DOI: https://doi.org/10.31351/vol31iss2pp271-282

Isolation of Catchin and Epigallocatchin From Iraqi *Rhus coriaria* By Preparative High-Performance Liquid Chromatography (PHPLC)

Nabaa M. Ibrahem [*,1], Enas J. Kadhim[*], Shihab Hattab Mutlag [**]

[*] Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad, Baghdad, Iraq
[**] Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Abstract

Two compounds were isolated from the fruit part of *Rhus coriaria* that grew wildly or cultivated in the north of Iraq. The compounds were separated by preparative high-Performance Liquid Chromatography and their structures were established based on detailed spectroscopic techniques like FTIR and LC-.MS/MS.
Keywords: *Rhus coriaria,* Preparative HPLC, LC-M/SMS, FTIR.

عزل كاتجين و ايبيكالوكاتجين من نبات السماك العراقي بواسطة PHPLC

نبأ محمد ابراهيم [*،١] ، ايناس جواد كاظم[*] و شهاب حطاب مطلك[**]

[*]فرع العقاقير والنباتات الطبية ، كلية الصيدلة ، جامعه بغداد ، بغداد ، العراق
[**] فرع الادوية والسموم ، كلية الصيدلة ، جامعه بغداد ، بغداد ، العراق

الخلاصة

تم عزل مركبين من ثمار السماك العراقي الذي ينمو بشكل عشوائي / او مزروع بالقرب من القرى في شمال العراق بواسطة (PHPLC) وتم اخضاع المركبات المعزوله للعديد من التقنيات التحليليه والكيميائيه والكروماتو جرافيه و الطيفيه للتعرف عليها مثل كروماتوغرافيا السائله العاليه الاداء (HPLC) , جهاز" الفوربيه" لتحويل طيف الاشعه تحت الحمراء (FTIR) , الكروماتوغرافيا السائله مع طيف الكتلة(LCMSMS).
الكلمات المفتاحية : نبات السماك , كروماتوغرافيا السائله عاليه الاداء التحضيري(PHPLC), طيف الاشعه تحت الحمراء (FTIR) , الكروماتوغرافيا السائلة مع مطياف الكتلة (LC-MS-MS).

Introduction

Medical plants have been used since ancient time for the treatment a variety of disorders, and in some countries, the use of medical plants is often associated with witchcraft and superstition, because people did not have the scientific insight to describe and predict the healing action of plants [(1)]. Local environmental purse derived from plants have play an important role in the provision of dietary and medical care for humans in many parts of the World. A very important factor buckle down to people's interest in wild plants as food are times of famine or food scarcity, but on the other hand, eating wild products is becoming neat in our modern society [(2)].

Sumac is the common name of the *Rhus* genus, which comprises 91 species names in the Anacardiacea family, represented in Iraq by one species namely ***R. coriaria* L**. which sprout wildly or cultivated in the north of Iraq [(3)] .The name "Sumac" comes from "summāq" which means "dark red" in Arabic and Syriac. ***R. coriaria*** has been used in spice blends and in traditional medicines for hundreds of years. The word "sumac" will be henceforth used to describe the spice product of ***Rhus coriaria* [(4)].**

Rhus coriaria has long been used as a condiment spice, either alone or in combination with other spices. Traditionally in Iraq, it is used as a spice especially along with famous rich dishes like Kabab and grilled meat as well as over salads that often accompany these dishes[(3)].The research efforts on sumac extracts to date show a promising potential for providing renewable bio products with the following reported bioactivities: antiinflammatory, antifungal, Antifibrogenic, antimalarial, antimutagenic and antimicrobial .Sumac had been used as traditional source of medication in different dietary cultures all over the world, the use of the plant in seasonings and flavoring agents has been the mainstay of indigenous remedies across the world [(5)].

R. coriaria is used as a spice, and has been used in cooking for millennia. About two thousand years ago, the Greek physician Pedaniu Dioscoride (40-90 A.D.) wrote in his voluminous "De Materia Medica" ("Of Medical Matters") about the benefit properties of Sumac, principally as a diuretic and anti-flatulent [(6)].

Traditionally ,Sumac has been used in the treatment of diarrhea [(7)], hemorrhoids [(8)], ulcer [(9)], liver disease [(10)], animal bites, pain [(11)], diuresis, dysentery, hemorrhage, hematemesis, hemoptysis, ophthalmia, conjunctivitis, leucorrhea, and stomach tonic. [(12)]. Medical practitioners have also used sumac for cholesterol reduction [(13)], in the treatment of sore throat, and as an abortifacient [(7)]. Other reports also show its use in wound healing and as an antimicrobial [(14)].

[1]Corresponding author E-mail: nabaaibrahim@copharm.uobaghdad.edu.iq
Received:23 /11 /2021
Accepted: 1/2 /2022

Different parts of the plant have been used in various preparations in traditional system of medicine.

Powdered bark of sumac is effective for whitening the teeth. Bark infusion is useful in early treatment of viral eye infections. Bark is contused in water and put on the forehead for the first-aid treatment of epistaxis [15].Sumac fruits Powder are sprinkled on boiled egg and orally taken for the treatment of diarrhea [16]. A decoction of sumac fruits is prepared and administered orally (150 cc) for the treatment of liver disease, diarrhea and urinary system disorders. Decoction is taken three times per day until improvement occurs [7].

The plant is globally distributed in temperate climate and tropical regions and can grow on marginal lands. The most common species of sumac is ***Rhus coriaria*** which is grown commercially on global scale in Mediterranean and Middle East. This species had been cultivated for several years to produce a material of high quality for tanning. It is growing wild in the region extending from the Canary Island over the Mediterranean coastline to Iran and Afghanistan. It is native to the Mediterranean and the Southeastern Region of Turkey [17].

In Iraq, sumac distributed mainly in North, North East and North West, in areas of an altitude more than 540m above sea level. ***R. coriaria*** sprout in mountain environments especially in Rawandowz city. Sulimania, which are the areas of most prevalent territories [18].

More than two hundreds compounds have been specified from the ***R. coriaria*** and most of them are physiologically active. These chemical constituents can be set to various classes of the tannins (condensed and hydrolysable), conjugated phenolic acids, phenolic acids ,anthocyanins, , organic acids ,coumarins, flavonoids, terpenoids, xanthones, steroids, essential oils , and other groups of compounds have been detected [19] . So in this study, an investigation for the phytochemicals in the fruit of this plant grown at Iraq north regions had been done using several hyphenated techniques include preparative HPLC, high-performance liquid chromatography coupled with diode array detector (DAD), and Liquid chromatography and mass spectroscopy is adopted. Preparative HPLC are used to separate a specified amount of pure compounds and HPLC-DAD is used to confirm the purity of collecting fractions. The molecular structure was assigned by FTIR and MS provides useful information about molecular masses and the structure of the isolated constituents of the crude methanolic plant extract.

Materials and Methods

Plant material collection.

Rhus coriaria fruit were collected from the north of Erbil in Rawendos at April 2020, . After collection of plant, it sent to be identified and authenticated by specialist Prof.(Dr. Sukaena Abass), Department of Biology /College of Sciences/ University of Baghdad .The plant parts were cleaned thoroughly, dried under shade, and ground in a mechanical grinder to a fine powder.

Extraction method

A-The air-dried powder of the fruit is weighted then defatted with N-hexane to get rid of chlorophyll and waxy material and then extracted in soxhlet with (1:10) aqueous methanol for 18hr then the extract is combined and dried by a rotary evaporator the dry residue is weighted and the yield of extraction is calculated.

The dry fruit extract is suspended in water, then treated with various reagents to screen the type of phytochemicals present and then partitioned 2-3 times with solvent of various hydrophilicity such as N-hexane, chloroform, ethyl acetate and N-butanol. Each fraction is dried and weighted and treated with screening tests to identify the phytochemicals present [20, 21].

The ethyl acetate fraction is dried and weighted to identify the phytochemicals present and Isolation different constituents using chromatographic techniques, preparative HPTLC. Identification of isolated compounds had been done by using different chromatographic and spectroscopic techniques like FTIR and LC/ MS/MS.

The preparative HPLC specification and conditions

Table 1 represents the preparative HPLC apparatus specification which consists of an autosampler, a pump, and a photodiode array detector coupled with an analytical workstation.

Table 1.Preparative HPLC apparatus specification

	Component	Model or version	Company and origin
1	Binary high pressure gradient pump	P6.1L	Knuaer , Germany
2	Diode array detector	DAD 2.1L	Knuaer , Germany
3	Sample loop (20 µl) and injector	D1357	Knuaer , Germany
4	Analyses and system control software	Claritychrom , V 7.4.2.107	Dataapex, Czech Republic
5	Fraction collector	Foxy R1	Teledyne Isco, USA
6	Mass spectrometry (LC/MS/MS)	SCIEX-ABI 3200 MS system	SCIEX Technologies, Germany

***The condition of HPLC-DAD analysis*.**

The High-Performance Liquid Chromatography was done by using stationary phase on Acclaim C 18 column (5 µm particle size,250 x 4.6 mm), Dionex Ultimate 3000 liquid chromatograph. The mobile phase consist of 1% aqueous acetic acid solution (Solvent A) and acetonitrile(Solvent B) using gradient elution.

The flow rate was kept to 0.7 ml/min, the column was thermostatically controlled at 280^0C and the injection volume was adjusted at 20 µl. The gradient elution was changed from 10 % to 40% B in a linear manner for duration of 28 min, from 40 to 60 % B in 39 min, from 60 to 90 % B in 50 min. The mobile phase composition back to initial condition (solvent B: solvent A: 10: 90) in 55 min and pliable to run for another 10 min, before the injection of another sample. Total time for analysis per sample was 65 min. The separation chromatograms were detected using a photo DAD array Ultraviolet detector at three different wavelengths (272, 280 and 310 nm) according to absorption maxima of analyzed compounds [(22)].

able 2. The gradient elution time and flow rate of HPLC analysis

Time	Mobile A concentration %	Mobile B concentration %	Flow rate ml/min
0	90	10	0.7
5	90	10	0.7
28	60	40	0.7
39	40	60	0.7
50	10	90	0.7
55	90	10	0.7

Fraction collector factors

Peak recognition: slope 0.2 Au/min , level 10 mAU
Fraction size: 5 milliliters

The detection of isolated compounds (compound I and II) were performed by matching retention time and absorbance spectrum of the standards (epigallocatechin and catechin). The quantitative analysis of the detected components were calculated by serial concentrations of standard materials to build calibration curve between concentration and its equivalent peak area, the calculation was done according to the straight line equation Y=aX +b, slop=y/x (Yand B: b: y-intercept), where R^2 regression factor.

Identification of compound by mass spectrometry

Apparatus and Instrumentation conditions of -mass spectrometry (LC/MS) direct infusion scan were applied as follow:

The- MS was performed using a SCIEX-ABI 3200 MS system (SCIEX Technologies, Germany).

The column jumper is used to connect Liquid Chromatography directly to the mass detector.

Using the auto sampling device, a sample volume of 20-µL was injected. Solvent A- Contained Millipore water with 5.0mM ammonium format/0.1%formic acid and Solvent B- consisted of HPLC-grade acetonitrile with 0.1% formic acid. The LC/MS was operated in the positive-ion mode with m/z scan range 50-900. The spectra of different peaks were studied in terms of LC-MS/MS chromatograms collected for 2 isolated fractions. The molecular masses of each product are calculated using a continuum. For molecular mass estimation, only molecular ion peaks were evaluated in each chromatogram. LC-MS/MS – spectra may be a valuable instrument to classify molecular mass components. The composition of a compound can be shown by the fragmentation patterns of different units. Unknown compound fragmentation results have been compared to the already available data in the literature.

Acquisition Method: \LCMS-ESI-POS. dam and all data had been shown in Table (3)

Table 3. Time and flow rate of analyzed compounds

Step Table:				
Step	**Total Time(min)**	**Flow Rate(µl/min)**	**A (%)**	**B (%)**
0	**0.00**	**1000**	**90**	**10**
1	**4** **15**	**1000**	**90**	**10**
2	**20**	**1000**	**10**	**90**
3	**22**	**1000**	**10**	**90**
4 **5**	**25**	**1000**	**90** **90**	**10**

A= 5.0mM ammonium format/0.1%formic acid , B= ACETONITRILE/0.1formic acid , Scan Type: Q1 MS (Q1) Range 50- 900 M+1, Polarity: Positive – ESI, Scan Mode: Profile .

Instrument specifications and details : Software = Analyst 1.6.4 version , Pump Agilent 1200 G1312A , Column Oven Agilent 1200 G1316A , AutoSampler Agilent 1200 G1367B , Mass Spectrometer API 3200 AB-Sciex

Fourier transforms infrared (FT-IR) Spectroscopy

Infra-Red (IR) spectra of isolated compounds were recorded in Shimadzu FTIR spectrometer in the range of wave number 500 to 4000 cm–1 and operated in the transmittance mode. The mold and press of a Pulver containing approximately 1 mg of substance were then used for preparing the thin disk under anhydrous conditions.

Results and Discussion

The results of the performed PHPLC analysis of ethyl acetate extract from the plant ***Rhus coriaria*** fruits, confirmed the sTable retention time of compound I and II in the extract which were comparable to retention times of authenticated reference standards at identical chromatographic conditions as shown in Figure (1). In addition to that, there is a matching between the UV spectra of the detected isolated compounds with corresponding standards UV spectra as shown in Figures (2 and 3) which demonstrate a good fitness between the detected compounds and the standards. These results revealed that sumac plant contain epigallocatechin and catechin.

The phytochemical constituents in plant extract are usually available in low quantity. For this reason a sensitive instrument was used in this study for detection and isolation. In this research, the PHPLC is a flexible, sTable, and strongly enhanced column chromatography method, a technique commonly used for the separation of natural materials. Preparatory high-pressure chromatography is an excellent purification technique and has been developed to ensure that important materials in the chemical, medicinal, biotechnological, and biochemical industries are insulated and identified [23] .

Gong and his colleagues have been isolated seven catechin compounds from fresh tea leaves using semi-preparative liquid chromatography technique [24] .

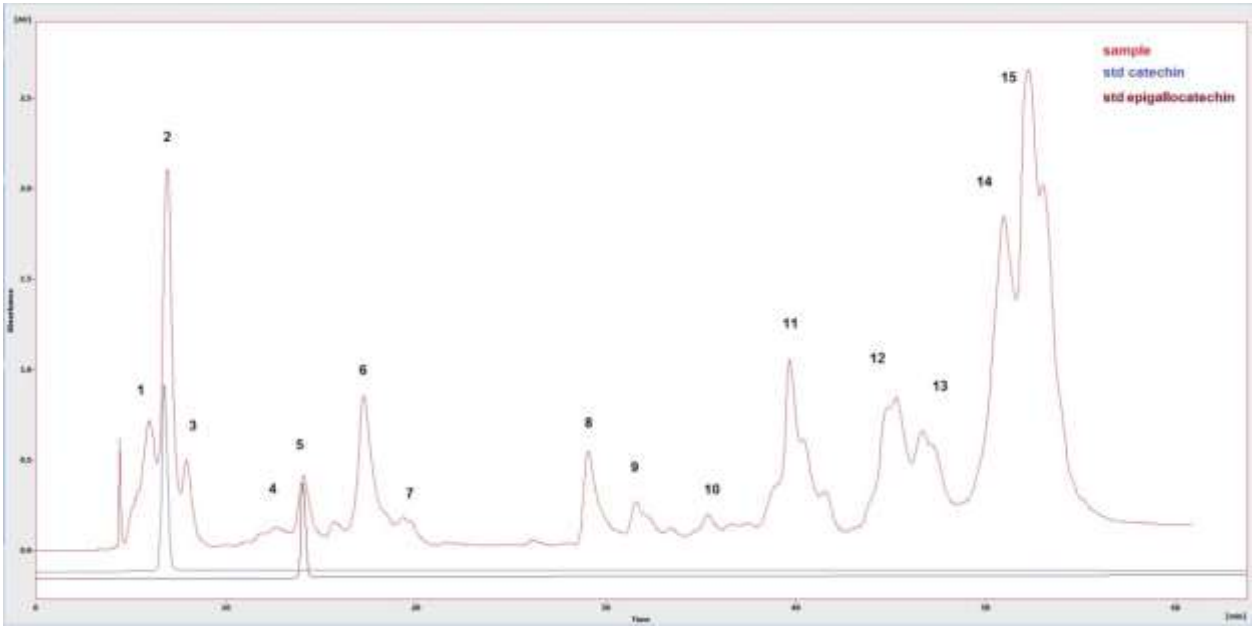


Figure 1. Preparative HPLC Chromatogram For ethyl acetate fraction

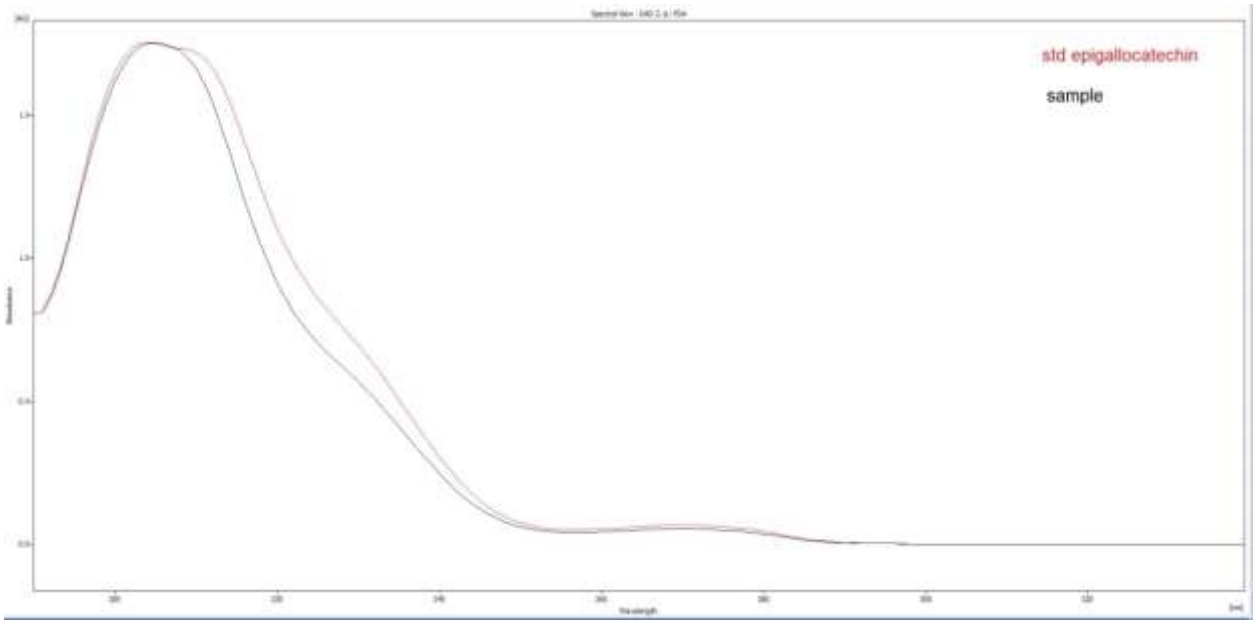


Figure2. UV spectrum of ethyl acetate fraction matched with standard UV spectrum of epigallocatechin.

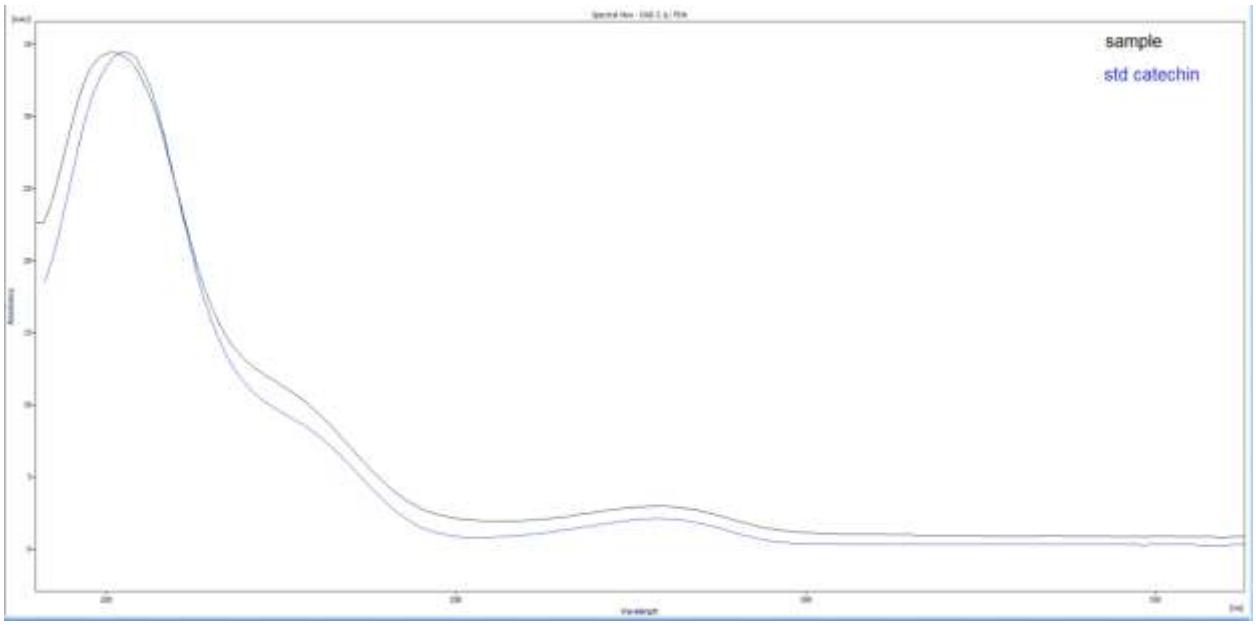


Figure 3. UV spectrum of ethyl acetate fraction matched with standard UV spectrum of catechin.

Quantities of the studied compounds were evaluated by constructing a calibration curve using plotted peak area versus mass concentration of each standard using 5 different values of concentration. Then applying the straight line equation of the standard curve as shown in Figures (4 and 5) .

The quantitative PHPLC analysis of the isolated compounds revealed that the catechin has higher concentration than epigallocatechin in fruit extract of ***Rhus coriaria*** plant as shown in Table (4).

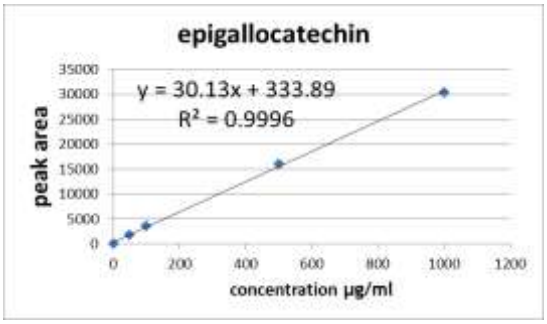


Figure 4 . Standard curves of epigallocatchin

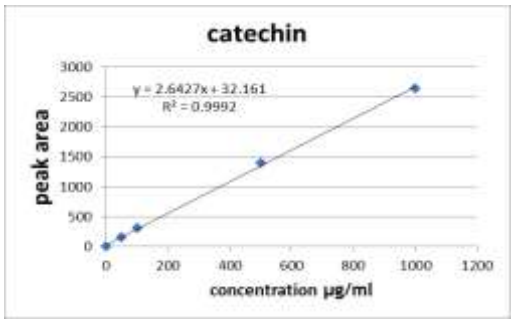
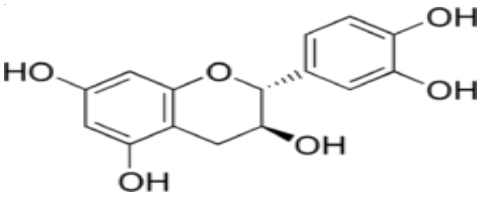


Figure 5 . Standard curves of catchin

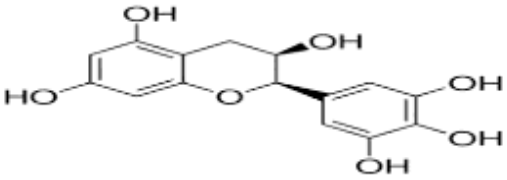
Table 4. Quantitative analysis of ethyl acetate fraction

Ethyl acetate fraction	Peak area (y)	µg/ml (X)	mg/ml
Epigallocatechin	661.58	109.90	0.1099
Catechin	3166.77	1198.66	0.19911

Catechin and epigallocatechin are belonged to Flavanols group of flavonoids. They have two chiral centers, one on C2 and other on C3. Structurally, catechin has trans configuration and epigallocatechin has cis configuration with additional hydroxyl group (Figure 6) [25].



Catechin



Epigallocatechin

Figure 6 . Chemical Structure of catechin and epigallocatechin

Identification of isolated compound by the spectroscopic techniques

Identification of an isolated compound is usually achieved by a combination of several important spectroscopic techniques, such as IR and LC-MS/MS. The compound of interest can be identified by comparing its characteristic features with literature values. (no need to mention Figure).

IR – spectroscopy

The Fourier transforms infrared spectroscopy of compound I (Table 5, Figure 7) and compound II (Table 6 and Figure 8) showed the appearance of characteristic peaks at 3197- 3375cm^{-1} due to Phenolic OH stretching. band (H-bonded). The peaks 1610 cm^{-1},1519 cm^{-1} for compound I and 1604 cm^{-1}, 1558 cm^{-1} attributed to the alkene stretching of aromatic ring . On the other hand, bending vibration band of phenyl O-H group is represent by 1350 cm^{-1} peak for compound I and 1365 cm^{-1} peak for compound II. The appearance of peak at (1276-1284 cm^{-1}) is related to the stretching of cyclic C-O.

It has been observed that all chemical compounds show marked selective absorption in the IR spectrum of any molecule and are the fingerprints for structural confirmation. IR Spectroscope technology is a very important tool for identifying several varieties of plant constituents in phytochemical studies. It also helps in the systemic elucidation of new compounds in plants [26].

Accordingly, isolated compounds were subjected to IR spectroscopy.

From interpretation of the IR spectra, We concluded that the IR spectra of compound I & II have been contrasted with that of authentic samples epigallocatechin and catechin respectively Figure 7 & 8).

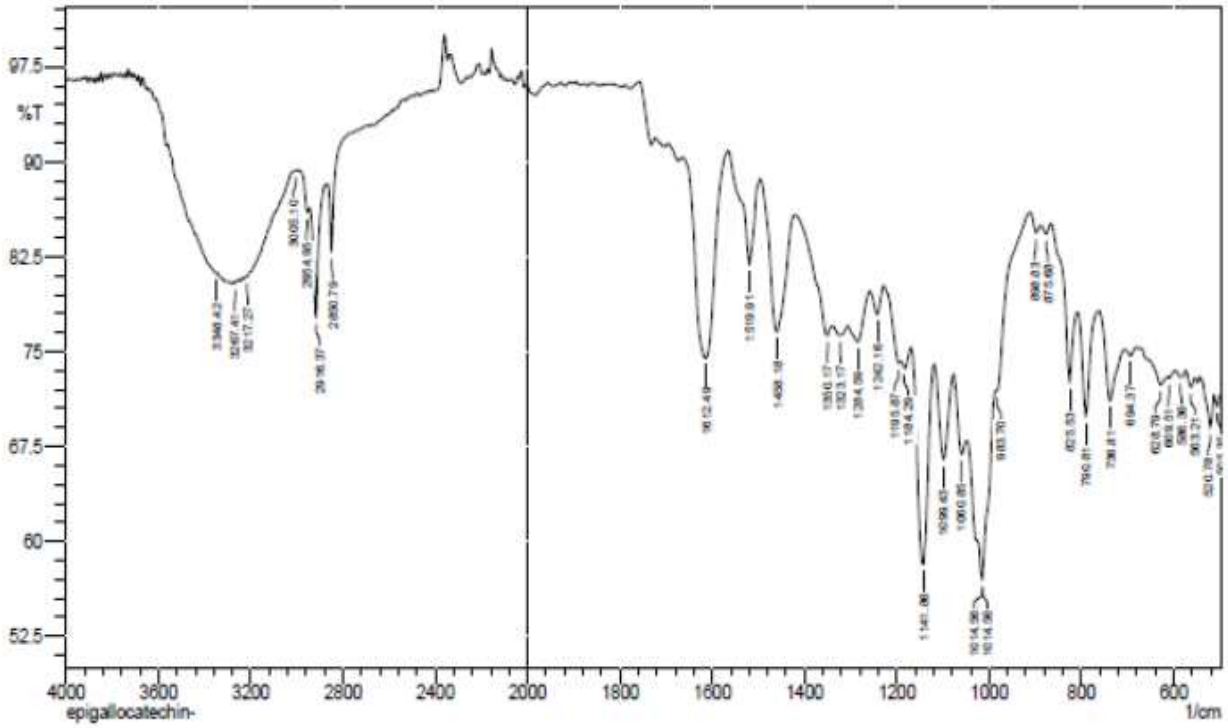


Figure 7. FTIR of Compound 1(epigallocatechin)

Table 5. FTIR Interpretation of Compounds I

Group frequency wave number in cm – 1	**Interpretation**
3217- 3348	Phenolic OH str. band (H-bonded)
3005	Aromatic C-H str.
2954	Asymmetric CH_2 str.
2850	symmetric CH_2 str.
1610, 1519	Ar. C=C str. Bands
1458	Bending vibration band of CH_2
1350	Bending vibration band of phenyl O-H
1284	Cyclic C-O str. vibration band

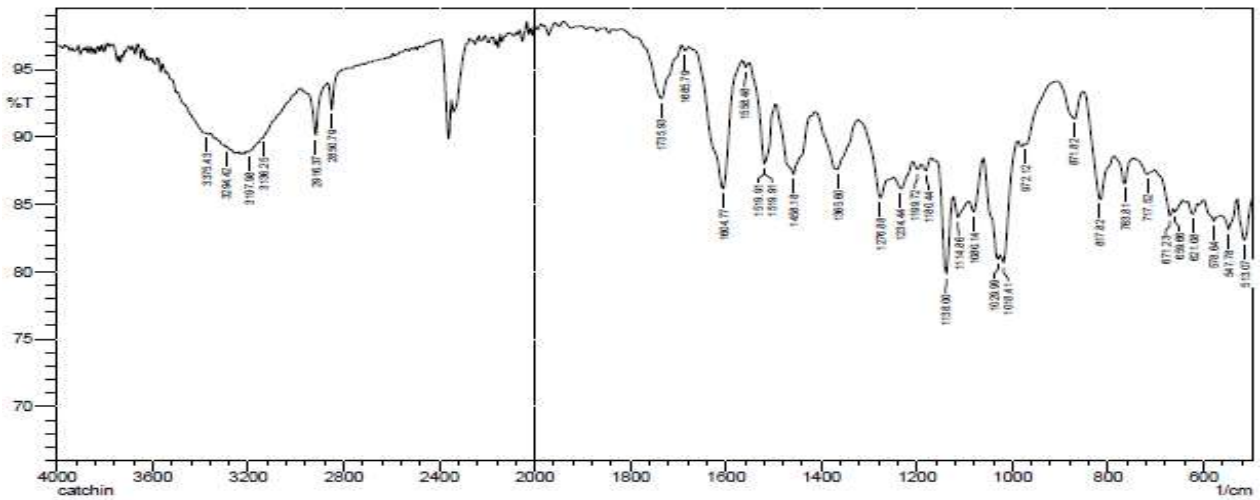


Figure 8. FTIR of Compound 1I (catechin)

Table 6. FTIR Interpretation of Compounds II.

Group frequency wave number in cm – 1	Interpretation
3197- 3375	Phenolic OH str. band (H-bonded)
2916	A symmetric str. Band of CH_2
2850	symmetric str. Band of CH_2
1604, 1558	Ar. C=C str. Bands
1365	Bending vibration band of phenyl-O-H
1276	Cyclic C-O str. vibration band
1458	C-H bending 0f CH_2

Identification of isolated compound by LCMS/MS spectroscopy

Identification of peaks of catechin and epigallocatechin isolated from *Rhus coriaria* were carried out by comparing mass spectra of standard data and literature [27] .

The mass spectrum of epigallocatechin was presented in(Figure 9,10). The peak of protonated ion [M+H]+ occurred at *m/z* 307. While, the mass spectrum of catechin was presented in (Figure 11,12) which show the *m/z* signal at 291 that represent a typical fragment ion detected in the mass spectrum of catechin [28,29].

From the result,Compound I according to FTIR and LC-MSMS identical to epigallocatechin and Compound II according to FTIR and LC-MSMS identical to catechin.

LC-MS/MS is also a powerful analytical method that combines the physical separating capacity of fluid chromatography with the mass analysis capacities of mass spectrometry, providing a powerful instrument for analyzing complex mixes of analytical chemistry techniques. Liquid chromatography will separate photo components based on the necessary time to appear at the end of appropriately packaged columns for individual components, while mass spectroscopy distinguishes each component based on the fragment mass that is produced when a beam of electrons bombarded the compound. LC-MS is used with high sensitivity and selectivity to analyze specific botanical extracts. It provides extensive information on structural elucidation of the substances generally and makes it easier to detect and identify possible chemical compounds in medicinal herbs, particularly when pure standards are not available. Herbal medicinal drugs are being standardized and characterized as an area of scientific concern in the herbal drug industry. HPLC is a good qualitative and quantitative technique, usually used for the evaluation of pharmaceutical Because of its unique properties like high resolutions, high sensitivity (ppm repeatability, small sample size, moderate analysis condition, no need to vaporise the sample as in the gas chromatography, easy to fractionate the sample and purify , The use of HPLC are increased day by day across the world[27] .

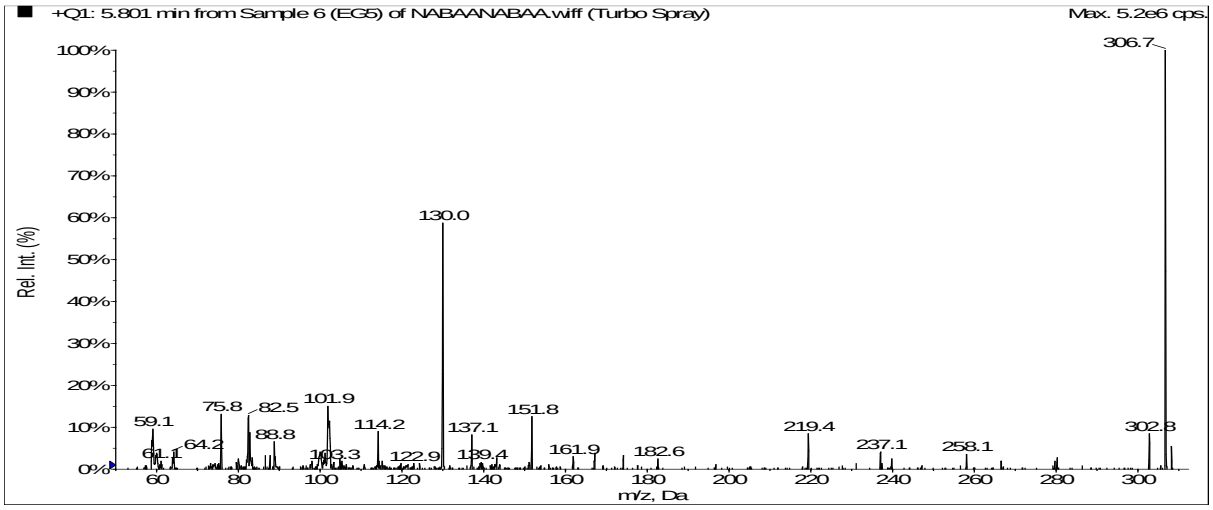


Figure 9.LC-MSMS of Compound 1I

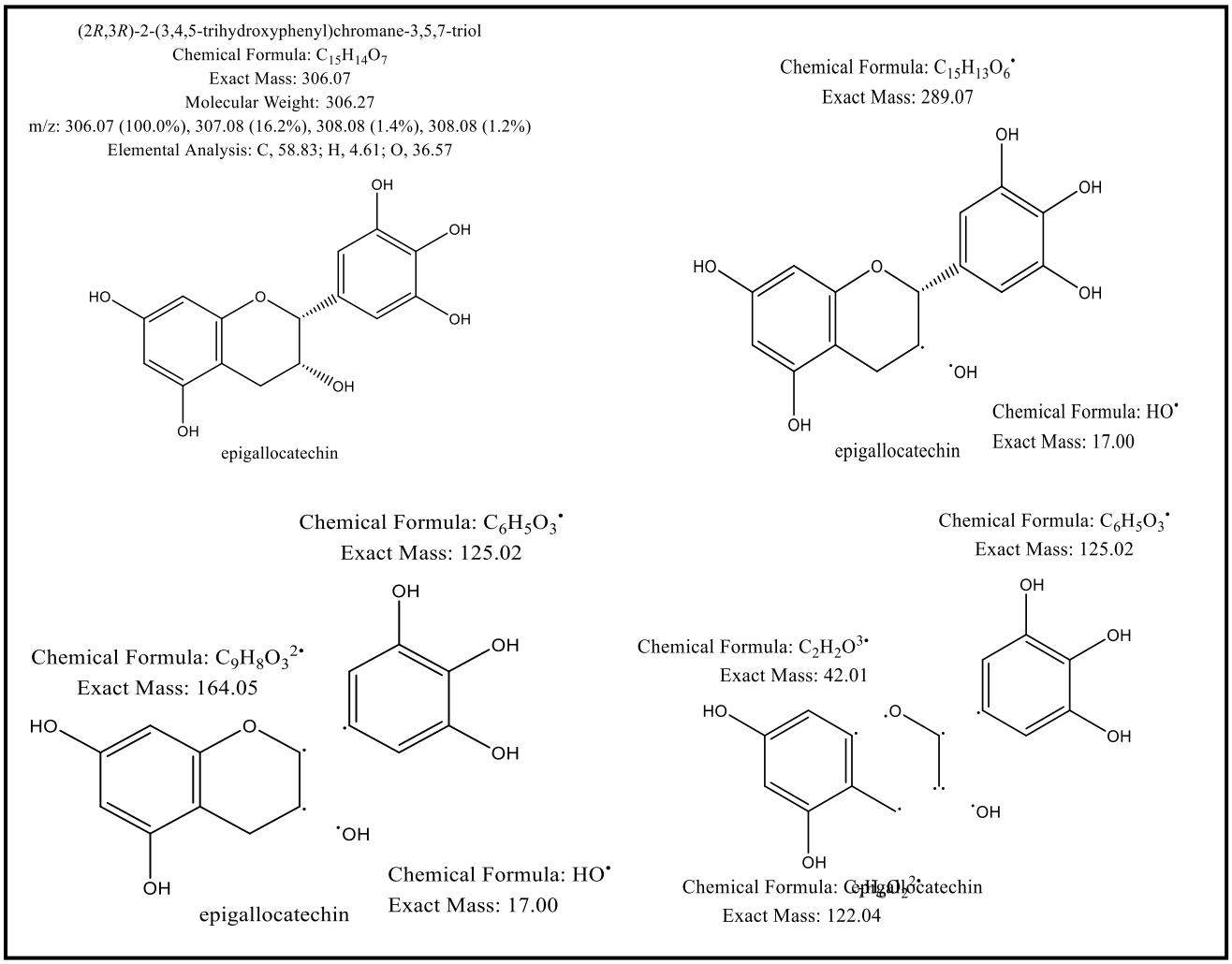


Figure 10. Mass Fragment of Compound I Epigallocatechin

Epigallocatechin = 306.27 g/mol

M\Z= 306.07

FRAGMENTS :

1.289

2.164

3.122-125

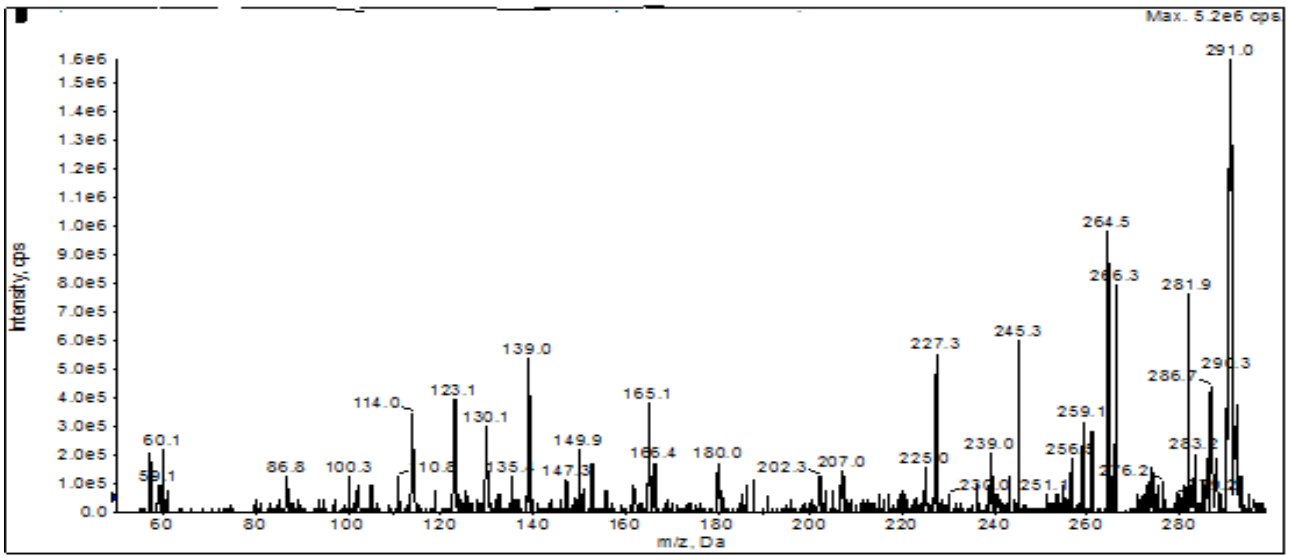


Figure 11. LC-MSMS of Compound 1I

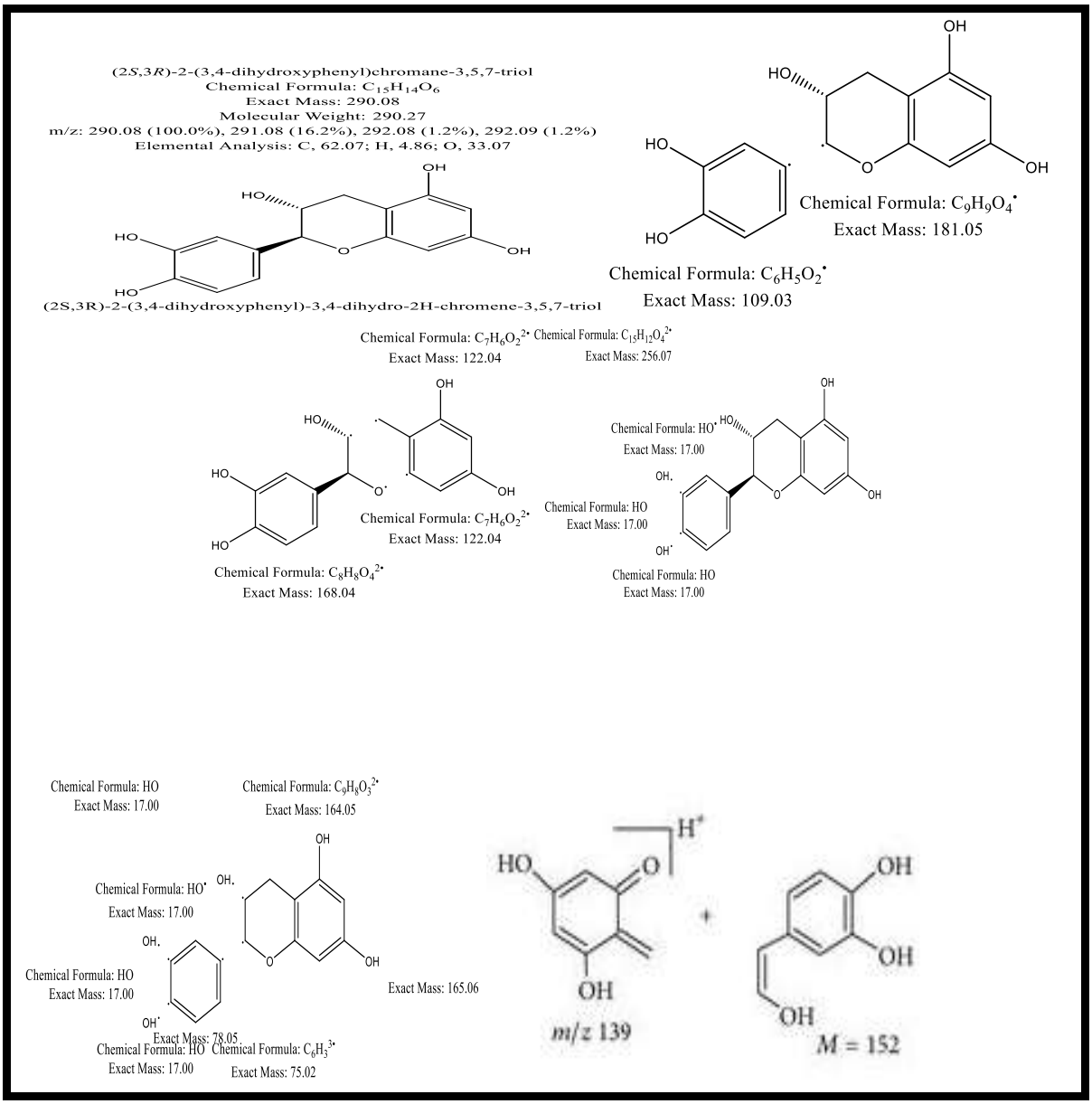


Figure 12.Mass Fragment of Compound II catechin

<u>catechin =290.27 g/mol</u>

M\Z= 291.0

FRAGMENTS :

1- 180-181
2- 123
3- 256
4- 165.1

Conclusion

From the above study two flavonoids are isolated from ***Rhus coriaria*** fruits (catechin and epigallocatechin) .phytochemical research is a scientific technique that is very important. This process identifies the main components found in the every plant element such as bark, leaves, stem, root and fruits. Although nobody know how many different plants in the world today are used, but we aware that both herbal and western medicine are very valuable medicinal plants.

References

1. Bagetta G. Herbal medicines: development and validation of plant-derived medicines for human health. Clinical pharmacognosy. 2012; p.27
2. Schunko, C, Grasser, S., Vogl, C.R.Explaining the resurgent popularity of the wild: motivations for wild plant gathering in the Biosphere Reserve Grosses Walsertal, Austria. Journal of Ethnobiology and Ethnomedicine. 2015;11(55): 1–14.
3. Thukaa Zuhair Abdul-Jalil, Rhus coriaria (Sumac): A Magical Spice. Herbes and spices book .2020
4. Abu-Reidah I M, Jamous R M and Ali-Shtayeh M S: Phytochemistry, Pharmacological Properties and Industrial Applications of *Rhus coriaria* L. (Sumac). Jordan Journal of Biological Sciences 2014; 7(4): 233 – 244.
5. Rayne S, Mazza G. Biological activities of extracts from sumac (*Rhus* spp.): A review. Plant Foods Hum Nutr 2007; 62:165-75.
6. Norton S and Lily Y B. :De materia medica by Pedanius Dioscorides. Hildesheim, Germany, Olms-Weidmann, J History Medicine and Allied Sci.2006; 61: 218-220.
7. Janbaz, K.H.; Shabbir, A.; Mehmood, M.H.; Gilani, A.H. Pharmacological Basis for the Medicinal Use of *Rhus coriaria* in Hyperactive Gut Disorders. *Br. J. Pharm.* **2014**; *9*: 636–644
8. Lev E. and Amar Z. : "Fossils" of practical medical knowledge from medieval Cairo. J. Ethnopharmacol 2008; 119:24-40.
9. Somayeh Zakeri, 1 Ayesheh Enayati , Fatemeh Kolangi , Sumac Can Be Offered as an Alternative Treatment for Controlling Abnormal Bleedings Based on Persian Medicine , Trad Integr Med. 2020; 5(4): 180-182 .
10. Samira Kazemi , Farzad Shidfar, Simin Ehsani , The effects of sumac (Rhus coriaria L.) powder supplementation in patients with non-alcoholic fatty liver disease: A randomized controlled trial, Complementary Therapies in Clinical Practice. 2020; 41(16):101259
11. Lev E: Reconstructed materia medica of the Medieval and Ottoman al-Sham. J.Ethnopharmacol 2002; 80:167-179.
12. Lev, E :Drugs held and sold by pharmacists of the Jewish community of medieval (11, 14th centuries) Cairo according to lists of *materia medica* found at the Taylor–Schechter Genizah collection, Cambridge. J. Ethnopharmacol 2007; 110:275-293.
13. . Lev, E. and Amar Z.: Ethnopharmacological survey of traditional drugs sold in the Kingdom of Jordan. J. Ethnopharmacol 2002; 82:131-145.
14. Rayne, S. and Mazza G.: Biological activities of extracts from sumac (Rhus spp.): A review; Plant. Food. Hum. Nut.2007; 62:165-175
15. Usmanghani, K., Saeed A., and Alam M.T: Indusyunic medicine. Karachi: University of Karachi Press, 1997; 378-379.
16. Sezik, E., Yeşilada E., Honda G., Takaishi Y., TakedaY., Tanaka T.: raditional medicine in Turkey X. folk medicine in Central Anatolia. J. Ethnopharmacol.2001; 75:95-115
17. **17.** Nabaa M. Ibrahem [1], Shihab Hattab Mutlag [2] , Enas J. Kadhem, The Cytotoxic Effect of Iraqi *Rhus Coriaria* against Breast and Esophagus Cancer Cells . Drugs and Cell Therapies in Haematology. 2021: 10(3) ; pp 115-129
18. AL-Maa'thidy A M: A systematic study of the genus *Rhus* L. (Anacardiaceae) in Iraq.2006; 10:100-114.
19. Asgarpanah J. and Saati S. : An overview on phytochemical and pharmacological properties of *Rhus coriaria* L. Research Journal of Pharmacognosy 2014; 1(3): 47-54.
20. Mustafa H. Alwan*, 1and Maha N. Hamad :* Phytochemical Investigation of the Aerial Part of Iraqi Convolvulus arvensis, Iraqi J Pharm Sci.2020; 29(2) .
21. Rabab H.Qassim*,1and Enas J. Kadhem : Phytochemical investigation and Anti-angiogenic Examination of Iraqi Vigna adiata L. Seeds and Sprouts , Iraqi J Pharm Sci. 2020 29(2)
22. Seal, T., Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, Sonchus arvensis and Oenanthe linearis of North-Eastern region in India. *Journal of Applied Pharmaceutical Science*. 2016: *6*(2), 157-166.
23. Kong W, Jin C, Xiao X, Zhao Y liu W, Li Z, *et al*. Determination of multicomponent contents in Calculus Bovis by ultra-performance liquid chromatography-evaporative light scattering detection and its application for quality control. J Sep Sci. 2010; 33(10); 1518-1527.
24. Gong, Zhihong & Chen, Si & Gao, Jiangtao & Li, Meihong & Wang, Xiaxia & Lin, Jun & Yu, Xiaomin. (2017). Isolation and purification of seven catechin compounds from fresh tea leaves by semi-preparative liquid chromatography.

Chinese Journal of Chromatography (Se Pu). 35. 1192-1197.

25. Colomer, Ramon & Sarrats, Ariadna & Lupu, Ruth & Puig Miquel, Teresa. Natural Polyphenols and their Synthetic Analogs as Emerging Anticancer Agents. Current drug targets, 2017; 18(2):145-157

26. Sasiidharan S, Chen Y, Saravanan D, Sundram KM and Lrtha LY: Extraction, isolation and chractarization of bioactive compounds from plants extract. Afr. J. Tradit. Compllement Altren. Med. 2011; 8(1): 1-10.

27. Erna Susanti, Ciptati, Retty Ratnawati, Aulanni'am, Achmad Rudijanto,:Qualitative analysis of catechins from green tea GMB-4 clone using HPLC and LC-MS/MS. Asian Pacific Journal of Tropical Biomedicine. 2015; 5(12): pp. 1046-1050.

28. Hamed A.L., Al-Ayed A.S., Moldoch J., Piacente S., Oleszek W. and Stochmal A. : profiles analysis of proanthocyanidins in the argun nut (Medemia argun- an ancient Egyptian palm) by LC-ESI-MS/MS. J.Mass spectrum 2014: 1-10.

29. Demir E, Serdar G and Sokmen M: Comparison of some extraction methods for isolation of catechins and caffeine from Turkish green tea. International Journal of Secondary metabolites 2015;2(5):16-25.