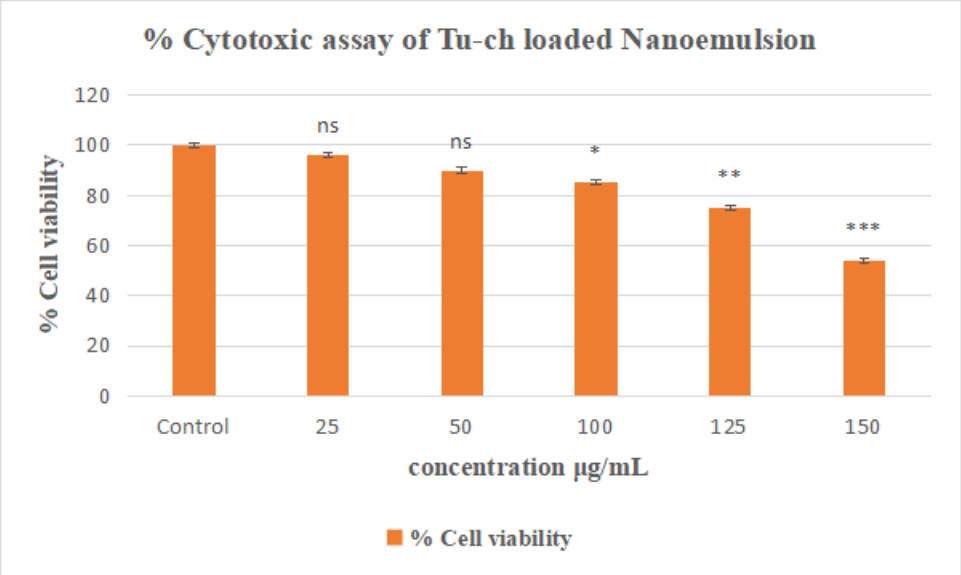

Fig. 9: Cytotoxicity assay of Tu-Ch (A2) loaded nanoemulsions at different concentrations against HeKa 293 cells. P-value *p<0.05,p<0.01, ***p<0.001,ns-not significant compared to a control group using two-way ANOVA in SPSS software.**

Discussions

The use of natural origin medications in the treatment of cancer involves extensive scientific research, including preclinical and clinical studies, in order to prove their safety, effectiveness, and ideal dose. Natural compounds can also be synthesised and modified to increase their potency and get around issues with availability and complexity. Alternative medicine is becoming more and more acceptable to modern man. More and more people are realising that adopting natural remedies is much more cost-effective and non-aggressive than using very potent drugs with synthetic origins (Marijanovic et al., 2021). Alkaloids, flavonoids, and glycosides are examples of important chemical compounds found in nature that can be used to create alternative medications. Numerous natural substances that were isolated from medicinal plants and herbs have both in vitro and in vivo antiproliferative and anticancer effects on a range of cancers. The most effective examples of produced anticancer medications include vinblastine, vinorelbine, vincristine, and vindesine (Mondal et al., 2019).

Several sesquiterpenes found in turmeric oil (Curcuma longa) have therapeutic effects. It has been determined that ar-turmerones are the primary constituents of petroleum fractions. According to Jacob and Toloue (2013), the non-cancerous cell line WI-38 exhibits less activity when exposed to the pure turmeric oil fractions

(TO), which have been shown to have anti-proliferative effects against breast cancer (SKBR-3), pancreatic cancer (PANC-1), and prostate cancer (PC-3). According to Gawlik-Dziki et al. (2013), chemopreventive and anticancer effects of chenopodium quinoa on oxidative stress and ROS-dependent intracellular signalling are exerted through synergistic effects, and the addition of Tween 80 as a surfactant enhances the encapsulation effectiveness and physicochemical stability of nanoemulsions. According to Kerdmuanglek et al. (2023), tween 80 is a non-ionic surfactant with a very low risk for causing irritation. Due to its special qualities and advantages over traditional targeted therapies, nanoemulsions have attracted a lot of attention in the field of cancer treatment. By strengthening targeting capabilities, optimising drug delivery, and offering therapies with regulated and prolonged release, nanoemulsions present a viable platform for the treatment of cancer. Cancer therapy may be revolutionised, and patient outcomes may be enhanced, with further research and development in this area (Pourmadadi et al., 2023). Because of the little droplets' increased surface area, penetration is made simpler. When the surfactant concentration was increased from 1:1, 1:2, and 1:3, the droplet diameter actually shrank around the time of sonication. Viscosity increases cause water and cross-link surfactant to become trapped. High levels of the Zeta potential reduce the coagulation It was shown that stability increased with longer emulsification times and higher surfactant ratios. The MTT test is useful for assessing anticancer efficacy. Tu-Ch laden Nanoemulsion demonstrated cytotoxicity that was concentration dependent. In several scientific domains, the MTT test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay) is a popular way to measure cell viability and cytotoxicity, including cancer research.

Conclusion

Tu-Ch oil-loaded nanoemulsions created using the ultrasonication approach resulted in uniformly sized droplets with sphere-like shapes at the nanoscale. Tongue cancer cells were cytotoxic to the formulation. Well-known traditional medicines turmeric oil and chenopodium oils together in nanoscale showed a concentration-dependent cytotoxicity.

Conflict of Interest: NIL

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