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DEVELOPMENT AND ASSESSMENT OF A NANOEMULSION CONTAINING CHENOPODIUM OIL AND TURMERIC FOR THE PURPOSE OF TREATING CANCER

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Abstract

Turmeric oil and Chenopodium oil are natural oils with much evidence for their therapeutic actions including anticancer activity. We explored the anticancer activity of nanoemulsion prepared by Turmeric and Chenopodium oil. Tu-Ch oil nanoemulsion was prepared using Tween 80 by ultrasonication. The anticancer activity of nanoemulsion estimated by MTT cell viability assay against HeKa tongue carcinoma cells. The developed Tu-Ch oil-loaded nanoemulsion has 172.3 nm particle size. Its viscosity was 28.55 ± 2.03 mP. The zeta potential was found to be 0.240 ± 0.05 with a PDI value of -28.0± 0.24. This formulation showed a concentration-dependent cytotoxicity effect. This study demonstrates that the combination of Turmeric and Chenopodium Oil nanoemulsion is a permissible natural medicine that has the potential to overcome the drawbacks in cancer treatment.

Keywords: Cancer, Evaluation, Formulation, Nanoemulsion, Optimisation.

1. INTRODUCTION

Oils are essential for a healthy diet. They frequently act as transmitters for vitamins that are fat-soluble, such as A, D, E, and K in addition to providing energy. In addition to improving food flavour, oils also include important fatty acids like linoleic, linolenic, and arachidonic acids. The organization and content of the fatty acids present, affect the physical properties of triglycerides, which are the main constituents of oil [1, 2]. Considering Natural oils' nutritional and medicinal importance, there has been growing interest in technologies for modifying these oils in recent years. Many 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 [3]. According to Pathania, nanoemulsions are actively stable emulsions in which surfactant molecules stabilize the aqueous and oily phases by lowering surface tension and occasionally by adding a co-surfactant [4]. 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 minimize 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 [5]. 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 characteristics of the substance. Alternatively, enlarging the unit's surface area 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 has shown the antibacterial power of essential oils on nanoemulsions [6]. 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. Nanoemulsions are used in many different industries because of their varied rheology, larger surface area, kinetic stability, and many other beneficial qualities [7].

The related mortality of cancer has reduced due to improved cancer treatment. Cancer treatment is mainly based on the targeted therapy of different signaling 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 of various natural compounds, primarily phytochemicals derived from plant extracts, in treating and chemoprevention of cancer [8].

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. These natural compound’s molecular mechanisms control the suppression of cancer by stopping the cell cycle, 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 effective cancer therapy because of the negative side effects of traditional medicines and the substantial increase in mortality to 8.8 million deaths per

year [9]. Turmeric and Chenopodium oils are predominantly significant essential oils used in traditional medicine. A nanoemulsion formulation was done in this investigation. using the above oils, and cytotoxicity was estimated by performing an MTT assay.

2. MATERIALS AND METHODS

Turmeric oil and Chenopodium oil was purchased from Sahibganj, Jharkhand, India. 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 Gattefosse graciously provided Tween 80 as a complimentary sample. (Mumbai, India).

2.1 Formulation and Optimisation

Tween 80 and water were used to create a nanoemulsion out of chenopodium and turmeric oil. The oil-in-water (O/W) emulsion system is more stable because Tween 80, a non-ionic surfactant, has adequate solubility with essential oils and the capacity to narrow droplet width by adhering to the droplet surface. O/W nanoemulsions are made by combining the right proportions of water, Tween 80, and oil (turmeric and cheno-podium 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). A PCI (Probe sonicator-Advanced model) 20 kHz ultrasonicator with a 750 W input power processor is then used to create the required nanoemulsions. The sonication times var-ied for each concentration and were 5, 10, and 15 minutes. By creating turmoil, the shock waves created with high energy can tear the droplets apart. To check for any slight heat produced, the sample was put in a container with ice [9].

2.2 Characterisation of Nanoemulsion

2.2.1 Measurement of Absorbances

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

2.2.2 Viscosity, Refractive Index, Conductivity, and ph

The viscosity of the nanoemulsion was determined using a Brookfield DV III ultra V6.0 RV cone and plate rheometer. An Abbe's refractometer was used to test the refractive index in triplicate for each of the different nanoemulsion formulations at 25 °C. A digital calibrated pH meter was used to determine the pH of the enhanced nanoemulsion in triplicate at room temperature. To measure conductivity and track current flow, a digital thermal conductivity meter was utilized [10].

2.2.3 Morphology of Surfaces Using Transmission Electron Microscopy

The shape and structure of the nanoemulsion were investigated using a Morgagni 268D transmission electron microscope (TEM) that runs at 70 KV and has a point-to-point resolution. Using a combination of diffraction modes and bright field imaging at increasing magnification, the shape and size of the nanoemulsion droplets were made apparent. The nanoemulsion drop containing 2% phosphotungstic acid was put on a carbon-coated grid and left for 30 seconds in order to conduct the TEM observations.

Over the dried coated grid on a slide, a cover slip was positioned and observed under the electron microscope [11].

2.2.4 Zeta Potential and the Polydispersity Index

With clean water, the emulsion sample was diluted 100 times. To measure the PDI and particle size, the produced Nanoemulsion was injected into a disposable zeta cell (DT1060C) and the measuring chamber of a dynamic light scattering instrument after being 100 times diluted. Samples were measured after equilibration at 25°C for 2 minutes [12]. 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 [13]. The Tu-Ch oil nanoemulsion’s zeta potential was calculated using a Zetasizer Nano ZS. 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 [14].

2.3 Anticancer Activity

2.3.1 Cell Culture

The media used was DMEM (Dulbecco's Modified Eagle Media) with 10% foetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 g/mL streptomycin added to grow HeKa 293 cell lines, while the same medium was used to culture K562 cells. Cancer cell lines were raised at 37°C in humidified incubators with 5% CO_2 [15].

2.3.2 Cell Viability Measurement

In 96-well plates, 10,000 HeKa293 cells were plated and incubated in RPMI 1640 medium at 37 °C for a day 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, by applying MTT solution (0.5 mg/ml) to each well, an MTT test was carried out and incubated for 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. The absorbance was assessed at 570 nm using a microplate reader. Calculating cell viability was done using the formazan uptake percentage [16]. Results were presented as the mean and standard deviation following the completion of each trial in triplicate throughout the three separate tests. The cell viability was measured against varying concentrations, and data were analyzed through two-way ANOVA using SPSS software.

3. RESULTS

3.1. Formulation and Optimisation

Using a 1:1 ratio of Chenopodium and turmeric oil, water, and Tween 80, a nanoemulsion was created. The chargeless surfactant Tween 80's adequate solubility with essential oils and ability to minimize droplet width by sticking to the surface of the droplet make the oil-in-water (O/W) emulsion system more stable. O/W nanoemulsions are produced by mixing the appropriate amounts of oil (Turmeric and Chenopodium oil), surfactant (Tween 80), and water using a magnetic stirrer at 500

rpm speed for 10 minutes. In Table 1, the formulation is indicated. The oil content was maintained at 6% (the same proportion of Chenopodium oil to turmeric oil). The required nanoemulsions were created utilizing a PCI (Probe sonicator-Advanced version) 20 kHz ultrasonication with a 750 W input power processor to create the emulsions. The sonication times for each concentration were 5, 10, and 15 minutes, respectively. The high-power shock waves split the droplets apart by producing turbulence. The smallest amount of heat generated was measured by placing the sample in a container with ice [9].

Table 1: Formulations of Turmeric and Chenopodium Oil Nanoemulsion for Optimization

Formulations	Oil:Surfactant	Tu-Ch oil: 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

Tu-Ch oil- Turmeric + Chenopodium oil

3.2 Characterisation of Nanoemulsion

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

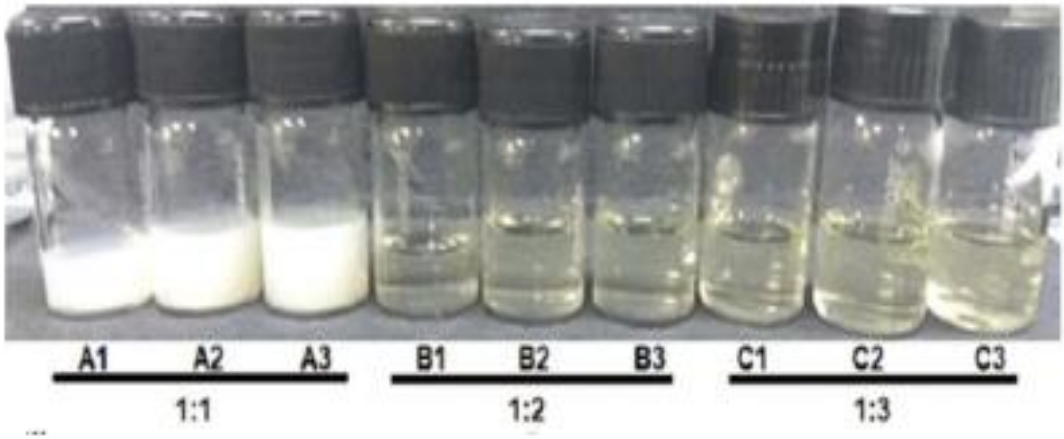

Figure 1: Color changes of Formulation from White to Clear Solutions after Sonication Time Intervals

3.2.1 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 figure 2.

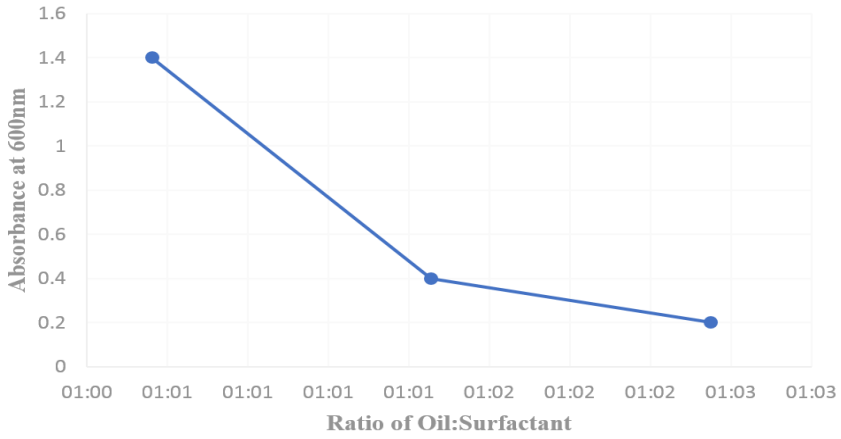

Figure 2: Absorbance of Formulations with Oil: Surfactants at ratios of 1:1 1:2 and 1:3 measured at 600 nm

The Effect of Sonication time on varying formulations of Mean Droplet size of Turmeric and Chenopodium oil based nanoemulsion are shown in figure 3.

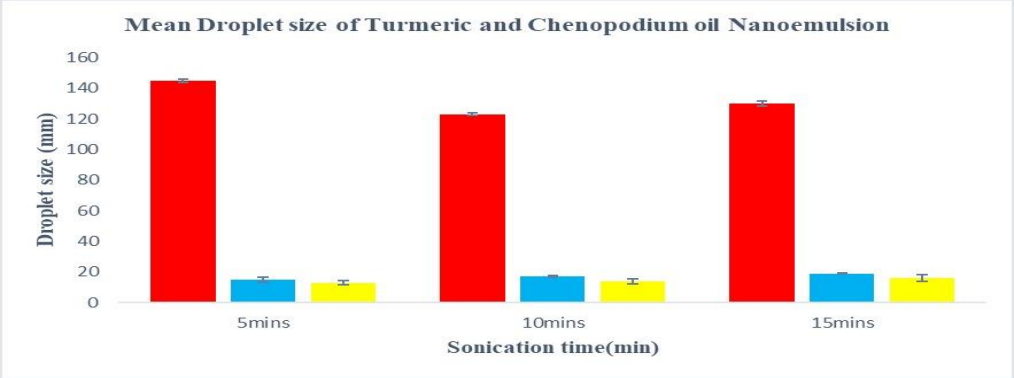

Figure 3: Effect of Sonication time on varying formulations of Mean Droplet size of Turmeric and Chenopodium oil based nanoemulsion

3.2.2 Viscosity, Refractive Index, Conductivity, and pH

With increased surfactant concentration, pH levels and viscosity rise, as shown in (Fig. 4 & 5).

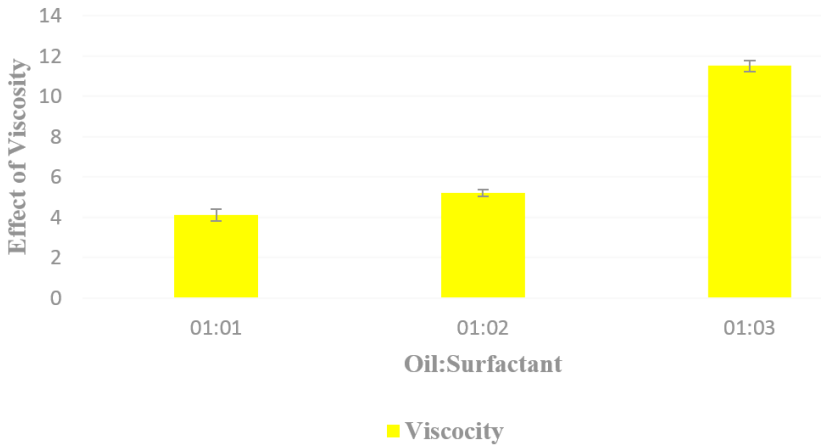

Figure 4: Effect of Viscosity ($kgm^{-1}s^{-1}$) on Various Formulations with Oil: Surfactant ratio 1:1 to 1:3

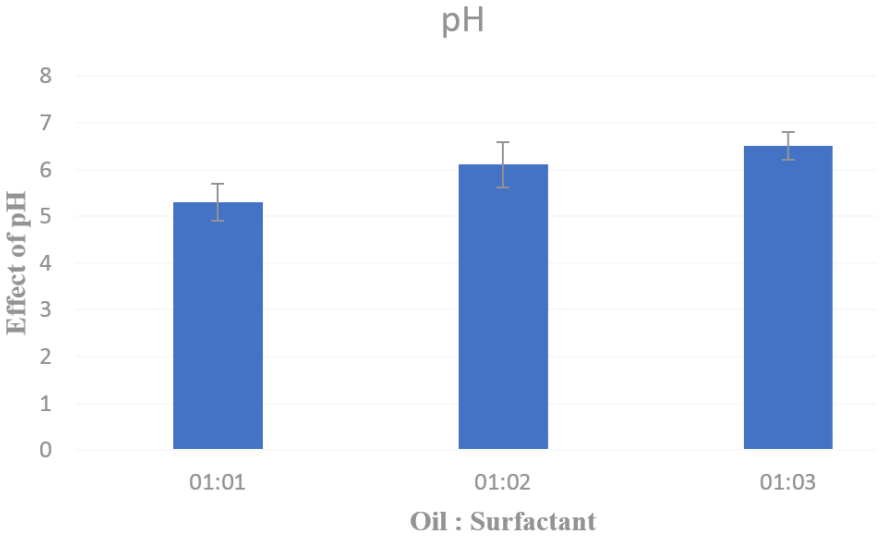

Figure 5: Effect of pH on Various Formulations with Oil and Surfactant ratio 1:1 to 1:3

As would be predicted for an o/w emulsion, the nanoemulsion (A2) formulation's viscosity was very low (28.55 ± 2.03mP). The refractive index, which indicates the formulation and represents the net value of an emulsion's component components, has an isotropic nature. The refractive index of the nanoemulsion was calculated using an Abbes-type refractometer (Nirmal International, New Delhi, India) at a temperature of 25 ± 0.5 °C. The refractive index for the formulation A2 had a mean value of 1.409. The nanoemulsion A2 has a specific conductivity of 10^{-4} s cm^{-1}. The apparent pH of the formulation was found to be 6.4 using a pH meter in triplicate at 25 1°C (Accument AB 15, Fisher Scientific, USA). (Table 2).

3.2.3 Zeta Potential and the Polydispersity Index

Table 2: Zeta Potential and Polydispersity Index of Optimized Tu-Ch oil Nanoemulsions

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).

3.2.4 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.

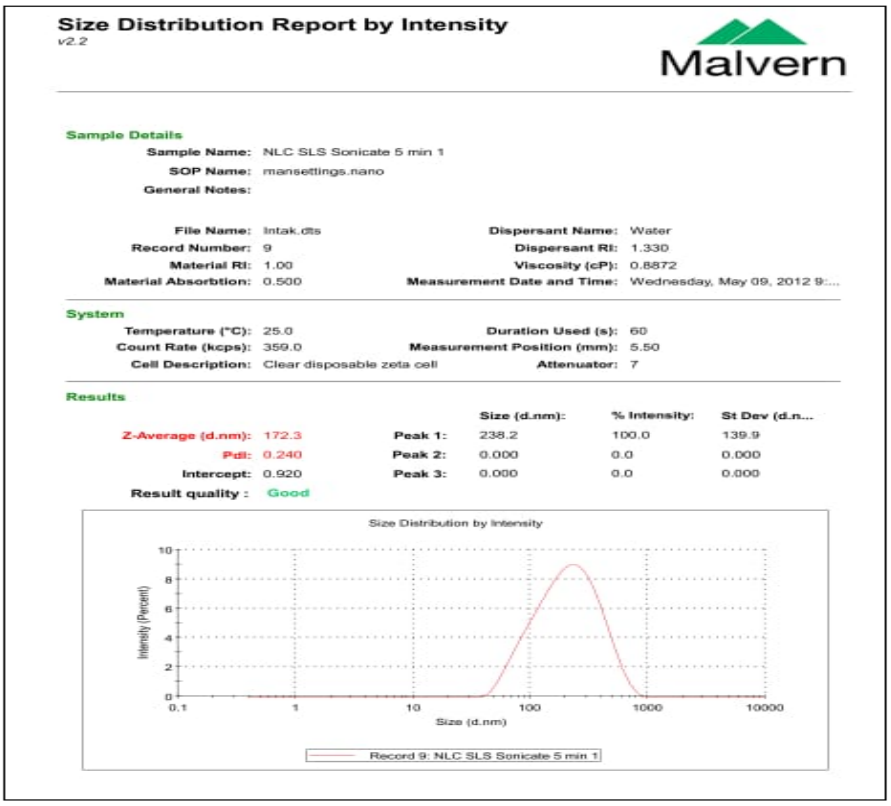

Figure 6: Particle Size Distribution of Nanoemulsion

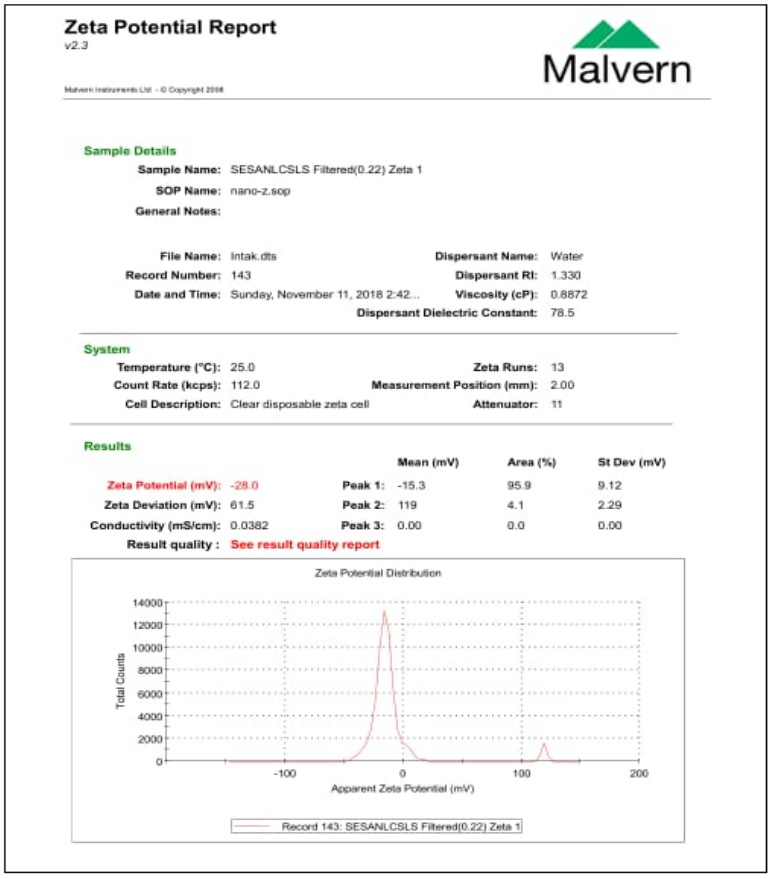

Figure 7: Zeta Potential of Optimized Tu-Ch oil-loaded Emulsion

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 [13]. 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.

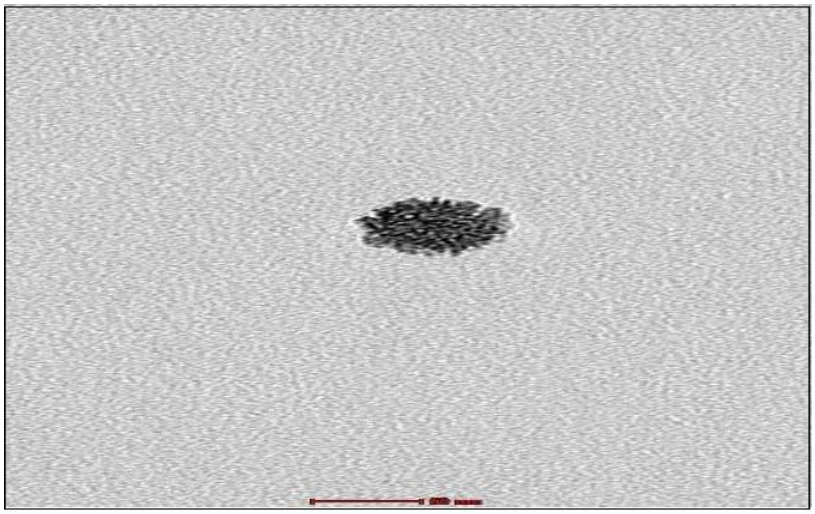

Figure 8: TEM Image of Tu-Ch oil-loaded Optimized Nanoemulsion

3.3 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 (Associated with cell activity and metabolism) methodology is a quantitative colorimetrical method for evaluating cell viability, growth, and cytotoxicity. HeKa 293 cell line, however, did not exhibit any cytotoxicity in response to the nanoemulsion (Figure 9), indicating that the nanoemulsion specifically targets and inhibits the growth and multiplication of tumour cells. With increasing exposure concentration, cell viability decreased.

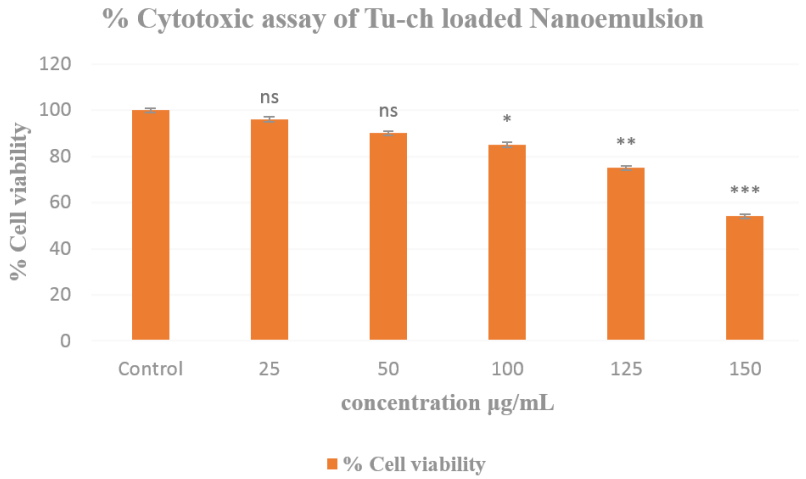

Figure 9: Cytotoxicity assay of Tu-Ch (A2) Loaded Nanoemulsions at Different Concentrations against HeKa 293 Cells in Comparison to a Control Group using two-way ANOVA in SPSS Software

*p<0.05, **p<0.01, ***p<0.001, ns-not significant

4. DISCUSSION

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 synthesized 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 realizing that adopting natural remedies is much more cost-effective and non-aggressive than using very potent drugs with synthetic origins [17]. 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 [18]. 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. Jacob and Toloue study showed that the healthy 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 neoplasms (SKBR-3), pancreatic neoplasms (PANC-1), and prostate neoplasms (PC-3) [19]. Gawlic et al observed 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 [20]. Kerdmuanglek et al found Tween 80 is a chargeless surfactant with a very low risk of causing irritation. Due to its special qualities and advantages over traditional targeted therapies, nanoemulsions have garnered a lot of interest in the field of cancer treatment [21]. 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 revolutionized, and patient outcomes may be enhanced, with further research and development in this area [22]. Because of the little droplets' increased surface area, penetration is made simpler. An increase in surfactant concentration shrank the droplet diameter around the time of sonication. Viscosity increases causing 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 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, also known as the MTT test, is a widely used method to assess cytotoxicity and cell viability in cancer research.

5. CONCLUSIONS

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.

Acknowledment

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