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Isolation and Structural Characterization of Quercetin 3-O-Rhamnoside and Essential oil Estimation from Leaves of Iraqi *Cupressus sempervirens* L (Conference Paper)#

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Abstract

Cupressus sempervirens L., Cupressaceae, which is known as evergreen cypress, Mediterranean cypress, and in Arabic called "al -Sarw. It is evergreen, has a medium-sized, and longevity, and is widely distributed over the world. The plant represents an important member of conifer plants which are characterized by aromatic leaves and cones. *Cupressus sempervirens* have been ethnobotanicals as an antiseptic, relief of cough, astringent, antispasmodic, wound healing, and anti-inflammatory. The aims of this work are phytochemical analysis, isolation, and structural identification of quercetin 3-O-rhamnoside (quercitrin) and essential oil in Iraqi *C. sempervirens*. Isolation of quercitrin was performed using a semi preparative HPLC from n.butanol fraction and extracted from *Cupressus sempervirens* leaves using ultrasound probe extraction, the structural identification of isolated quercitrin done by TLC, HPLC, UV spectrophotometry, FT-IR characterization according to a variety of frequency ranges, and LC-MS/MS revealed a molecular ion at 448 m/z and base peak m/z 301. Furthermore, isolation of essential oil using hydro-distillation and estimated by GC-MS shows a good essential oil yield of 0.9% with an interesting concentration of alpha-pinene 44%, Carene10%, Cedrol 4.86%, and β- myrcene 3.67%. Hence, the isolation of a new Quercetin-glycoside and 0.9% essential oil yield from leaves of *Cupressus sempervirens species* is considered an important valuable source of quercetin 3-O-rhamnoside and essential oil in the Iraqi cypress plant and might prefer the agriculture of this plant and widespread it in different regions.

Keywords: Cupressus, Conifers, Al Sarw, Mass, Pinene, Quercitrin, Spectroscopy.

عزل وتشخيص مركب الكويرسيترين quercetin 3-O-rhamnoside وتقدير الزيت العطري المعزولة من أوراق نبات السرو العراقي (بحث مؤتمر)#

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الخلاصة

نبات السرو من عائلة السرويات وايضا معروف ب السرو دائم الخضرة و السرو المتوسطي . النبات دائم الخضرة ، متوسط الحجم معمر ، ومنتشر على نطاق واسع في جميع أنحاء العالم. يمثل النبات عضوا مهما في النباتات الصنوبرية التي تتميز بالأوراق والمخاريط العطرية. نبات السرو لديه استخدامات نباتية عرقية كمطهر ، وتخفيف السعال ، وقابض ، ومضاد للتشنج ، والتئام الجروح ومضاد للالتهابات. أهداف هذا العمل هي التحليل الكيميائي النباتي والعزل االكيميائي وتشخيص المركب المعزول quercitrin والكشف عنه وتحليل الزيت العطري لاوراق السرو المستزرع في العراق. تم إجراء عزل quercitrin بواسطة HPLC من جزء n-butanol الذي تم استخلاصه من أوراق نبات السرو باستخدام جهاز الموجات فوق الصوتية ، تشخيص المركب المعزول بواسطه TLC, HPLC، قياس الطيف الضوئيUV، FT-IR ، تشخيص بواسطهLC-MS/MS الذي اظهرالأيون الجزيئي عند ٤٤٨ m/z واعلى قمه 301 m/z.علاوة على ذلك ، عزل الزيت العطري باستخدام التقطير المائي وتقديره بواسطة GC-MS . تظهر النتائج عائدا جيدا من الزيت العطري ٠,٩٪ مع اعلى تركيز لماده ال باينين ٤٤٪ ، كارين ١٠٪ ، سيدرول ٤,٨٦٪ ، وميرسين 3.67 .٪لذا،عزل مركب جديد من quercetin-glycosideو ٠,٩٪ من إنتاج الزيت العطري من اوراق صنف نبات السرو, يعتبر مصدر مهم في نبات السرو العراقي،وقد تفضل زراعة هذا النبات وانتشاره في مناطق مختلفة .

الكلمات المفتاحية: السرويات ، الصنوبريات ، نبات السرو ، كتله ، باينين، كويرسيترين، التحليل الطيفي

Introduction

Medicinal plants are considered an indigenous source of a wide variety of compounds possessing different therapeutic applications [(1)]. Sumerian and Babylonian civilizations employed clay tablets to document the usage of medical plants in Iraq for thousands of years, and they used a variety of treatment methods for medicinal plants [(2)]. The Conifers are woody plants formed from varieties of genera and species. The plants are generally characterized by needle-shaped leaves, single-veined, and consist of both female and male unisexual cones categorized with bract scales [(3)]. The conifers consist of eight families, the most common families were Cupressaceae, Pinaceae, Podocarpaceae, Cephalotaxaceae, Taxaceae, and Phyllocladanecea [(4)]. *Cupressus sempervirens* L. (C.S) is an evergreen, aromatic, longevity, and widest extant tree among the Cupressaceae family [(5)]. It is usually known as evergreen cypress in different regions, while in Arabic called "al -Sarw" [(6,7)].

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The origin of this plant is back to several ancient Greek mythology legends according to a man named Cypresses, the Provence cypress was used as a coating for the wells and the cypress wood was considered as "dowry of the daughter"[8]. The previous literature review indicates that various parts of Cupressus around the world including wood, cones, leaves, stem and bark different ethnomedicinal applications and have been used as a topical to relieve muscle pain and rheumatoid arthritis, relief of gout, have an expectorant effect, relief whooping cough, asthma [9,10], bronchitis, diabetes and have a diuretic effect. Furthermore, the essential oil of coniferous plants is used as an antiseptic for wound healing, as an anti-scarring, and astringent effect [11,12]. The major chemotaxonomic secondary metabolites are polyphenols; flavonoids, bi-flavonoids, phenolic acids and terpenes, fatty acids, carbohydrates, and resins [13]. Amongst the polyphenols reported in the Cupressus genus are flavonoid glycosides such as 3,5,7-trihydroxy-4-O-methoxy flavone, quercetin-3-D-xyloside, and kaempferol-7 neo-hesperidin - side [14,15]. Chemically, Quercitrin is also known as Quercitroside or quercetin O-glycoside; Figure 1 has a potential role in biological activity such as antioxidant, control of cancer, relief of rheumatoid disorders antiangiogenic, hepatoprotective, and antiproliferative activities [16-18]. In addition, these constituents were revealed to have acted as protection from skin ageing through the cytoprotective effect of this constituent on UVB-induced cell injury in humans [19]. Furthermore, essential oil (EO) is considered one of the major components in *C.sempervirens* and almost conifer plants. The chemical unit of monoterpenes containing ten carbon atoms with unsaturated carbon atoms (the building unit isoprene) joined head-to-tail and is considered important volatile oil, which is widely spread in medicinal plants with potential biological activity [20,21]. Moreover, pharmacologic activities of *C.sempervirens* includes; anti-inflammatory[22], antimicrobial, antiplatelet, and anticoagulant activity [23], and hepatoprotective activity[24]. Our work aimed to phytochemically investigate Iraqi *Cupressus sempervirens* leaves, isolation of quercitrin (quercetin 3-O-rhamnoside), and structural identification by UV, FT-IR, and LC-MS/MS analysis moreover, GC-MS analysis of estimation of essential oil.

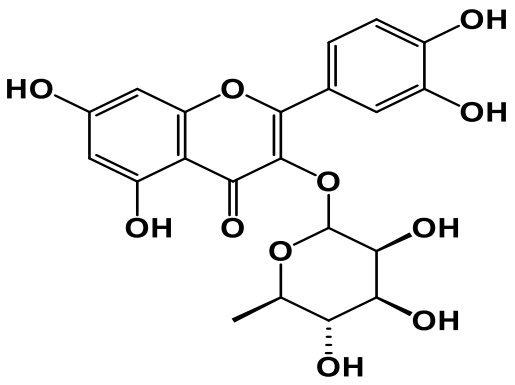

Figure 1. Quercetin 3-O-rhamnoside [16]

Materials and Methods

Plant material collection and authentication

Cupressus sempervirens L leaves were collected from Baghdad in September 2020. Prof. Dr Khansaa Ghazi Rasheed identified and authenticated this plant at the Iraqi Natural History Research Center and Museum -Plant and Environment Department- Baghdad. Leaves were cleaned, air-dried in shade, ground in an electrical grinder to a fine powder, and subsequently used for extraction and phytochemical analysis.

Equipment and chemicals

A rotary evaporator was used in this work from BÙCHI Rotavapor R-205, Swiss). All chemicals used in this study were of high purity the solvents and chemicals were used in analytical grade from BDH, Ltd. Poole, England, and the reference standard was purchased from Chengdu Biopurify Phytochemicals, China (purity >97). Silica gel GF254 TLC plates belonging to Merck trademark Ltd., India.

Extraction and isolation of essential oil (EO)

The extraction and isolation of (EO) from the leaves of *C. sempervirens* were performed with the hydro distillation method using the Clevenger apparatus. The procedure for the extraction of (E.O) was applied as follows: for every 200 grams of air-dried leaves extracted with 1500L water in Clevenger-apparatus for 4 -5 hours. A pale-yellow oil was isolated and then it was dried using anhydrous sodium sulfate. The storage was at about 4 °C in an air-tight and dark glass container until analysis by Gas chromatography/Mass spectrometry (GC/MS) [25].

Preparation of plant extract and standard

Ultrasound-assisted extraction (UAE) is an efficient, one of the new non-conventional extraction methods, and a favoured technique to isolate phytochemicals from botanical sources. Sonication realizes a complete extraction and enhanced extraction yields are obtained quickly [26]. Ultrasonic extraction was performed using a probe Sonicator. 300 gm of powdered leaves of *C. sempervirens* L was defatted with hexane, then the

defatted leaves powder was extracted with 85 % methanol using Ultrasound-assisted extraction (probe) with the following parameters;20 min, solid - solvent ratio 1:8mg/ml, temperature 25 ^{0}C, and frequency 20 kHz;), then concentrated under reduced pressure and suspended with water for fractionation method three times with 150 ml of different solvents polarity in ascending manner (chloroform, ethyl acetate, and n. butanol fraction). The n.butanol fraction was dried and subjected to chemical screening tests for the detection of flavonoids and flavonoid glycosides. On other hand, the target component is determined and isolated by semi-preparative HPLC compared with standard. the sample to be analyzed by HPLC was dissolved in methanol -HPLC grade and filtered through a 0.45µm Millipore membrane filter. Furthermore, the standard solution; quercitrin was prepared by dissolving 1 mg /1 ml of methanol (analytical HPLC grade) to compare with the sample to determine of isolated compound.

Preliminary phytochemical screening

Chemical screening tests were used for the detection of flavonoids and to reduce sugar in n. butanol fraction of leaves extract [27-29].

Alkaline reagent test

The alkaline reagent was used for detection of flavonoids, 3ml of the extract was mixed with approximately 1.5 ml of alkaline reagent (5% KOH), and the yellow color was considered an appositive indication of flavonoids, and when the addition of a few drops of dilute acid the solution became colourless.

Ferric chloride test

Phenolic groups were screened using 1% alcoholic ferric chloride added to 2ml of extract (1:1) the color change to deep green or deep blue color indicates the presence of the phenolic compound.

Reducing sugar test

Benedict's reagent was used to screen the reducing sugar in n. butanol fraction. Approximately 2 ml of sample and 3 ml of reagent were added in the test tube and then the mixture was heated in a water bath for 10 minutes. The greenish brown precipitate according to the concentration of sugar was an indication of the presence of the reducing sugar.

Salkowski test

Salkowski test was used to detection of terpenoids in *Cupressus Sempervirens* leaves. 5ml of extracts were dissolved in 1.5 ml of chloroform then added 2 ml of concentrated H_2SO_4 carefully. Reddish brown color indicates a positive result.

Libermann-burchard test

Sterols and steroids were detected using acetic anhydride (2 ml) and 2ml of H_2SO_4 was added in a test tube with 0.5 gm of extract. The appearance of green or blue color confirmed steroids in the sample.

Gas chromatography-mass spectrometer (GCMS) conditions

GC/MS -SHIMADZU -QP-2010 ULTRA with Scan mode -ACQ was used at the University of Basrah/ College of Agriculture/ Department Food Science, for this analysis and adjusted at these conditions: The capillary column that was used to separate constituents was (30 m× 0.25mm, with a thickness of 0.25µm) at a flow rate of 1.0 mL/min. in addition, the carrier gas that was used is helium, the split ratio was 2.0, the Injector port was 250˚C, oven temperature: was from 80 º C for I min, then rise to 240 ºC at a rate of 10 ºC /min and the detector was 280º C. electronic impact mode (SEI) was used as an ionization mode at 70e [30]. Structural identification was based on the comparison of component mass spectra with components in the NIST mass spectral library.

Chromatographic analysis for an n. butanol fraction

Thin-Layer Chromatography (TLC)

In this work, a qualitative analysis of the n. butanol fraction was performed using silica gel 60 F_{254} plates with 0.25mm thickness and activated at 110 °C (Merck), using the mobile phase system EtOAc/ H_2O/ HOAc (glacial) (10:10:40) v/v/v [31]. The target compound compared with the quercitrin standard was detected under UV light at 254 nm.

Semi preparative high-performance liquid chromatography

In this work, n. butanol fraction of leaves extract was subjected to an analytical HPLC system equipped with a fraction collector unit. HPLC -UV system, Shimadzu, 10AV-LC, Japan was carried out for separation and isolation of target compound (quercitrin) in n.butanol fraction compared with quercitrin standard the chromatographic separation done by using specific chromatographic conditions [32,33]: HPLC Column: C18- reverse phase, 3 nuclear µm particle size (50 x 2.0 mm). The mobile phase consisted of two solvents mixture in gradient Mode; (A) 0.1% formic acid in water and (B) methanol with gradient elution program from 10% A to:95% A(v/v).

The flow rate was 1 ml /min Injection volume was 20 µL and the detection was at ƛ 280 nm -310 nm.

Chromatographic analysis for Isolated compound (quercitrin)

Analytical HPLC and TLC for identification of isolated compound

Regardless of HPLC, the purity and identification of the isolated compound were directly checked compared with the standard by using the same analytical HPLC (without a fraction collector unit) with the observant of the same chromatogram conditions used in isolation of the quercitrin as mentioned above.

TLC was considered a simple and also the rapid method used for further screening of purity and simple identification of isolated compound compared with quercitrin standard using the same mobile phase system that was used above; EtOAc/ H_2O/ HOAc (glacial) (10:10:40 v/v/v).

Characterizations for isolated compound (quercetin-3-O-rhamnoside)

Ultraviolet spectroscopy (UV)

The Ultra Violet spectra analysis was done using a double beam Shimadzu-spectrophotometer (UV-1700) at Al-Mustansirya University - College of pharmacy. The isolated compound and quercetin 3-O- rhamnoside standard were dissolved in methanol and then analyzed between a range of 200 nm to 600 nm.

Fourier transforms infrared spectroscopy

FT-IR spectroscopy was used for structural identification of isolated quercitroside (quercetin 3-O- rhamnoside) and carried out at Baghdad University-College of Pharmacy. FTIR spectra Shimadzu were performed using the KBr disk method and the results were recorded in the range of 400–4000 cm^{-1}.

Liquid Chromatography /Mass /Mass Spectrometry (LC/MS/MS)

The mass spectrometric analysis was done using API (ion trap mass spectrometer) /3200/ LC-MS/MS system with Ion Spray Ionization (ESI) source Ion spray, ESI is more frequently used in flavonoid and flavonoid glycosides analysis [34]. The liquid chromatographic separation of the target compound was carried out on a Phenomenex Gemini C_{18} (250 mm x 4.6 mm i.d.; 5 µm particle diameter), column temperature was kept at 250 C^0, and 20µl was injected and a flow rate (FR) was 1 ml/min. Full scan spectra were acquired in ESI-MS spectra and were carried out in the positive /negative ionization mode. The mass range was measured, m/z 50-900m/z; drying nitrogen gas nebulizing N_2 30 psi and the flow rate was 45 ml/min. MS/MS fragmentation experiments were performed on the selected precursor ion and the conditions were; Ion spray voltage, 4500 V; Data Analysis (Analyst 1,6.3 version –Germany-Darmstadt) was used to analyze the mass spectra.

Results and Discussion

In this work, the results of phytochemical screening of the *C.sempervirens* plant indicate the presence of flavonoids, phenolic groups, steroids, reducing sugar, and terpenoids [35].

GC-MS analysis of essential oil (EO) of C.sempervirens

The chemical components of the essential oil of *C.sempervirens* leave estimated in this study and identified by GC-MS analysis as in Figure 2. In this work, the extraction yield of the EO from the leaves of Iraqi Cupressus sempervirens L. was 0.9%. Table 1 shows the chemical components of the essential oil obtained by GC-MS analysis and revealed 50 compounds;46 compounds identified and 4 unidentified., the results show the essential oil (EO) contains monoterpenes(C10) and sesquiterpenes(C15) as the major constituents in the essential oil at different retention times (R_t) and concentrations.

Hence, the reported previous studies, GC-MS analysis revealed the essential oil components of *C.sempervirens* leaves in different geographical regions such as in Turkey;α-pinene was; 35.60 %, Δ-2-carene concentration was 22.7, 14.9 %, trans-pino-carveol (5.22 %), α-phellandrene-8-ol percentage was 4.56 %, β-pinene (3.06 %), D-limonene[36]; in Iran different concentration in this plant that is identified in both parts leaves and fruits such as α-pinene (46.2, 59.2 %) highest one, while its isomer β-pinene was (3.3, 2.8 %), germacrene-D (6.3-2.1 %), in addition, myrcene percentage value was (4.6, 3.4 %), isoterpinolene (3.7, 3.2 %), sabinene (2.2, 2.0 %), limonene (2.8, 2.4 %) as cyclic hydrocarbon, and as ester was α-terpenylacetate (2.6, 3.2 %) [37], and in North of Tunisia were α-pinene 47.51%, δ-3-carene7.40,% α-terpinyl acetate4.11%, β-caryophyllene, 4.53%, and α-cedrol4.99% [38]. However, these different qualitative and quantitative of volatile oil composition belongs to many factors such as environment, soil composition, and plant organ [39].

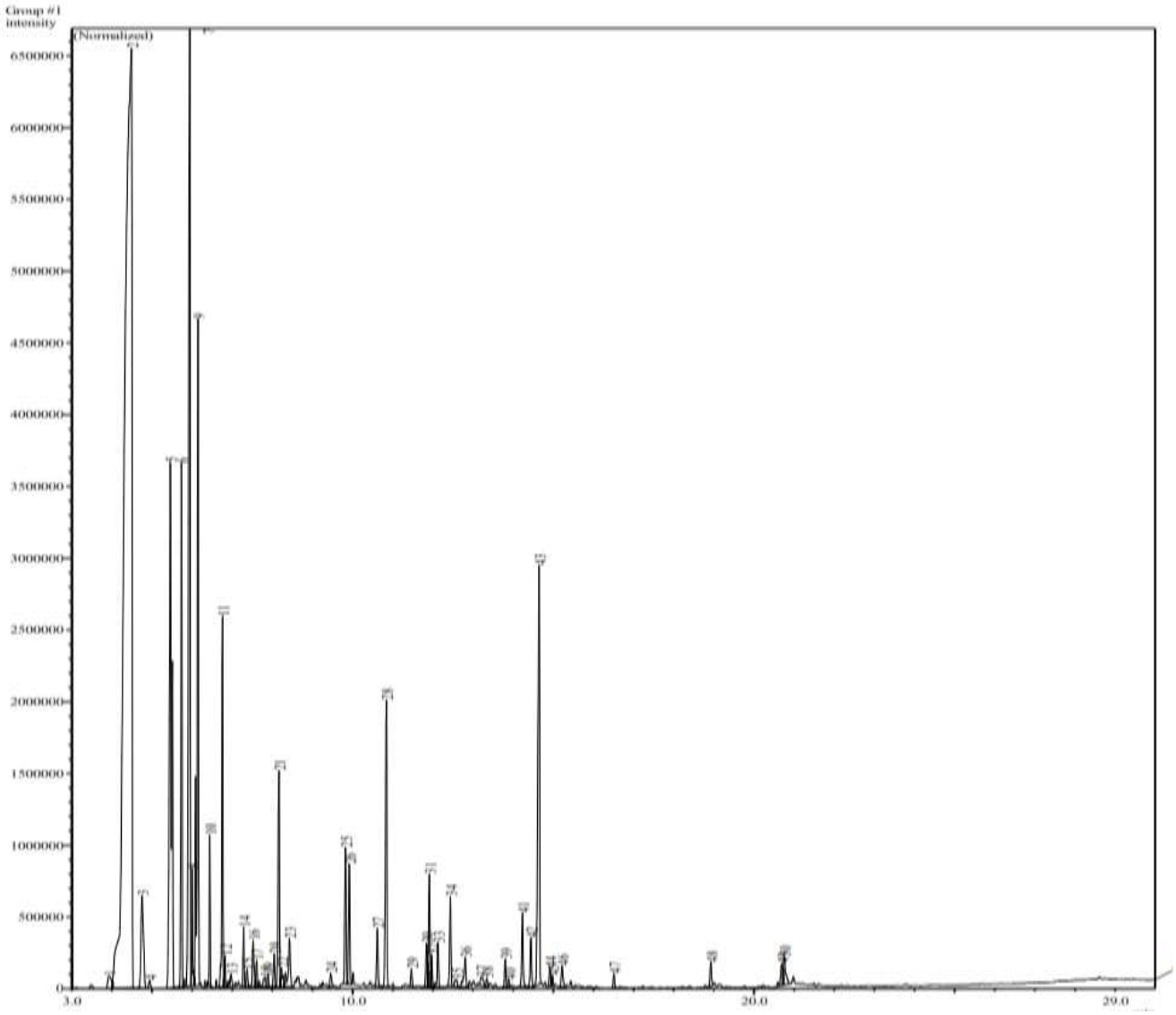

Figure 2. GC-MS chromatogram of essential oil from Iraqi *C. sempervirens* leaves

Table 1. Essential oil composition from leaves of Cupressus sempervirens L.

Peak NO.	R. Time	Area%	*SI	Name
2	4.488	44.09	95	alpha.-Pinene
3	4.752	1.44	93	Camphene
5	5.459	7.49	96	Thujene , Sabinene
6	5.737	3.67	90	beta.-Myrcene
7	5.939	10.01	95	3-Carene
9	6.146	6.41	90	m-Mentha-6,8-diene, (R)-(+)- (+)-m-Mentha-1(6),8-diene
10	6.437	0.89	95	gamma.-Terpinene
11	6.757	2.63	94	(+)-4-Carene
12	6.817	0.23	91	alpha.-Terpinene
13	6.967	0.18	85	beta.-Terpineol
14	7.283	0.41	86	alpha.-Campholenal
16	7.519	0.48	90	L-trans-Pinocarveol,L-pinocarveol
21	8.163	2.07	94	p-Menth-1-en-4-ol, Terpinen-4-ol
25	9.820	1.16	95	Bornyl acetate
28	10.840	2.64	90	alpha.-Terpineol acetate
29	11.464	0.16	91	beta.-Elemene,

Continued table 1.

Peak NO.	R. Time	Area%	*SI	Name
30	11.842	0.48	82	beta.-Cedrene
31	11.911	1.03	93	Caryophyllene
32	11.967	0.23	81	beta.-Cedrene
33	12.126	0.40	93	Thujopsene, Sesquichamene
34	12.434	0.76	95	alpha.-Caryophyllene
36	12.804	0.39	87	alpha.-Cubebene
37	13.202	0.16	92	alpha.-Curcumene
39	13.801	0.25	93	Hedycaryol
43	14.646	4.86	92	Cedrol
44	14.915	0.19	86	Cubenol
46	15.21	0.24	87	beta.-Selinenol

SI: similarity index

Separation and detection of quercitrin in an n. butanol fraction by TLC

Thin-Layer Chromatography was applied for the separation of components in n. butanol fraction and screening of quercitrin in the extract. The result under UV light at 254 nm shows the separation of different components of n. butanol Extract (E), and one component shows identical R_f values with quercitrin standard(Q) at 0.8, as seen in Figure 3.

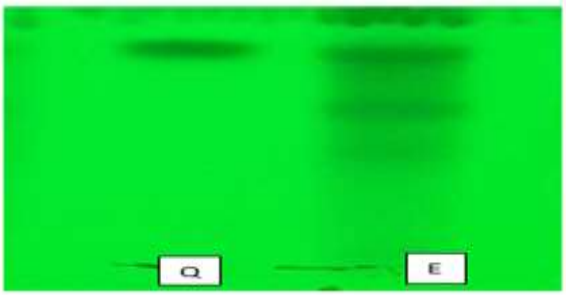

Figure 3. TLC analysis of n. butanol Extract (E)compared with quercitrin standard(Q)

Isolation and identification of (target compound) quercitrin

The results of The Semi-preparative HPLC for separation and isolation of chemical components in n. butanol fraction of *C.sempervirens* leaves was indicated at three peaks with different retention times and the concentration was at R_t 6.44 for the target compound to be isolated compared with quercitrin standard as in figures 4 (A) and (C). After that, the analytical HPLC was also performed for identification, and checking the purity of the isolated target compound (quercitrin) was achieved at R_t 6.55 min as a single peak as in Figures 4 :(B) and (C).

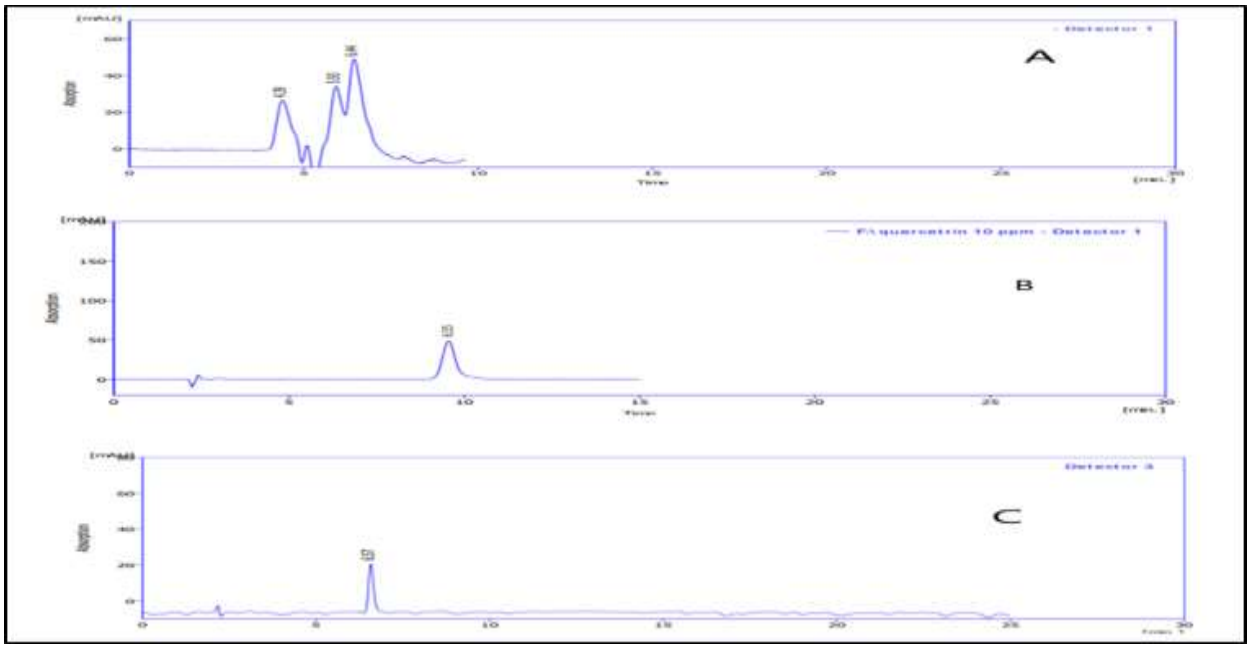

Figure 4. The HPLC chromatogram (A): n. butanol fraction of C. sempervirens leaves; (B): Isolated target compound (quercitrin); (C): quercitrin standard.

Furthermore, A thin-layer chromatographic (TLC) method is considered simple and fast screening for separation and simple identification of organic compounds [40]. The results revealed the purity of the isolated compound (one spot) and optimized compatibility with the standard at R_f value 0.52 and detected under UV light at 245nm as shown in Figure 5.

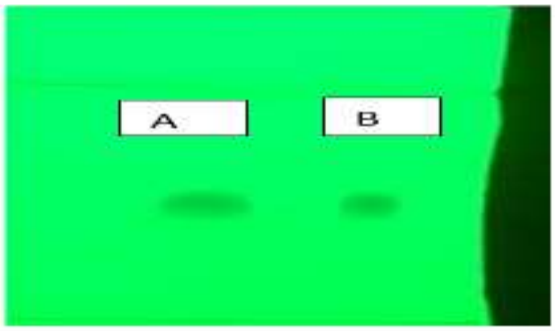

Figure 5. Analytical TLC for identification of the isolated compound and checking the purity: (A)quercitrin standard; (B) the isolated compound.

Structural elucidations of quercetin-3-O-rhamnoside

For structural characterization, Figure 6 shows the UV spectra characteristic of the isolated compound with quercetin-3-O-rhamnoside (quercitrin) at the following spectra of UV max: λ 210, 250, and 350 nm. Taken together, these data indicated the binding of quercetin to sugar molecules.

The characteristics of FT-IR spectra were in the range of 400–4000 cm −1as seen in Figure 7. The results show, that the band at 3160 cm−1and broadband range at 3414 -3340 cm - 1 represented the OH stretching vibration of the phenolic O-H and alcoholic O-H due to the intramolecular hydrogen bonding. The strong band observed at 1651 cm - 1 assigned to conjugated carbonyl C=O stretching vibration, A prominent peak at 1500 –1454cm - 1 represents C=C of an aromatic ring; stretching vibration, the prominent peak at 1056 and 1288 cm -1 represent stretching v. of conjugated ether linkage. The bands near 802 cm -1 indicate the bending vibration of the Ar– H group. However, the spectral patterns are in agreement with those data reported previously [41,42].

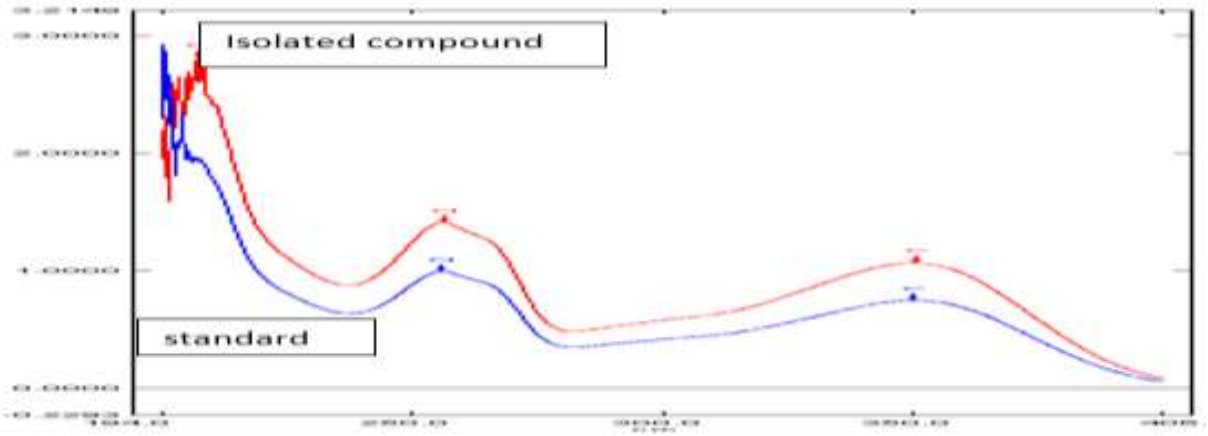

Figure 6. UV- Spectra of quercetin-3-O-rhamnoside compared with standard.

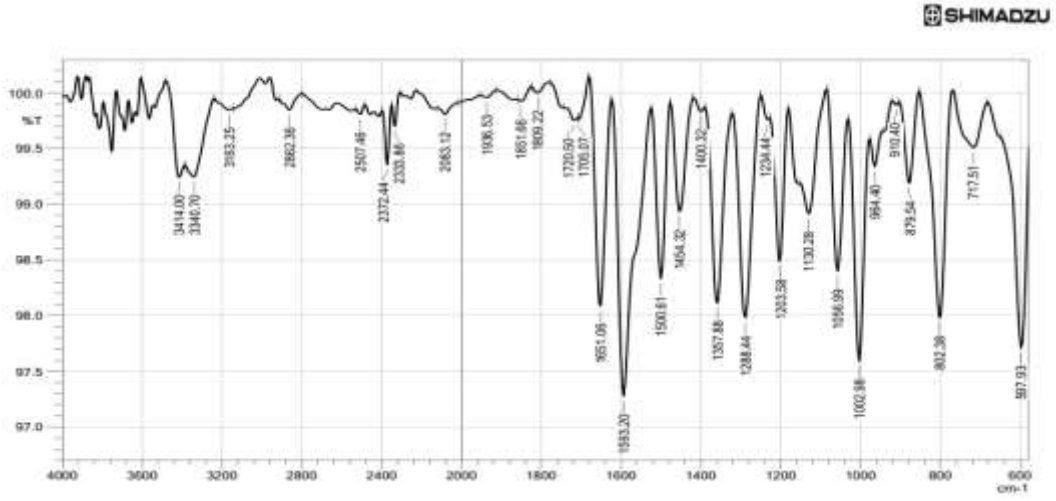

Figure 7. FTIR spectra of the isolated quercetin-3-O-rhamnoside

Furthermore, for further characterization, full scan product ion liquid chromatography coupled with negative ES ionization spectra was carried out of the isolated quercetin-3-O-rhamnoside. The scan mode for the identification of isolated compounds was MRM (multiple reaction monitoring). The retention time at 19.11 min and MS-MS of the isolated compound was compared with the standard in library Data. Figure 8 shows MS/MS spectra of the target compound showing different fragment ions at m/z 448 indicating a precursor ion $[M+1]^+$ and base peak at m/z 301 because of loss of $C_6H_{11}O_4^{\cdot}$ that represents quercetin. In addition, the characteristic product ions at m/z 177 represent $C_6H_{11}O_4^{\cdot}$-CH_2O, and at m/z 194 represent $C_6H_{11}O_4^{\cdot}$ – CO +H_2O and at m/z 181 represent $C_6H_{11}O_5^{\cdot}$ -OH (L-rhamnose - H_2O). However, all the fragmentation patterns are in agreement with those data reported previously [43].

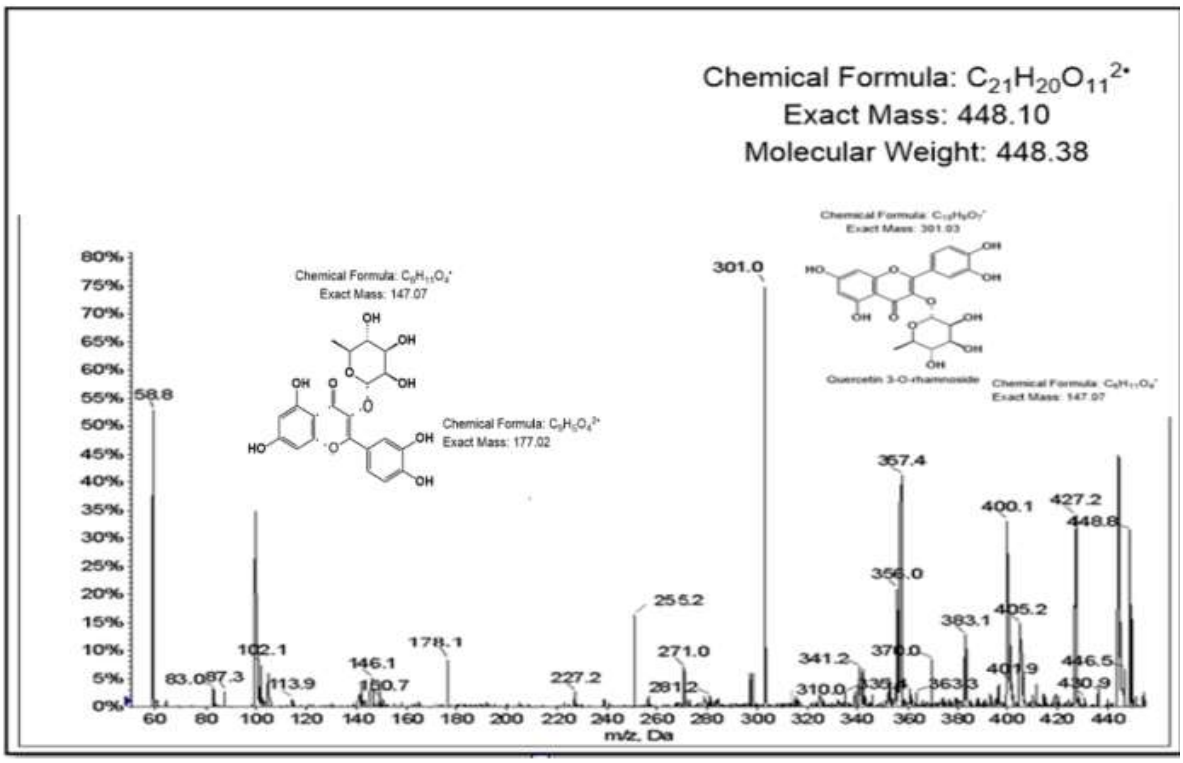

Figure 8. LC/MS-MS Spectra of isolated quercetin 3-O-rhamnoside

As a final consideration, the above findings approved that the isolated compound from n. butanol fraction of Iraqi *C.sempervirens* was quercetin 3- O- rhamnoside . This compound was reported in previous studies in conifer plants and in different species of the genus Cupressus such as; *C. funebris and C. lusitanica, Cupressus junebris* L, Cupressus glabra L., *Cupressus goveniana*, and C. macrocarpa, while it was not detected previously in species *C.sempervirens* [44,45]. However, an important flavonoid glycosyl; quercetin 3- O-rhamnoside was determined in this species (*C.sempervirens*)which is cultivated in Iraq.

Conclusion

The findings of this work evidenced a new Quercetin-glycoside from the species *Cupressus sempervirens* L. cultivated in Iraq. quercetin 3-O-rhamnoside was isolated as a major compound in an n-butanol fraction extracted by ultrasonic extraction. Moreover, the study also provides a good concentration of 0.9% essential oil yield. Hence, the Iraqi cypress plant might be considered a valuable source of quercetin 3-O-rhamnoside and essential oil.

Conflict of interest

The authors declare no conflict of interest.

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References

1. Mujeeb F., P. Bajpai, and N. Pathak. Phytoche mical evaluation, antimicrobial activity , and determination of bioactive components from leaves of *Aegle marmelos*. BioMed Res. Intl. 2 014; 6 (1): 1–11.
2. Nedhal A. Al-douri.A survey of medicinal plants and their traditional uses in Iraq. Pharmaceut. Biol. 2000; 38(1): 74-79.
3. Neale, D.B., Wheeler, N.C. The conifers in the conifers: genomes. In chapter one of variation and evolution. Cham, Springer Nature Switzerland .2019: 1-21.

4. Bhardwaj K, Islam MT, Jayasena V, Sharma B, Sharma S, Sharma P, et al. Review on essential oils, chemical composition, extraction, and utilization of some conifers in Northwestern Himalayas. Phyto. Res., Wiley. 2020 ;34(11):2889–910.

5. Farahmand, H. et al. Characterization and evaluation of genetic diversity of some old cypress genotypes (*Cupressus sempervirens L.*) in Iran using leaf mineral concentration, biochemical characteristics and SSR markers. Pl. Syst. Evol. 2014;301(2):761–772.

6. Eldalawy R, Naser NM, Naqqash ZA. Antimicrobial and antioxidant activity of *Iraqi Cupressus empervirens* cones. AIP. 2020 ;. 2213(1): 020006.

7. Goetz, P. & Ghedira, K. *Cupressus sempervirens L*. (Cupressaceae): Cyprès. Phytothérapie anti-infectieuse. 2012: 253–257

8. D'Auria A, Vingiani S, Marzaioli F, Teobaldelli M, Di Pasquale G. Recent history, use and forgetfulness of the cypress forest of Fontegreca (Southern Italy). Diver., MDPI.2020; 12(12):461-7.

9. Thukral SK, Singh S, Sharma SK. Pharmacognostical standardization of leaves of *Cupressus macrocarpa* Hartweg. ex Gordon. J.App. Pharm.Sci. 2014 May ;4(5):071-4.

10. Kuiate, Jules-Roger, Jean Marie Bessière, Paul Henri Amvam Zollo, and Serge Philibert Kuate. Chemical composition and antidermatophytic properties of volatile fractions of hexanic extract from leaves of *Cupressus Lusitanica* Mill. from Cameroon. J.Eth.pharma. 2006;103 (2): 160–165.

11. Sharma, Hitender, and Munish Garg. A Review of traditional use, phytoconstituents and biological activities of himalayan yew, Taxus Wallichiana. J. Integ. Med. 2015;13 (2): 80–90.

12. Kunwar RM, Shrestha KP, Bussmann RW. Traditional herbal medicine in Far-west Nepal: a pharmacological appraisal. Journal of ethnobiology and ethnomedicine. 2010 ;6(1):1-8.

13. N, Heimler DH. HPLC quantification of flavonoids and biflavonoids in Cupressaceae leaves. J. Chromatog.. 2002;56(7):469-74.

14. Romani A, Galardi C, Pinelli P, Mulinacci N, Heimler DH. HPLC quantification of flavonoids and biflavonoids in Cupressaceae leaves. Chromatographia. 2002;56(7):469-74.

15. Elmongy EI, Negm WA, Elekhnawy E, El-Masry TA, Attallah NG, Altwaijry N, Batiha GE, El-Sherbeni SA. Antidiarrheal and antibacterial activities of Monterey cypress phytochemicals: in vivo and in vitro approach. Molecules. 2022;27(2):346-20.

16. YÜKSEL TN, Bozgeyik E, Yayla M. The effect of quercetin and quercetin-3-d-xyloside on breast cancer proliferation and migration. J.Basic Clin. Heal. Sci. 2021;6(2):235-44.

17. Luo J, Si H, Jia Z, Liu D. Dietary anti-aging po lyphenols and potential mechanisms, antioxida nt, MDPI AG; 2021;10(2):283-87.

18. Abdulrazzaq MH, Khadeem EJ, Al-Muhammadi SS, Hepatoprotective Effect of *Echinops tenuisectus* (Compositae) on CCl4 Induced Hepatic Damage in Rats. IJPS, 2008;17(1):16-24.

19. Pandey A, Kumar R, Mishra A, Pandey A, Pandey AK., A systematic review on synthetic drugs and phytopharmaceuticals used to manage diabetes. Current Nutr., 2020; 16(4):340-356.

20. Yang D, Wang T, Long M, Li P., Oxidative medicine and cellular longevity, Hindawi Limited; 2020 Dec;2020:1–13.

21. Gonzalez - Burgos E, Gomez-Serranillos MP. Terpene compounds in nature: A review of their potential antioxidant activity. C.Med.Ch em., Bentham Science Publishers Ltd. 2012 N ov;19(31):5319–41.

22. Karkabounas S, KoRTsIs DN, Zelovitis J, Skafida P, DEMETzos CO, Malamas M, Elisaf M, Ev.angelou A. Effects of *Cupressus Sempervirens* cone extract on lipid parameters. in vivo. 2003; 17:101-4.

23. Al-Sayed E, Gad HA, El-Shazly M, Abdel-Daim MM, Nasser Singab A. Anti-inflammatory and analgesic activities of cupressuflavone from *Cupressus macrocarpa*: Impact on pro-inflammatory mediators. D. Dev. Res., Wiley. 2017;79(1):22–8.

24. Ulusal BG, Arikan S, Durusoy C. Anticoagulant effect of *Cupressus sempervirens*. Phytotherapy Research. Wiley.2007;21(11):1116–16.

25. Ibrahim NA, El-Seedi HR, Mohammed MMD. Phytochemical investigation and hepatoprotective activity of *Cupressus sempervirens L.* leaves growing in Egypt. Nat.Prod. Res. 2007;21(10):857–66.

26. Degenhardt RT, Farias IV, Grassi LT, Franchi GC, Nowill AE, da S. Bittencourt CM, et al. Characterization and evaluation of the cytotoxic potential of the essential oil of *Chenopodium ambrosioides*. Revista Bras. de Farm. . Springer. 2016;26(1):56–61

27. Zahari NAAR, Chong GH, Abdullah LC, Chua BL. Ultrasonic-Assisted Extraction (UAE) Process on Thymol Concentration from *Plectranthus Amboinicus* Leaves: K. Mod. and Opt. MDPI; 2020 9;8(3):322.

28. Altemimi A, Lakhssassi N, Baharlouei A, Watson D, Lightfoot D. Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. Plants, MDPI. 2017 ;6(4):42

29. Ibrahim NM, Kadhim EJ. Phytochemical investigation and antioxidant activity of Iraqi

Tribulus terrestris. IJPS. Sci.2015;24(1):68-73.
30. Sahagun G, Pobre RF. Sahagun G, Pobre RF. Effects of ultrasonic waves on breast cancer cell line (MCF-7) coupled with Zinger officinale (GINGER) extract using cell viability and comet assay tests. In International Conference on Photonics and Imaging in Biology and Medicine 2017 ; 7(1):18.
31. M'barek K. Chemical composition and phytotoxicity of *Cupressus sempervirens* leaves against crops. J.Ess. Oil.Plants. 2016;19(7):1582-99.
32. Trineeva OV, Safonova II, Safonova EF, Slivkin AI. Quantitative determination of rutin by thin-layer chromatography. Pharmaceutical Chemistry Journal. Springer Science and Business Media. 2014 ;48(2):134–8.
33. Karapandzova M, Stefkov G, Cvetkovikj I, Stanoeva JP, Stefova M, Kulevanova S. Flavonoids and other phenolic compounds in needles of Pinus peuce and other pine species from the macedonian flora. Nat.Prod. Comm. 2015 ;10(6): 1934578X1501000
34. Yang B., Kortesniemi M., P. Liu, et al., Analysis of hydrolyzable tannins and other phenolic compounds in Emblic leaf flower (Phyllanthus emblica L.) fruits by high-performance liquid chromatography-electrospray ionization mass spectrometry, J. Agric. Food Chem. 60 (2012) 8672–8683.
35. Corradini E, Foglia P, Giansanti P, Gubbiotti R, Samperi R, Laganà A. Flavonoids: chemical properties and analytical methodologies of identification and quantitation in foods and plants. Natural Product Research. 2011 ;25(5):469–95.
36. Azzaz NA, Hamed SS, Kenawy TA. Chemical studies on cypress leaves (Cupressus sempervirens) and their activity as antimicrobial agents. Al-Azhar J.Agricul.Res. 2019 ;44(2):100-9.
37. Ulukanli Z, Karabörklü S, Ates B, Erdogan S, Cenet M, Karaaslan MG. Chemical composition of the essential oil from *Cupressus sempervirens* L. Horizontalis Resin in conjunction with its biological assessment. J. E.Oil-Bearing Plants. 2014;17(2):277–87.
38. Milos M, Radonic A, Mastelic J. Seasonal variation in essential oil compositions of *Cupressus sempervirens L*. J. E. Oil Res .2002 ;14(3):222–3.
39. Ben Nouri A, Dhifi W, Bellili S, Ghazghazi H, Aouadhi C, Chérif A, et al. Chemical composition, antioxidant potential, and antibacterial activity of essential oil cones of tunisian *Cupressus sempervirens*. J. Chemist. 2015; 2015:1–8.
40. Tawfeeq AA, Mahdi MF, Abaas IS, Alwan AH. Isolation, quantification, and identification of rosmarinic acid, gas chromatography-mass spectrometry analysis of essential oil, cytotoxic effect, and antimicrobial investigation of Rosmarinus officinalis leaves. AJPCR2018 ;11(6):126.
41. Glavnik V, Simonovska B, Albreht A, Vovk I. TLC and HPLC screening of p-coumaric acid, trans-resveratrol, and pterostilbene in bacterial cultures, food supplements, and wine. JPC-J. of Planar Chromatog.Modern TLC. 2012 ;25(3):251-8.
42. Karakurt S. Modulatory effects of rutin on the expression of cytochrome P450s and antioxidant enzymes in human hepatoma cells. Acta Pharmaceutica. 2016;66(4):491-502.
43. Gopi K, Anbarasu K, Renu K, Jayanthi S, Vishwanath BS, Jayaraman G. Quercetin-3-O-rhamnoside from *Euphorbia hirta* protects against snake Venom induced toxicity. Biochimica et Biophysica Acta (BBA).2016 ;1860(7):1528-40.
44. Kumar S, Singh A, Kumar B. Identification and characterization of phenolics and terpenoids from ethanolic extracts of Phyllanthus species by HPLC-ESI-QTOF-MS/MS. J. pharmac. anal. 2017 ;7(4):214-22.
45. Romani A, Galardi C, Pinelli P, Mulinacci N, Heimler DH. HPLC quantification of flavonoids and biflavonoids in Cupressaceae leaves. Chromatographia. 2002 ;56(7):469-74.
46. Attallah NG, Negm WA, Elekhnawy E, Elmongy EI, Altwaijry N, El-Haroun H, El-Masry TA, El-Sherbeni SA. Elucidation of phytochemical content of *Cupressus macrocarpa* leaves: in vitro and in vivo antibacterial effect against methicillin-resistant Staphylococcus aureus clinical isolates. Antibiotics. 2021;10(8):890.