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Preparation and characterization of nanoemulsion prepared and loaded with *Hypericum perforatum* extract (St. John's Wort)

[1] Hadeel Rashid Wali, [2] Buthaina Abdulhameed Abdullah

1, 2 Department of pharmacology, biochemistry, and physiology. College of Veterinary Medicine, Tikrit University, Tikrit, Iraq.

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Corresponding Author:
Name:
Hadeel Rashid Wali
E-mail:
elina.arther.special.x2.mine.only@st.tu.edu.iq
Tel:07700578131

ABSTRACT

Background: Nanoemulsions, also known as nanometric-sized emulsions, are fine water-in-oil (w/o) and oil-in-water (o/w) dispersions of two immiscible fluids, as opposed to the milky-white hue concomitant with coarse dispersion. The current study aimed to prepare and characterize a noanoemulsion loaded with *Hypericum perforatum* extract using different phytochemical techniques. Also evaluate the effect of nanoformulation on particle size distribution, physical properties, and herbal extract stability.

Methods: Dried *Hypericum perforatum* (St. John's Wort) plants were obtained and extracted and studied its phytochemical compounds were studied by using High – Performance Liquid Chromatography (HPLC), then the nanoemulsion of *Hypericum perforatum* was prepared. The Characterization of nano emulsion and plant extract was performed by using X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Fourier Transform Infrared Spectroscopy (FTIR) and Surface Potential Analysis (Zeta Potential Analysis).

Results: Zeta potential values obtained were −1.3, −1, and −1.1 mV. The FTIR examination of the nanoemulsion showed multiple absorption peaks, indicating functional groups and bioactive chemicals. A board peak (3390 cm-1) indicates (O-H) stretching vibrations, confirming phenolic compounds and alcohol groups. Peaks at 2924 and 2857 cm-1 from (C-H) stretching of aliphatic chains showed lipids in nanoemulsion. Esters from fatty acids were confirmed by significant absorption bands at 1734cm-1, indicating C-O stretching. Peaks at 1647cm-1 (C-C stretching of aromatic compounds), 1400-1500cm-1 (aromatic rings), and 1100-1040cm-1 (alcohol and ether C-O stretching) were also examined. Examination using the transmission electron microscope showed that the nanoparticles emulsion formed from the plant extract ranged in diameter from 100–200 nm and were of a regular spherical shape. X-ray diffraction analysis of the nanoemulsion showed distinct peaks at 2θ angles ≈ 22.9° with d- spacing of 3.87°. Infrared spectra analysis of *Hypericum perforatum* displayed several absorption peaks. The intense and broad band was 3500-3300 cm-1 which corresponds (O-H) stretching vibration and indicates phenolic and flavonoid compounds. The predominant constituent in the plant extract was hyperforin with

(5.772 min) retention time and significant peaks (20003.378). The other component was hypericin which was investigated with retention time (8.988) and peak amount (1000.276); it was present in lower concentrations than hyperforin

Conclusion: The study synthesized and characterized successfully nanoemulsion loaded *Hypericum perforatum* extract. The nanoemulsion development was confirmed by several tests, SEM, XRD, FTIR and zeta potential which confirmed that nanoemulsion encapsulated with preserved bioactive compounds. The study concluded that nanoemulsion improved plant extract phytoconstituent stability, suggesting new herbal formulation and pharmacological application.

Introduction

Hypericum perforatum, commonly referred to as St. John's Wort, and known by other names such as Tipon's weed, chase devil, rosin rose, etc, belongs to the Hypericum genus, which encompasses about 484 species globally. It is indigenous to Madeira, Europe, North Africa, West Asia, India, China, and the Azores, and is currently disseminated globally [1]. It is a perennial shrub that attains a height of 1–3 feet and reproduces both vegetatively and sexually [2].

St. John's wort has a complex and diverse chemical makeup. Constituents include volatile oils (0.05 to 0.3%, including α-pinene, and cineole), anthraquic nones, carotenoids, cumarine, flavonoids (0.5-1.0%, including hyperoside, quercetin, and rutin), naph Tho dianthrones (0.1-0.3% of which 80-90% are hy pericin and pseudo hypericin), carbolic acids, phloroglucins (up to 3% hyperforin), xanthones, and proanthocyanidins [3] . The ancient Greeks employed this species as a herbal remedy to treat several ailments, including anxiety, wound healing, pulmonary issues, urinary tract infections, hysteria, hemorrhages, and moderate stomach discomforts and infections [4]. Numerous pharmacological investigations have shown that H. perforatum L. possesses significant nutraceutical qualities, including antifungal, anti-mycobacterial, anti-inflammatory, and antiviral activity [5].

In contrast to the milky-white color associated with coarse dispersion, nanoemulsions, sometimes called nanometric-sized emulsions, are fine dispersions of two immiscible fluids, such as water-in-oil (w/o) or oil-in-water (o/w). The addition of suitable amphiphilic emulsifiers or emulsifiers stabilizes these 20-200 nm droplets. So, nanoemulsions are referred to as small emulsions as well [6]. Unlike microemulsions (ME), nanoemulsions (NE) remain stable in heterogeneous systems because of their kinetic stability. While nanoemulsions don't seem to assemble or flocculate, their protracted physical constancy gives them the distinctive name "potential thermodynamic stability" [7].

Biphasic (O/W or W/O) or multiple nanoemulsions were classified according to the relative composition and dispersion of the internally distributed phases and the more widespread continuous phase [8]. The *Hypericum perforatum* plant has little solubility and absorbance in body, therefore it loaded into nanoemulsion. Several research has examined herbal nanoformulations, but little is known about *Hypericum perforatum* extract-loaded nanoemulsion systems. Characterization utilizing integrated analytical methods is also understudied. therefore, the current study aimed to prepare and characterize a noanoemulsion loaded with *Hypericum perforatum* extract using different phytochemical techniques. Also evaluate the effect of nanoformulation on particle size distribution, physical properties, and herbal extract stability.

Methods

Ethical approval

The College of Veterinary Medicine, Tikrit University council committee approved the research project proposed by the researcher with postal code TU. Vet.148, on 16/11/2026.

Preparation of *Hypericum perforatum* (St. John's Wort) extract

Dried *Hypericum perforatum* (St. John's Wort) plant were obtained from an herbalist in Kurdistan/Iraq. The plant extract was prepared according to [9] as described below:

1.Plant material collection: About 111–211 g of the aerial parts (flowers and leaves) of *Hypericum perforatum* were collected during the flowering period.

2.Drying: The plant material was air-dried in a shaded, well-ventilated area at a temperature not exceeding 41 °C to preserve active compounds.

3.Grinding: The dried samples were ground using an electric grinder to obtain fine powder.

4.Solvent extraction preparation: 11 g of the dried plant powder was used per 111 mL of solvent (71% ethanol or methanol).

5.Extraction: The plant powder and solvent were placed in a beaker equipped with a magnetic stirring rod.

6.Stirring: The mixture was stirred at moderate speed until a gentle vortex formed.

Heating: The mixture was heated to 41–51 °C only.

7.Extraction duration: Stirring and heating were continued for 2–4 hours, covering the beaker to minimize evaporation.

8.Filtration: The extract was filtered using Whatman filter paper.

9.Evaporation: The filtrate was concentrated using a rotary evaporator at 41 °C.

10.Storage: The resulting extract was stored in a dark bottle in the refrigerator until further use.

Preparation of *Hypericum perforatum* nanoemulsion

The nanoemulsion of *Hypericum perforatum* was prepared according to [10] using a high-energy emulsification method.

1.Initially, 5 g of plant extract was mixed with 40 g of Tween 80 using a magnetic stirrer for 15 minutes until a homogeneous mixture was obtained.

2.The aqueous phase was prepared separately by dissolving 20 g of glycerol in 35 g of distilled water, then added gradually to the prepared mixture under continuous stirring to form a coarse emulsion.

3.To reduce droplet size and obtain a nanoemulsion, the mixture was subjected to vortex for 10 minutes (30 son / 10 s off cycles) to ensure proper dispersion and prevent overheating.

4.For formulation optimization, a pseudo-ternary phase diagram was constructed using Tween 80 and Transcutol HP (2:1 ratio) with oil phase mixtures, followed by gradual addition of distilled water to determine the nanoemulsion region.

5.The optimized nanoemulsion was prepared by mixing the extract (1.5% w/v) with the oil phase, followed by addition of the surfactant/co-surfactant mixture, and finally distilled water was added dropwise under continuous stirring until a clear nanoemulsion was formed.

6.The stability of the prepared nanoemulsion was evaluated by centrifugation (3500 rpm for 30 min) and dilution tests, where no phase separation or turbidity was observed and nanoemulsion observed under microscope.

Characterization of nano emulsion and plant extract

1.X-ray Diffraction (XRD)

X-ray diffraction (XRD) was used to examine the crystalline quality of the biosynthesized nano emulsion. The diffraction pattern was acquired using a powdered sample in the scanning mode, which was run at 30 mA current with 40 kV voltage and Cu/Kα radiation with 20°–70° in 2θ angles. The Debye-Scherrer equation was used to determine the average crystalline size. The following is the equation:$D = k\lambda\beta cos\theta$ where k = shape factor (0.94).

- λ = X-ray wavelength (λ = 1.5418 Å);
- β = full width at half maximum (FWHM) in radians.
- and θ = Bragg's angle.

2.Transmission Electron Microscopy (TEM)

Transmission electron microscopy provides superior imaging capabilities, enabling the investigation of the morphological structure of nanoparticles at nearly the atomic scale. Microscopic droplets of nanoemulsion suspension were deposited onto copper grids coated with a thin layer of carbon and then allowed to dry at room temperature to preserve the original particle structure. This examination enabled the acquisition of high-resolution images revealing the geometry, size distribution, and degree of agglomeration, allowing for the evaluation of the bio fabrication process.

3.Ultraviolet–Vis Spectroscopy

The most important and simple technique for confirming the formation of nanoparticles is ultraviolet visible (UV Vis) spectrophotometry. nano emulsion formation was verified using a UV visible spectrophotometer, which monitored the band (200–800 nm) of surface plasmon resonance.

4.Fourier Transform Infrared Spectroscopy (FTIR)

The chemical compositions of plant extract and the synthesized nano emulsion were studied using FTIR spectrometer (Thermo Scientific Nicolet 380). The solutions were characterized in the range 4000–4000 cm−1 using KBr pellet.

5.Surface Potential Analysis (Zeta Potential Analysis)

The surface charge of the particles was measured using a Zetasizer at room temperature. The results are expressed in millivolts (mV). Samples are considered colloidally stable if the potential exceeds ±30 mV, indicating the presence of an electrostatic repulsive force that prevents agglomeration.

High – Perfotmance Liquid Chromatography for *Hypericum perforatum* extract

HPLC was used for the determination of phenolic compounds in *Hypericum perforatum* extract. The quality and quantity of the compounds were analyzed using a High-Performance Liquid Chromatography system (SYKAM, Germany) equipped with a C18-ODS column (250 × 4.6 mm, 5 µm).

A volume of 20 µL of the sample was injected into the system. The mobile phase consisted of water (Solvent A) and acetonitrile (Solvent B) under gradient conditions at a flow rate of 1 mL/min.

The gradient program was as follows: 10% B from 0–5 min, 25% B from 5–7 min, 40% B from 7–13 min, then returning to the initial conditions. The absorption was measured in the range of 200−800 nm, with the UV−visible detector

working at 260 nm for hyperforin's and 350 and 590 nm for hypericin's. The chromatographic data were recorded and processed with Agilent Open LAB Control Panel software. For UPLC-ESI QTOF-MS analysis, a Waters Acquity high-performance liquid chromatograph (UPLC, Waters) coupled with a time-of-flight Q-TOF micro-mass spectrometer (Waters) and equipped with electrospray ionization (ESI) was used. Mass spectra were recorded in the positive ion mode at 3 kV capillary voltage and 230 °C desolvation temperature [11].

Statistical analysis

Physicochemical properties of prepared nanoemulsion and plant extract were evaluated by using descriptive analysis. FTIR, TEM, XRD and HPLC were interpreted qualitatively based on the spectral pattern, diffraction peaks, morphology of particles and chromatographic retention times respectively. Values obtained by zeta potential and XRD measurements were directly reported from the analytical instruments. Qualitative analytical techniques such as FTIR, TEM, XRD and HPLC were interpreted in terms of spectrum pattern, diffraction peaks, particle shape and chromatographic retention periods. The values acquired from zeta potential and XRD measurements were immediately reported from the analytical instruments.

Results and Discussion

Characterization of Nano emulsion

1.Zeta Potential Analysis

zeta potential values (−1.3, −1, and −1.1 mV) obtained revealed the relatively low electrostatic stabilization of the nanoemulsion system produced. These near-neutral values show that steric stabilization may be more significant than electrostatic repulsion in maintaining dispersion stability. The nanoemulsion was physically distributed even though the surface charge was low. This may be attributable to the surrounding surfactant molecules around the droplets. Surface charge properties are recognized as critical criteria impacting particle interaction, aggregation behavior and the possible delivery efficiency of encapsulated phytochemicals [12].

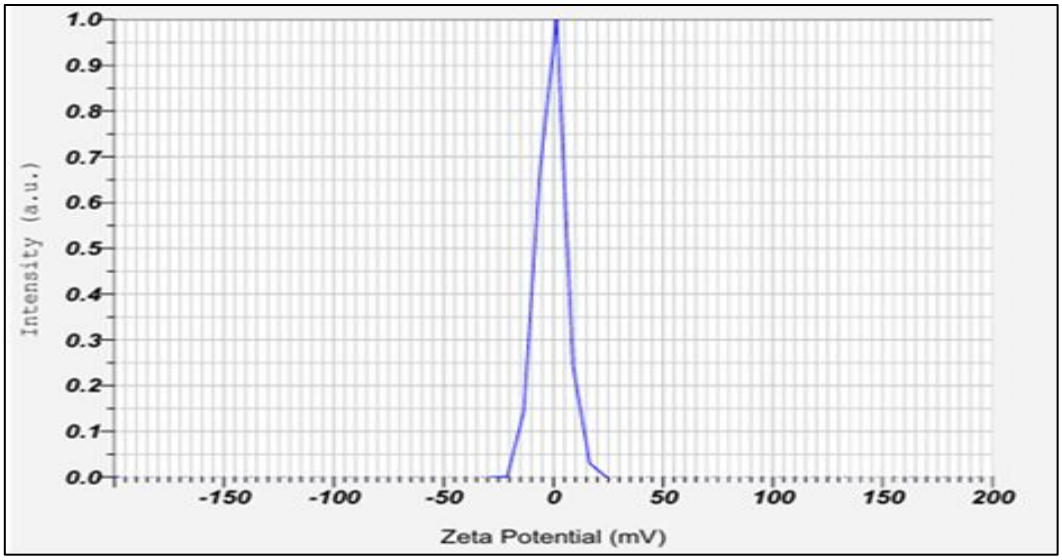


Figure (1): Zeta potential distribution of H. perforatum loaded nanoemulsion showing surface charge values and colloidal stability characteristic.

2.Fourier Transform Infrared Spectroscopy (FTIR) analysis

The fourier transform infrared spectroscopy analysis of nanoemulsion revealed several absorption peaks which indicate the presence of different functional groups and bioactive compounds in nanoemulsion. A board peak observed (3390 cm-1) corresponds (O-H) stretching vibrations which confirm the presence of phenolic compounds and Alcohol groups. The presences of lipid components in nanoemulsion were indicated by peaks at 2924 and 2857 cm-1 which attributed to(C-H) stretching of aliphatic chains. The presence of ester groups from fatty acids were confirmed by presence of strong absorption bands at 1734cm-1 which means C-O stretching. Also, peaks at 1647cm-1 (C-C stretching of aromatic compound), peak range 1400-1500cm-1 (aromatic rings) and peaks range 1100-1040 cm-1 (C-O stretching of Alcohol and ethers) were investigated. The persistence of the peaks of the active ingredient in the nanoemulsion

with little displacement demonstrates compatibility between the drug and the carrier without any detrimental chemical reaction.The observation of phenolic and flavonoid compounds confirms bioactive compounds stability within the system of nanoemulsion [13].

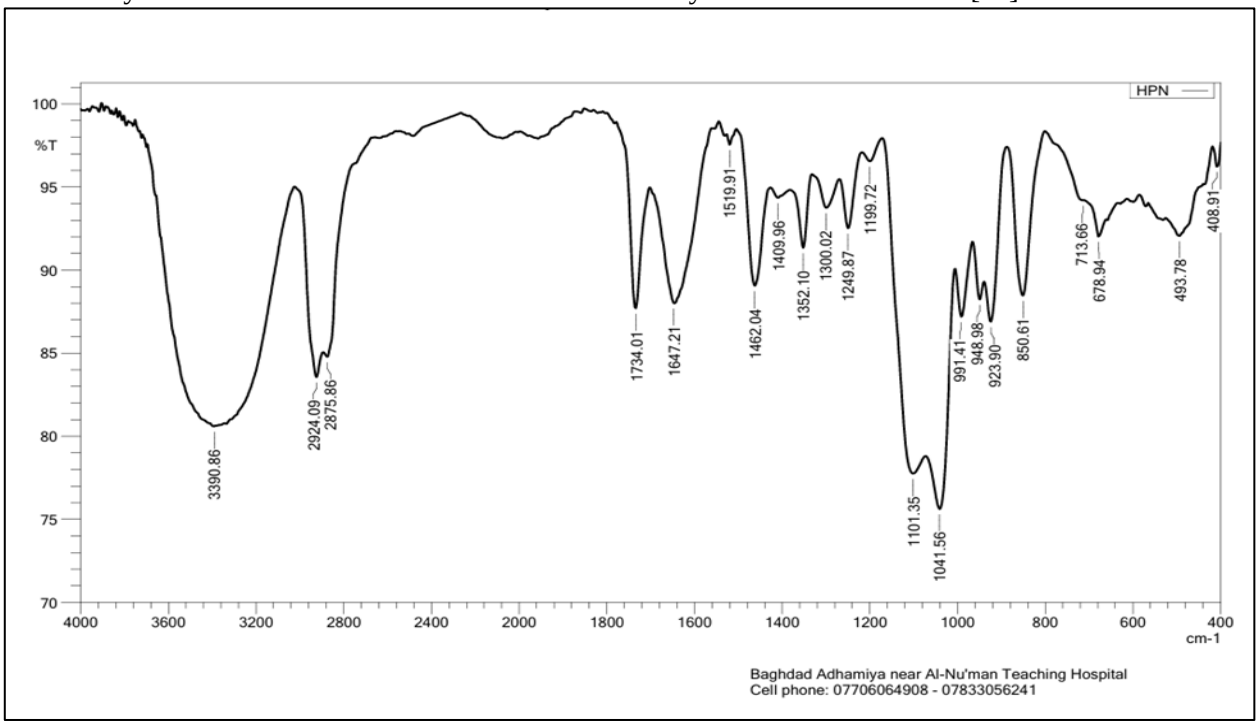
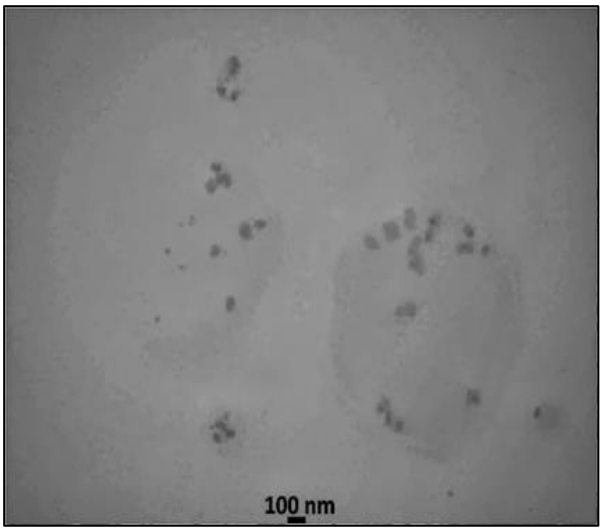


Figure (2): Fourier transform infrared spectroscopy of the nanoemulsion of plant extracts

3.Transmission Electron Microscopy (TEM)

Examination using the transmission electron microscope showed that the nanoparticles emulsion formed from the plant extract ranged in diameter from 100–200 nm, and were of a regular spherical shape with a similar size distribution, indicating the high effectiveness of the extracts in controlling the formation process without the occurrence of agglomeration or interference between the particles. Nanoscale dimensions may improve dispersion and surface area, which may improve lipophilic phytoconstituent encapsulation and delivery efficiency like hyperforin. No aggregation indicates nanoemulsion system stabilization throughout preparation [14].



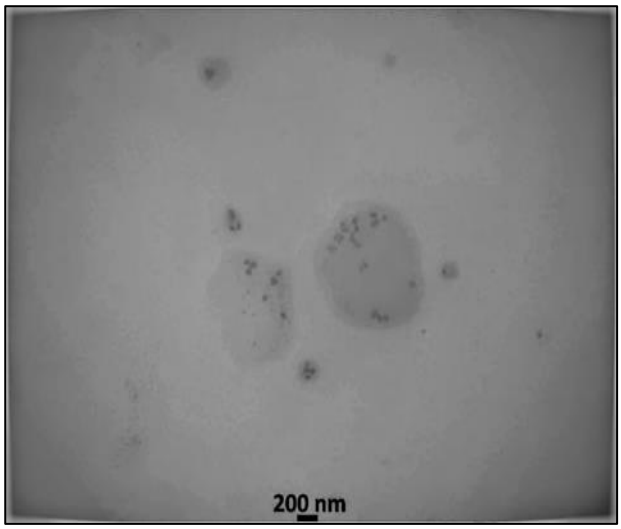


Figure (3): Transmission Electron Microscopy micrograph of H. perforatum nanoemulsion

showing spherical nanoparticles with 100-200 nm particle size.

4.X-ray diffraction (XRD)

X-ray diffraction analysis of the nanoemulsion showed distinct peaks at 2θ angles ≈ 22.9° with d-spacing of 3.87°. The peak indicates a lack of sharp structure of crystalline because it exhibited a full width at half maximum (≈ 6.7) as shown in figure (4). This structural change from crystalline to amorphous form could be a major factor in the nanoemulsion's improved therapeutic efficacy [15].

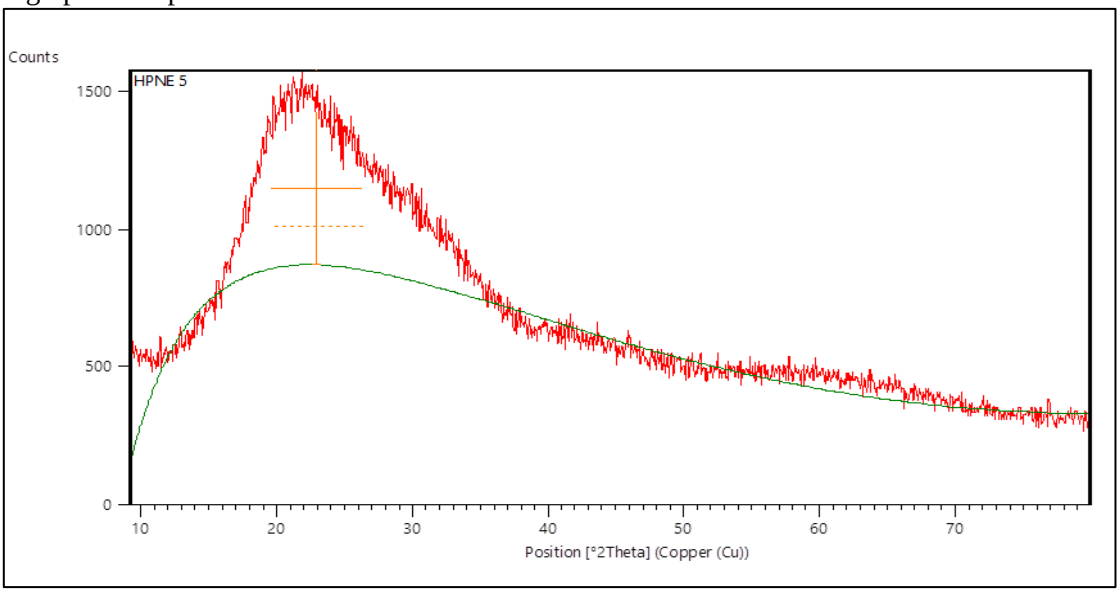


Figure 4: X-ray diffraction (XRD) of the nanoemulsion of plant extract

Table 1: X-ray diffraction (XRD) parameters of H. perforatum loaded nanoemulsion

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Tip Width
22.92(4)	558(11)	6.7(1)	3.87755	100.00	8.0880
22.97(4)	279(11)	6.7(1)	3.87755	50.00	8.0880

5.Fourier Transform Infrared Spectroscopy (FTIR) analysis for plant extract

Infrared spectra analysis of *Hypericum perforatum* displayed several absorption peaks. The intense and broad band was 3500-3300 cm-1 which corresponds (O-H) stretching vibration indicates phenolic and flavonoid compounds. A peak of 2779 cm-1 corresponds to the aliphatic group at (C-H strechting). The strong absorption peak which means aromatic compounds (C=O or C-C stretching) was recorded at 1637cm-1, Also, peaks at 1072 cm-1 revealed C-O stretching was mean presences of ethers or alcohol compounds. The last peaks (750-500 cm-1) indicate complex aromatic compounds.

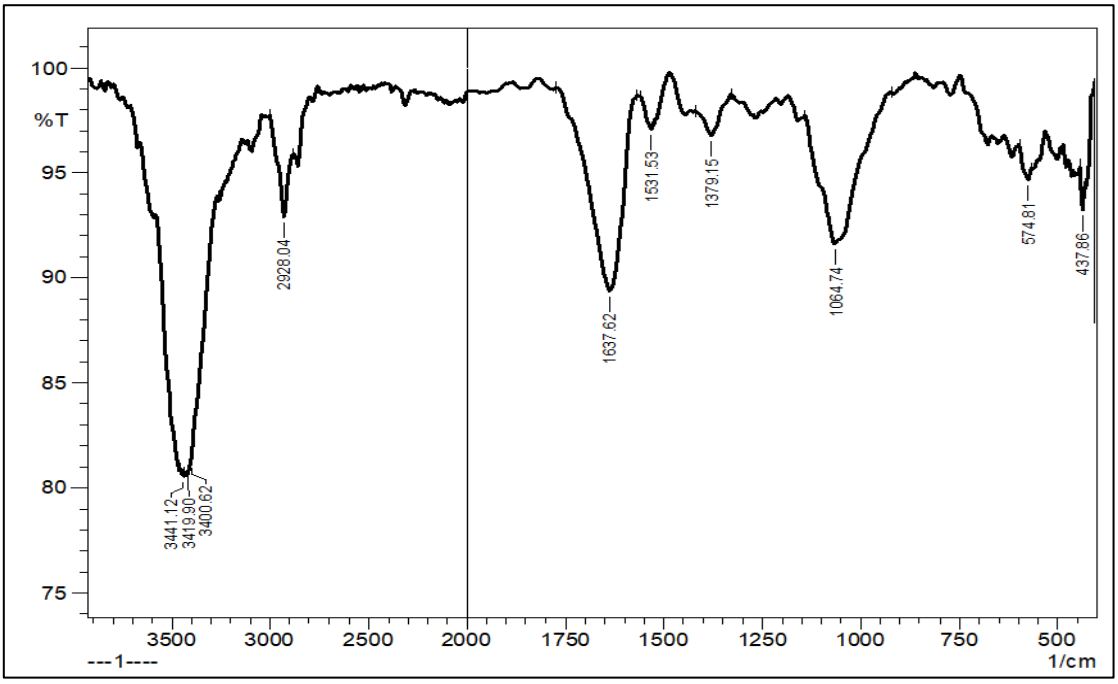


Figure 5: Fourier Transform Infrared Spectroscopy (FTIR) analysis for plant extract

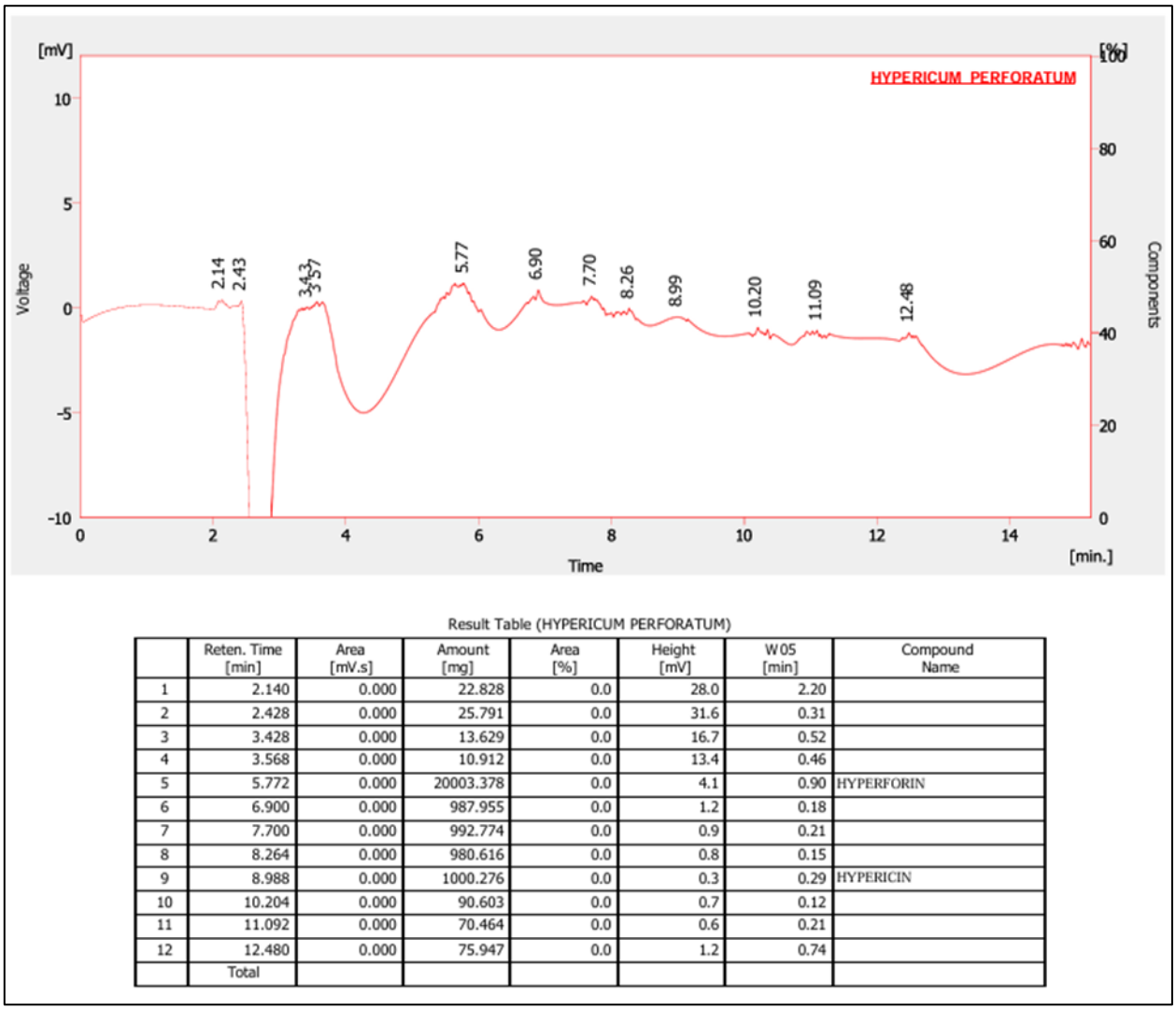
HPLC for *Hypericum perforatum* plant

The High-performance liquid chromatography analysis of the plant extract exhibited a complex phytochemical profile marked by multiple distinct peaks which showed plant principal secondary metabolites. The figure (6) showed that the active principles separated in 15 minutes. Hyperforin and hypericin are important therapeutic compounds that were found based on retention time.

The predominant constituent in plant extract was Hyperforin with (5.772 min) retention time and significant peaks (20003.378). Hyperforin was known as primary lipophilic component which was responsible for the antidepressant activity of H. perforatum [16] claims that hyperforin works by preventing neurotransmitters including dopamine, serotonin, and norepinephrine from being reabsorbed. This sample's high yield indicates that the extraction procedure was tuned to successfully retain this chemically labile phloroglucinol derivative.

The other component was hypericin which was investigated with retention time (8.988) and peak amount (1000.276), it was present in lower concentrations than Hyperforin and its detection is essential for quality standardization. Hypericin including pseudo hypericin and hypericin are important chemotaxonomic indicators for Hypericum according to study of [17]. Because of its hydrophobicity, it elutes later than more polar phenolic compound in HPLC procedure which appear at 9 minutes.

Also, there were a group of secondary peaks (6.90-8.26min) which represents the flavonoid fraction of Hypericum extract including some compounds such as rutin, hyperoside and quercetin. It is well-known that these flavonoids increase the pharmacological activity and bioavailability of hypericin by acting in a synergistic manner. Because of the synergistic effect, phytotherapists frequently favor "full-spectrum" extracts—those with several minor peaks—over isolated chemicals [18].

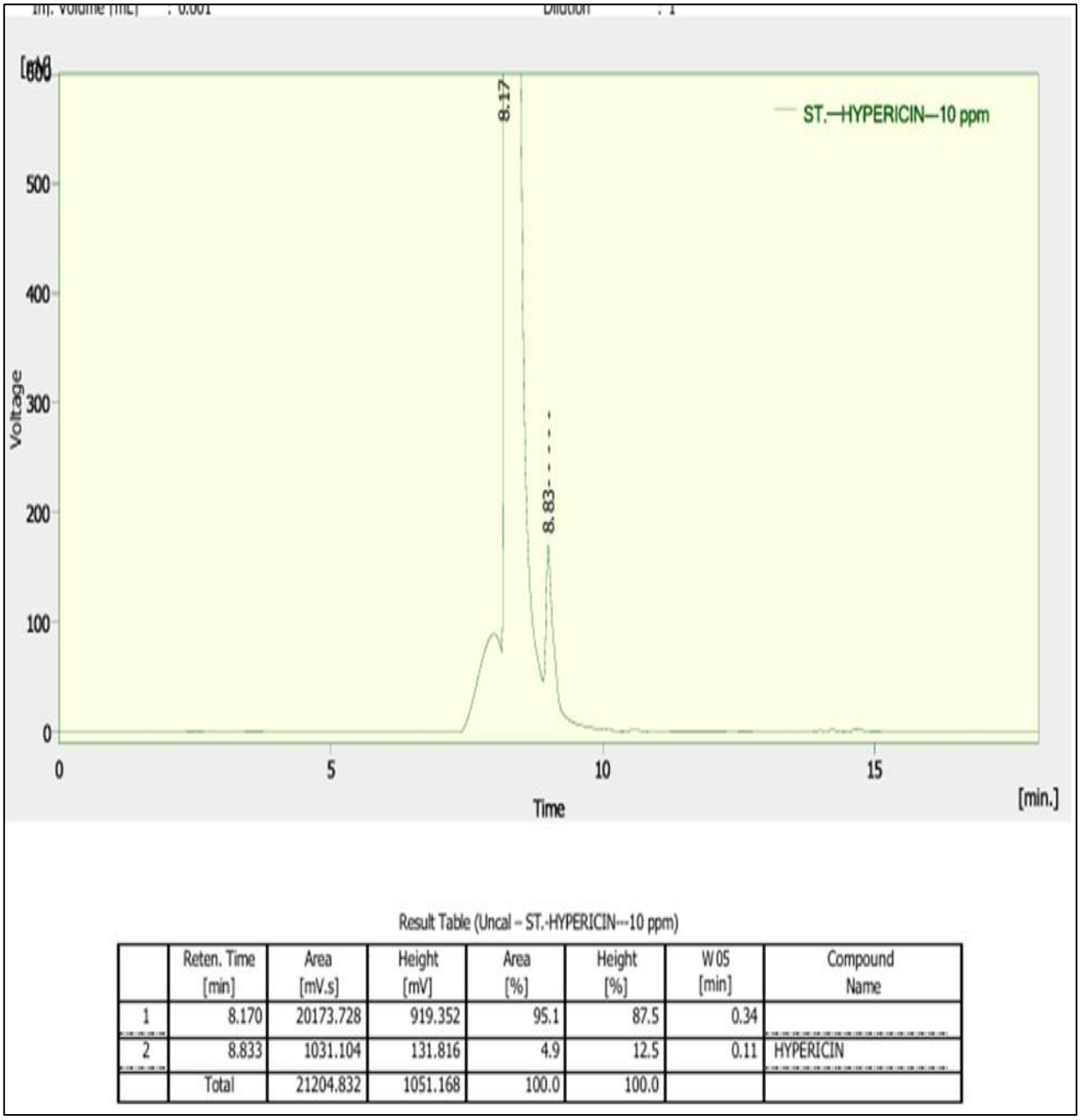


Result Table (HYPERICUM PERFORATUM)

	Reten. Time [min]	Area [mV.s]	Amount [mg]	Area [%]	Height [mV]	W05 [min]	Compound Name
1	2.140	0.000	22.828	0.0	28.0	2.20	
2	2.428	0.000	25.791	0.0	31.6	0.31	
3	3.428	0.000	13.629	0.0	16.7	0.52	
4	3.568	0.000	10.912	0.0	13.4	0.46	
5	5.772	0.000	20003.378	0.0	4.1	0.90	HYPERFORIN
6	6.900	0.000	987.955	0.0	1.2	0.18	
7	7.700	0.000	992.774	0.0	0.9	0.21	
8	8.264	0.000	980.616	0.0	0.8	0.15	
9	8.988	0.000	1000.276	0.0	0.3	0.29	HYPERICIN
10	10.204	0.000	90.603	0.0	0.7	0.12	
11	11.092	0.000	70.464	0.0	0.6	0.21	
12	12.480	0.000	75.947	0.0	1.2	0.74	
	Total						

Figure (6): HPLC chromatogram of *Hypericum perforatum* extract showing the major phytochemical constituents including hypericin and hyperforin.

A comparative analysis using a pure Hypericin standrard(10ppm) was conducted to confirm the identification of the phytochemicals isolated from Hypericum plants with peaks at retention time of (8.833 min). a high degree of correlation was shown when compared this with retention time of plant extract (8.988 min). In addition, the standard peak showed a relative area percentage of 95.1%, which means it is very pure and guarantees that the quantitative estimate is robust. In order to prove that the phytochemical screening results shown here are accurate, this validation process is necessary.

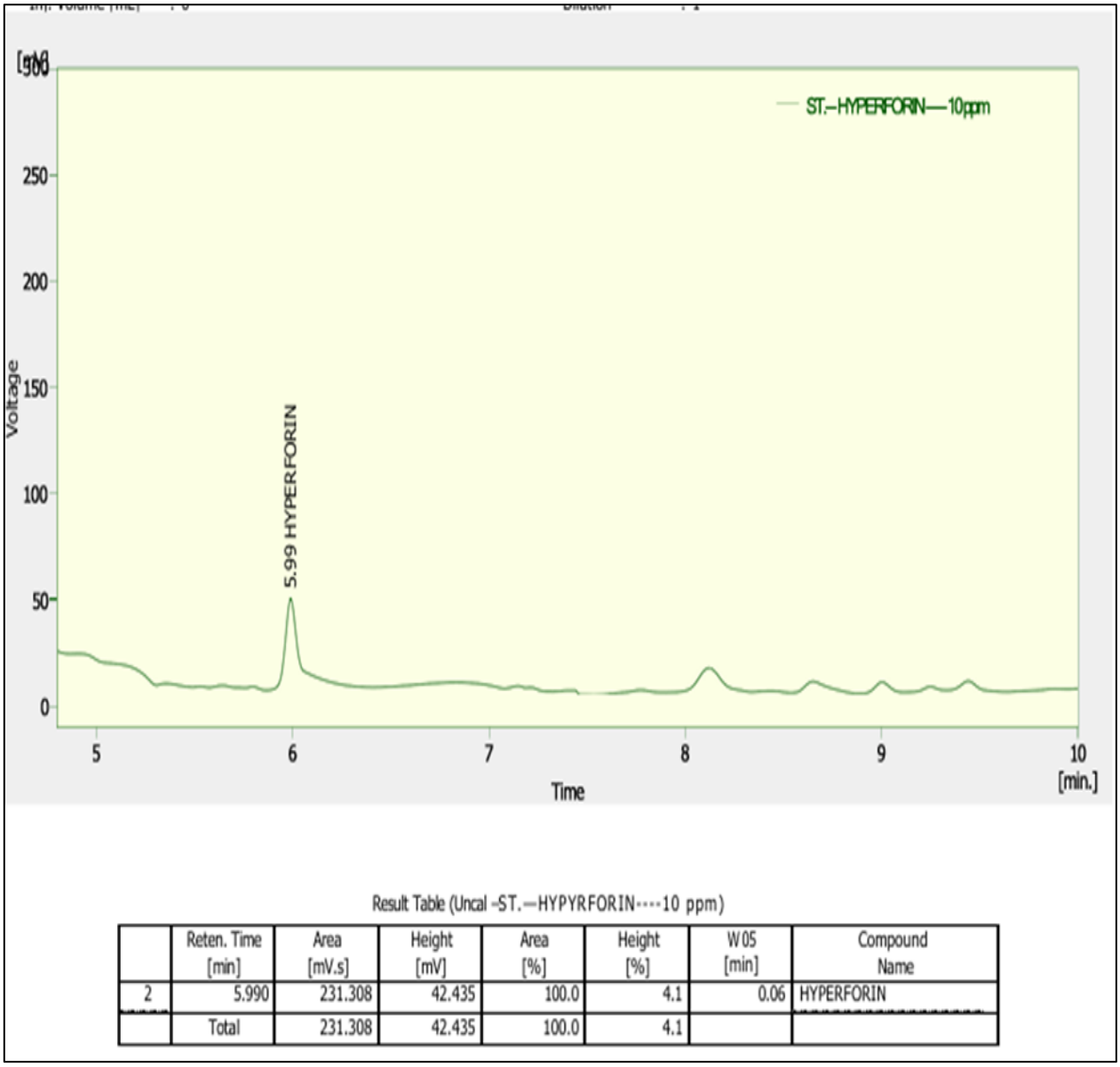


Result Table (Uncal - ST.-HYPERICIN---10 ppm)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	8.170	20173.728	919.352	95.1	87.5	0.34	
2	8.833	1031.104	131.816	4.9	12.5	0.11	HYPERICIN
	Total	21204.832	1051.168	100.0	100.0		

Figure (7): HPLC for pure Hypericin standrard

A standard hyperforin(10ppm) were measured under chromatographic condition for more validation of chemical profile. It showed a sharp peak at Rt (0.990 min). the major peak showed in plant extract (5.772 min) was closely aligned with this peak value. The minor deviation retention time is typically attributed and expected analytically to matrix effect in which co extraction of phytochemicals in crude extract influence the interaction between the stationary phase and the analyte compared with pure standard. As the hyperforin is a bioactive molecule documented for its synaptic neurotransmission interaction, its identification confirms the pharmacological potential of hypericum extract [19,20].



Result Table (Uncal -ST.—HYPYRFORIN----10 ppm)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
2	5.990	231.308	42.435	100.0	4.1	0.06	HYPERFORIN
	Total	231.308	42.435	100.0	4.1		

Figure(8): HPLC for pure Hyperforin standrard

4.Conclusion

The study synthesized and characterized successfully nanoemulsion loaded *Hypericum perforatum* extract. The nanoemulsion development was confirmed by several tests, SEM, XRD, FTIR and zeta potential which confirmed that nanoemulsion encapsulated with preserved bioactive compounds. The study concluded that nanoemulsion improved plant extract phytoconsitituent stability, suggesting new herbal formulation and pharmacological applications. The current study examined plant physicochemical properties, not toxicity or biological activity, therefor, the therapeutic application pf nanoemulsion and pharmacological potential must be studied.

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Declaration of interests

The authors declare that they have no conflicts of interest.

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Data and material availability
All data generated and analyzed during this study are included in this published article.

Author contribution
Hadeel Rashid Wali**:** Sample collection, prepared the plant extract and nanoemulsion, analyzed the data, manuscript writing, and revising. Buthaina Abdulhameed Abdullah**:** Suggested the title of the research, supervised the study, reviewed the methodology, interpreted the results, manuscript writing, and revising and approved the final version.

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تحضير وتوصيف مستحلب نانوي مُحضر ومُحمل بمستخلص عشبة القديس (العرن المثقوب) *Hypericum perforatum*

هديل رشيد ولي محمد [1], بثينة عبدالحميد عبدالله [2]

1,2 فرع الادوية والفسلجة والكيمياء الحياتية، كلية الطب البيطري، جامعة تكريت، العراق

الملخص

الخلفية: المستحلبات النانوية، والمعروفة أيضًا بالمستحلبات النانوية الحجم، هي عبارة عن تشتتات دقيقة من الماء في الزيت (w/o) والزيت في الماء (o/w) لسائلين غير قابلين للامتزاج، على عكس اللون الأبيض الحليبي المصاحب للتشتتات الخشنة. هدفت هذه الدراسة إلى تحضير ودراسة خصائص مستحلب نانوي من مستخلص نبات نبتة القديس (Hypericum perforatum).

طرق العمل: تم الحصول على نبات عشبة القديس المجففة (Hypericum perforatum)، واستُخلصت مركباتها الكيميائية النباتية ودُرست باستخدام كروماتوغرافيا السائل عالي الأداء (HPLC)، ثم حُضّر المستحلب النانوي من نبتة عشبة القديس. تم توصيف المستحلب النانوي والمستخلص النباتي باستخدام حيود الأشعة السينية (XRD)، والمجهر الإلكتروني النافذ (TEM)، ومطيافية الأشعة تحت الحمراء بتحويل فورييه (FTIR)، وتحليل جهد السطح (تحليل جهد زيتا).

النتائج: كانت قيم جهد زيتا المُقاسة -1.3، -1، و-1.1 ملي فولت. أظهر فحص FTIR للمستحلب النانوي قمم امتصاص متعددة، مما يشير إلى وجود مجموعات وظيفية ومواد كيميائية نشطة بيولوجيًا. تشير قمة عريضة (3390 سم$^{-1}$) إلى اهتزازات تمدد الرابطة (O-H)، مما يؤكد وجود المركبات الفينولية ومجموعات الكحول. أظهرت القمم عند 2924 و2857 سم$^{-1}$، الناتجة عن تمدد الرابطة (C-H) للسلاسل الأليفاتية، وجود الدهون في المستحلب النانوي. تم تأكيد وجود إسترات الأحماض الدهنية من خلال نطاقات امتصاص واضحة عند 1734 سم$^{-1}$، مما يشير إلى تمدد الرابطة C-O. تم فحص قمم عند 1647 سم$^{-1}$ (تذبذب تمدد الرابطة C-C للمركبات العطرية)، و1400-1500 سم$^{-1}$ (الحلقات العطرية)، و1040-1100 سم$^{-1}$ (تذبذب تمدد الرابطة C-O للكحولات والإيثرات). أظهر الفحص باستخدام المجهر الإلكتروني النافذ أن مستحلب الجسيمات النانوية المتكون من مستخلص النبات يتراوح قطره بين 100 و200 نانومتر، ويتخذ شكلاً كروياً منتظماً. أظهر تحليل حيود الأشعة السينية للمستحلب النانوي قممًا واضحة عند زاوية $\theta \approx 22.9°2$ بمسافة بين المستويات البلورية (d) تبلغ 3.87°. أظهر تحليل طيف الأشعة تحت الحمراء لنبات عشبة القديس (Hypericum perforatum) عدة قمم امتصاص. كان النطاق العريض والكثيف عند 3300-3500 سم$^{-1}$، والذي يتوافق مع اهتزاز تمدد الرابطة (O-H)، مؤشرًا على وجود مركبات فينولية وفلافونويدية. كان الهايبرفورين المكون الرئيسي في مستخلص النبات، حيث بلغ زمن احتجازه 5.772 دقيقة، وظهرت قمم واضحة عند 20003.378 نانومتر. أما المكون الآخر، الهايبريسين، فقد تم فحصه بزمن احتجاز 8.988 نانومتر وكمية قمة 1000.276 نانومتر، وكان موجودًا بتركيزات أقل من الهايبرفورين.

الاستنتاجات : خلصت هذه الدراسة إلى نجاح تحضير مستحلب نانوي من مستخلص نبات عشبة القديس (Hypericum perforatum)، حيث تراوح حجم الجسيمات بين 100 و200 نانومتر. كما أظهر التحليل الكروماتوغرافي أن الهايبرفورين هو المكون النشط الرئيسي، بينما ظهر الهايبريسين بتركيزات منخفضة.

الكلمات المفتاحية: مستحلبات نانوية، عشبة القديس، كروماتوغرافيا سائلة عالية الأداء (HPLC)، مطيافية الأشعة تحت الحمراء بتحويل فورييه (FTIR).