مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد الرابع والعشرون - المجلد الخامس - علوم الحياة - كانون أول 2024 م
doi.org/10.52866/esj.2024.05.24.27

Study the antihemolytic activity of biologically synthesized nanoemulsion of *Cuminum cyminum* L. essential oils

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Abstract:

The use of nanoencapsulated essential oils is interesting much consideration for medical applications. Numerous of the nanoemulsion production methods depend on toxic chemicals as a reducing agent. For the reasons aforementioned, it is important to investigate plant resources with biocompatible and robust antioxidants activity occurring already within the human food chain with non-toxic properties, this brings this study to incorporate plant with nanotechnology to explorer the antioxidant and anti-hemolysis action for the cumin nanoemulsion. The cumin nanoemulsion was formulated with tween 80 as non-ionic surfactant using ultra-sonication technique. Gas Chromatography-Mass Spectroscopy (GC-mass) was achieved for study the cumin oil phytochemicals. The major component detected in cumin essential oils was 4-(1-methylethyl) benzaldehyde (cuminaldehyde). Transmission Electron Microscope (TEM) used to determine the particle shape, size and distribution, the nanoemulsion revealed uniform and homogenous spherical droplet with size range ٢٠.91nm. the Dynamic light scattering (DLS) results shows droplets for cumin nanoemulsion was 155.9 nm with the polydispersity index 0.28. Furthermore, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay was studied, it has been shown that cumin nanoemulsion exhibit antioxidant activities, it was (76.57 %) as compared with cumin essential oil. Nanoemulsion biocompbatibilty was studied, it was exhibiting no blood hemolysis for all concentrations (0.2, 0.01,0.02,4.7,4.8,24,50,75). These nanoemulsion could be candidate as a drug delivery vehicle due to their natural ingredients and potential biocompatibility efficacy.

Keywords: Nanoemulsions, cumin, DPPH test, biocompatibilty, Drug Delivery.

دراسة فعالية المستحلب النانوي المضادة لتحلل الدم المنتج بايولوجيا من الزيوت الاساسية لنبات الكمون

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مستخلص:

اصبح استخدام الزيوت الاساسية النباتية المغلفة من الاهتمامات الحالية في مجال التطبيقات الطبية. تعتمد العديد من طرق إنتاج المواد النانوية على المواد الكيميائية السامة كعامل اختزال. لذلك، من المهم الكشف عن موارد النباتات التي تتمتع بنشاط مضاد للأكسدة قوي ومتوافق حيوياً والتي تكون موجودة ضمن سلسلة الغذاء البشرية بخصائص غير سامة، لذلك دمجت هذه الدراسة بين علم النباتات وتكنولوجيا النانو لأستكشاف فعالية للمستحلب النانوي للكمون المضادة للأكسدة والمضادة لتحلل كريات الدم الحمراء، حيث تم تحضير المستحلب النانوي للكمون باستخدام توين 80 كعامل سطحي غير أيوني باستخدام تقنية الصوتنة العالية. استخدم تحليل الغاز الكروماتوغرافيا- الكتلة (GC-mass) لدراسة المواد الكيميائية النباتية الزيتية للكمون وظهر اعلى قمة لمركب الديهايد الكمون بالإضافة الى مركبات اخرى. تم دراسة شكل قطيرات المستحلب النانوي وحجمها وتوزيعها بالمجهر الإلكتروني الناقل (TEM) ، حيث ظهرت بشكل كروي متجانس بنطاق 20.91 نانومتر. أظهرت نتائج تشتت الضوء الديناميكي (DLS) أن قطرات مستحلب الكمون النانوي كانت 155.9 نانومتر مع مؤشر تعدد التشتت 0.28. علاوة على ذلك، تمت دراسة مقايسة 2،2-ثنائي فينيل-1-بيكريل هيدرازيل (DPPH)، وقد تبين أن مستحلب الكمون النانوي له نشاط مضاد للأكسدة، وبلغت (76.57٪) مقارنة مع زيت الكمون العطري. تمت دراسة التوافق الحيوي للمستحلب النانوي، ولم يظهر أي انحلال للدم لجميع التراكيز (0.2، 0.01،0.02،4.7،4.8،24،50،75). يمكن أن يكون مستحلب النانو هذا مرشحًا كوسيلة لتوصيل الأدوية نظرًا لمكوناته الطبيعية وفعاليته المحتملة للتوافق الحيوي.

الكلمات المفتاحية: المستحلب النانوي ، نبات الكمون ، التوافق الحيوي،اختبار DPPH ، توصيل الدواء.

Introduction

Cumin *(Cuminum cyminum L.),* renowned for its aromatic fragrance and therapeutic properties, originated in South Asia, the Mediterranean, and Egypt before spreading to tropical regions. Its extensive history encompasses culinary, cultural, and medicinal uses, making it a versatile spice. Cumin is rich in beneficial compounds like alkaloids, flavonoids, and terpenoids, with cumin aldehyde playing a pivotal role in its medicinal effects. Mainly derived from its seeds, cumin is recognized for its significant medical potential, positioning it as the second most favored spice globally within the Apiaceae family [1-4]. The use of plant materials, such as herbal extracts and essential oils, in the creation of healthy and functional foods has garnered significant attention. This is due to their abundance in biologically active ingredients, which possess a diverse range of health-promoting attributes that contribute to human well-being (5). Plant extracts have been explored for their medicinal properties, including antimicrobial, anti-inflammatory, and anticancer activities (6). Plant extracts rich in antioxidants contribute to cellular protection, potentially delaying aging processes and reducing oxidative stress (7,8). Plant extracts contribute to the development of nutraceuticals and functional foods, providing natural sources of bioactive compounds with potential health benefits (9). Nanotechnology is the study of matter at the nanoscale. It offers enormous promise across multiple sectors by integrating the natural sciences materials in comparison to bigger sizes. nanotechnology focuses on producing and stabilizing different types of nanomaterials and Nano particles (10,11), making it a innovation of the 21st century. Falling within droplet size ranges of 20 to 500 nm represent a specialized class of emulsions. Their utilization in biomedical contexts is broad due to their small droplet sizes, which confer notable characteristics like strong stability and adjustable rheology. These Nanoemulsions find widespread application in pharmaceutical formulations for various delivery methods such as topical, ocular, and intravenous routes. particularly concerning white cumin seed oil, frequently entails forming Nanoemulsions or nanoparticles. These size reductions may induce alterations in the properties, bioavailability, and potential uses of the substance. (12) Nano-sized formulations of cumin seed oil may exhibit improved stability due to reduced aggregation and enhanced solubility. Nanoemulsions designed for intravenous administration, which do not cause hemolysis, hold significant potential in drug delivery. They offer enhanced solubility and bioavailability for hydrophobic drugs. These Nanoemulsions consist of tiny droplets stabilized by surfactants, enabling the encapsulation of lipophilic therapeutic agents (13).

2- Materials and Method

2-1 Cumin Nanoemulsions preparation

Cumin seed oil (*Cuminum cyminum* L.) was purchased from local market in Baghdad-Iraq. The non-ionic surfactant, (polyethylene, glycol sorbitan mono-oleate also commonly known as tween 80 (Labo Cheme. India) deionized water was used in analytical grade. Cumin Nanoemulsions were prepared using the three components: cumin seed oil, distilled water, and tween 80. Tween 80 was chosen due to its favorable water-in oil (W/O) characteristics. Nanoemulsions formulation process is a two-step process, start configuring of W/O macroemulsion by mixing cumin seed oil at different concentrations (0.2, 0.01,0.02,4.7,4.8,24,50,75), tween 80 and deionized water and using a magnetic stirrer at a speed of ١٠٠٠ rpm for 10 min and then ultra-sonication for 20 minutes at 25 ^{0}C then they were kept in dark vial to prevent light from reaching them at 4 ^{0}C for further characterization.

2-2 Gas chromatography-mass spectrometry (GC–MS)

GC-MS analysis of cumin essential oil and its nanoemulsions was carried out GC equipped with HP-5MS capillary fused silica column (30m x0.25 micrometre), the oven temperature was held at 45 C for 1 min then programmed at 5 C/min to 250C, and held for 20 min. Other operating conditions were as follows: carrier gas, Helium (99.999%), inlet pressure 85.4 Kpa, linear velocity 44.2 cm/sec, injector temperature 280c, detector temperature 250 C, and split ratio1:50.

2-3 Characterization of Nanoemulsions

The droplet size and poly disparity index of emulsions were analyzed using the dynamic light scattering technique (DLS) (Horiba, Nanopartical SZ-100 series), all the samples were diluted to 10% with deionized water to reduce the effects caused by multiple scattering effects.

2-4 DPPH Radical-Scavenging Activity

The DPPH scavenging assay using cumin essential oil and its nanoemulsions was studied. The cumin essential oil was diluted with ethanol. For positive control, firstly, 750 ml of both DPPH and Ascorbic acid was mixed, then shaken vigorously for 20 min. For negative control, 750 ml of DPPH and ethanol was mixed and then shaken vigorously for 20 min, then add essential oil and nanoemulsions with 750 ml of both DPPH. The absorbance was measured at 515 nm by the microplate reader. The percentage inhibition free radical scavenging rate of DPPH was calculated as follows:

DPPH scavenging activity (Inhibition%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

2-5 Anti-hemolysis activity

To study cumin annoemulsion biocompatibility, blood samples was obtained from healthy volunteers and anticoagulated using EDTA tubes 20 µl of blood samples were mixed with cumin essential oil and cumin nanoemulsions and shaken in a roller mixer (karl Kolb). Next, the incubator was 37 ˚C for 60 min for CBC analysis (systmex).

3- Results and discussion

Cuminum cyminum oil are light yellow solution as shown in fig. (1).

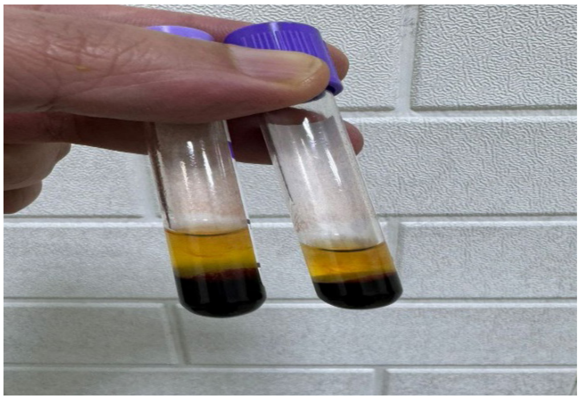

Figure 1: cumin oil

Figures (2,3) illustrate chromatograph for the highest peak area is cumin aldehyde by GC-Ms analysis as well as for another active component in GC-MS of *Cuminum cyminum* essential oil and its nanoemulsions components which are important as anti-oxidant agents (14). Figure 3, Identification of *Cuminum cyminum* by GC.MS was performed for nanoemulsions at concentration 4.7v/v the peak area and retention time.

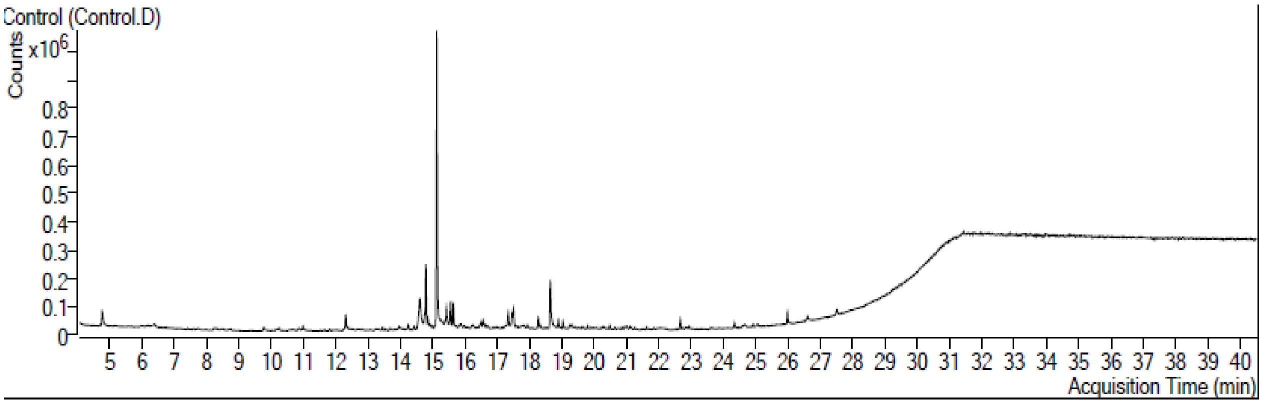

Figure 2. GC-MS revealed cumin aldehyde with highest Peak for the cumin cumin oil

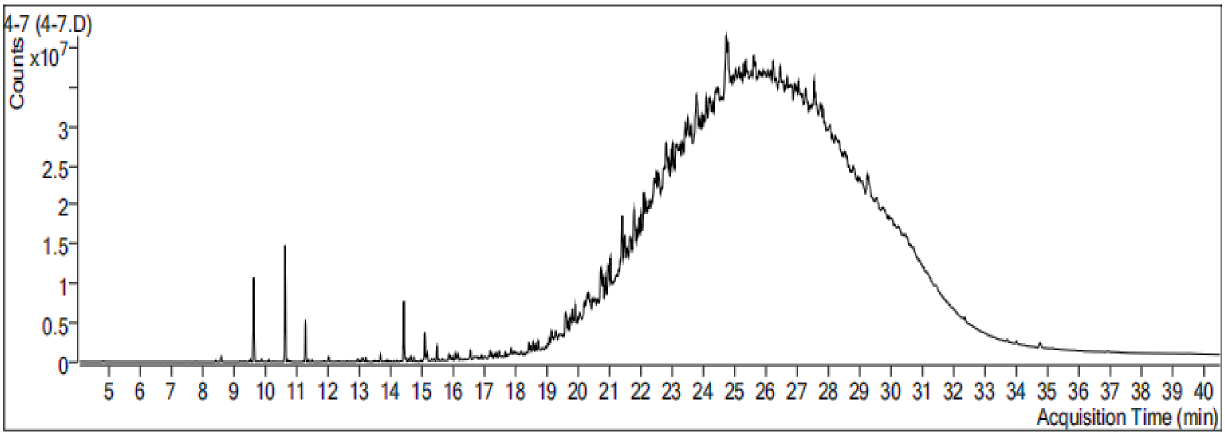

Figure 3. the test revealed cumin aldehyde (4-(1-methylethyl) benzaldehyde) with highest Peak for Nanoemulsions concentration 4.7v/v

Dynamic light scattering (DLS) is a method used to identify small particles in a suspension or polymers in a solution, when interpreting the particles formed in Cumin oil found the mean size of particle nanoemulsion prepared from cumin oil was 207 nm as shown in fig. (4).

The average particle size of 207.4 nm indicates the average diameter of the particles in the cumin oil emulsion. The predominant size is 207.3 nm, but experimentally the size of the most common particles is slightly smaller at 182.4 nm. This may mean a buffered distribution with many smaller particles, but there are also many larger particles, increasing the average. Fig. (4) shows data of a size distribution curve with a peak mode at 182.4 nm, a spread indicating a particle size range around an average of 207.3 nm. The width of this spread is related to the standard deviation, which indicates the extent of size variation within the sample.

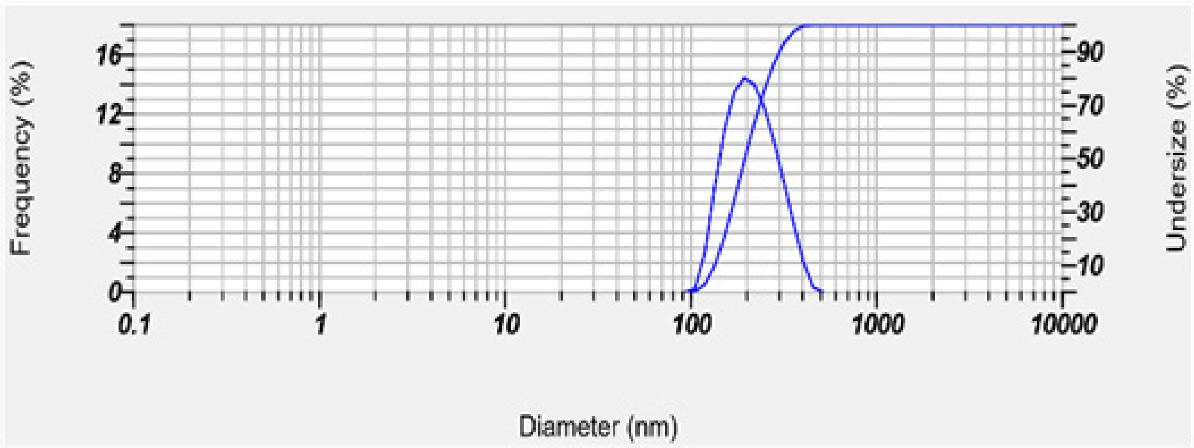

fig (4) shows the average particle size of nanoemulsion prepared from cumin oil

In this study, scanning electron microscopy (SEM) revealed an average nanoemulsion droplet size of 49.235 nm for a concentration 0.2 v/v. The SEM images (Figure 5) show mostly spherical droplets, which indicates a stable and uniform emulsion that confirms the size distribution of the droplets. The small droplets size of nanoemulsion reflect the behavior well-formulated nanoemulsions. This finding is compatible with previous report (15) where a similar formulation resulted in droplet sizes of 2.59 nm. The implications of achieving a droplet size of 49.235 nm are important for the intended application in targeted drug delivery, as smaller droplets can enhance bioavailability. In Figure (6), the SEM examination revealed an average nanoemulsion droplet size of 122.966 nm, within the typical size of nanoemulsions, which usually ranges from (20-200) nm, which indicates that the emulsion has been successfully formulated (16).

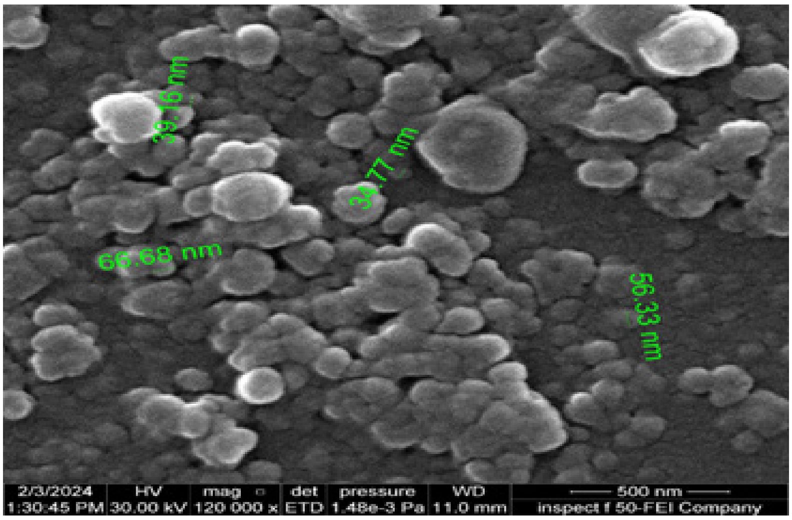

Figure (5) shows the shape and size of nanoemulsion particles prepared from cumin oil with a concentration of 0.2 d/d

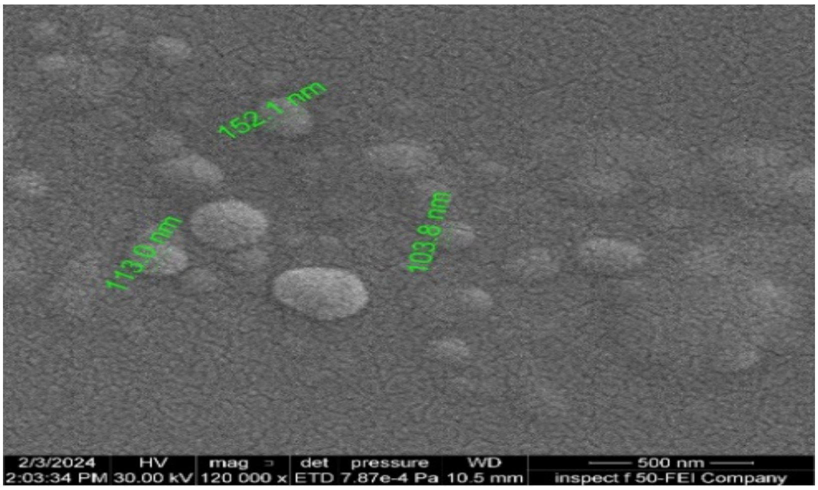

Figure (6) shows the shape and size of the nanoemulsion prepared from cumin oil at a concentration of 0.02 d/d.

DPPH Anti-oxidant test

The DPPH test is a widely used method to evaluate the ability of compounds to scavenge free radicals and is an indicator of their antioxidant activity. It is radical and stable. The DPPH radical has a charcteristic absorption at 517 nm, which decreases upon reduction by an antioxidant. The degree of color change indicates the ability of the antioxidant compound to Suppression. Ascorbic acid, one of the known powerful antioxidants, was used as a reference compound in this study. The activity of the acid in suppressing free radicals was 87.09%. The high value indicates the high effectiveness of the acid in suppressing free radicals. The antioxidant activities in the test samples were compared with the activities of ascorbic acid whenever it increased. The percentage of DPPH inhibition is the stronger the antioxidant activity in the sample. Antioxidants of 75.65 in the concentration v/v of 2% indicate that they are stronger than the concentration of 1%, which has antioxidants of 65.15, indicating the important effect of the oil concentration on the percentage of antioxidants as shown in Table 1 and Figure 7.

The stronger antioxidant activity of the essential oil nanoemulsions can be related to the smaller size, greater solubility, and better permeability of droplets than essential oil emulsions; as a result, more free radicals are involved by the scavenging effects of essential oil nanoemulsions. Moreover, pure essential oil is not able to dissolve in aqueous systems (17) which reduces its antioxidant activity compared with the nanoemulsion essential oil (18). However, nanoemulsions can dissolve in aqueous systems, which leads to the efficient release of activated compounds; subsequently, they can more effectively scavenge radicals (19).

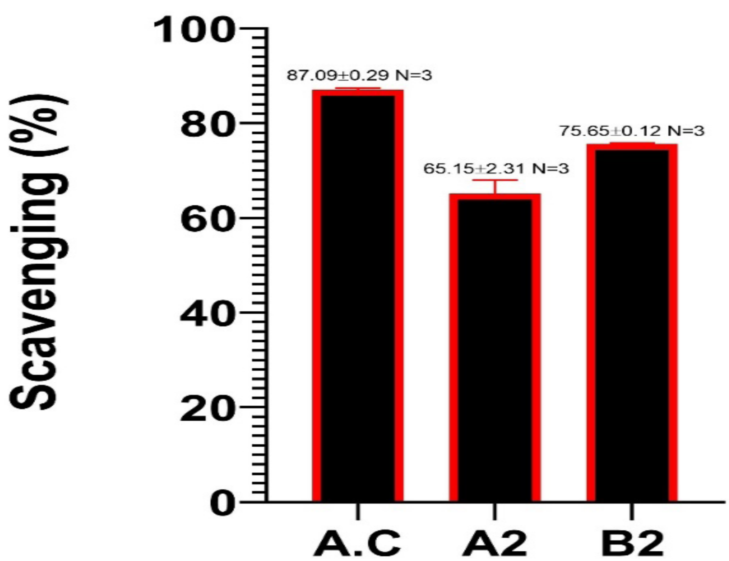

figure (7) shows the free radical suppression values for nanoemulsions prepared from cumin oil for concentrations v/v 1% and 2%

Anti-hemolysis test

The results of the red blood cell lysis revealed that the nanoemulsion was not lysis red blood cells at various concentrations shown in Table (1) and Fig. (8), the result demonstrated the cumin oil at a concentration of 4.8 significantly packed RBC from haemolysis as well as haemoglobin levels. These results are supported by findings of (20) that explore the antioxidant properties of the extracts of Cumin in a cell-based system, the extracts of human erythrocytes from healthy adult volunteers. Inhibition of human erythrocyte hemolysis was differently inhibited by cumin exhibiting approximately 38% and 54.6% cell lysis. Cumin oil contains compounds such as cuminaldehyde and phenolic compounds that show antioxidant activity that helps neutralize free radicals, which reduces oxidative stress associated with conditions such as hemolysis.

Table (1): antihemolytic activity of cumin oil with different concentrations

Test	Blood Control Negative	Control Positive	1%	2%	50%	75%	4.7	4.8
RBC	µl/10^3*4.55	µl/10^3*4.5	µl/10^3*4.49	µl/10^3*4.49	µl/106*4.17	µl/10*4	µl/10^3*4.55	µl/10^3*4.50
HGB	14.1g/Dl	13.9g/dL	13.7g/dL	13.8g/dL	g/dL 13	g/dL 12.8	14g/dL	14g/dL

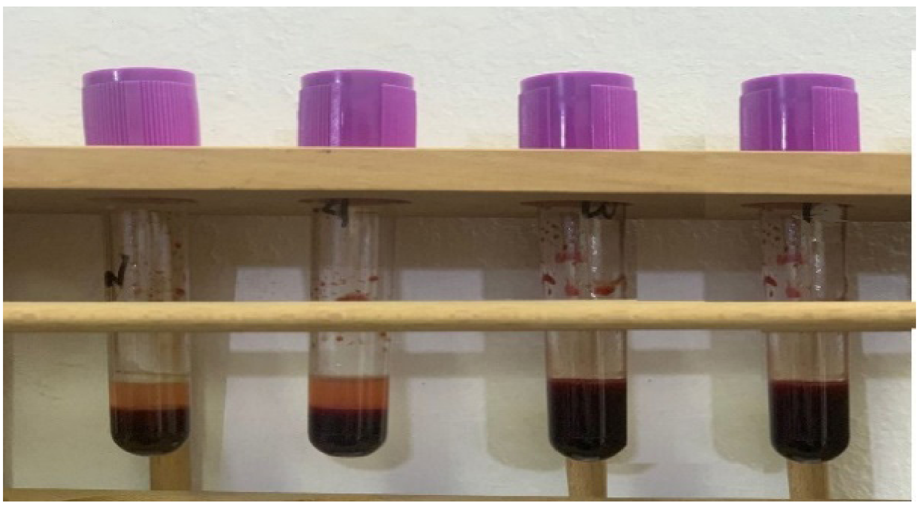

Figure (8): Demonstrates hemolysis assay for nanoemulsions prepared from cumin oil in various concentrations

Conclusion

Cumin nanoemulsion can serve as effective carriers for delivering natural therapeutic agents. The small droplet size and enhanced stability and the bioavailability of cumin nanoemulsion as well as the anti-oxidant properties that could be beneficial in inflammatory conditions remedies. The cumin nanoemulsion does not lysis red blood cells which make it candidate using for intravenous injection due their biocompatibility.

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