LC/MS Characterization of Flavonoid Compounds From Fruits of *Vitex Agnus-Castus Cultivated in Baghdad-Iraq*

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ABSTRACT

Objective: the study was conducted to evaluate the qualitative and quantitative properties of flavonoid compounds from the fruits of Vitex Agnus cultivated in Baghdad-Iraq. Methods: plant fruits were collected from two famous spots at different sporadic regions in Baghdad where the plant famously grows. Powered plant fruit was extracted with 95% ethanol. The extract was filtered, and then evaporated to dryness using rotary evaporator. Results: The LC-chromatogram obtained from the fruit of Vitex Agnus represented the peaks which were labeled according to the order of their retention time. The highest peaks of the major compounds belong to flavonoids; vitexin, quercetin, luteolin, taxifolin, eupatilin, iso-orientin and baicalien. Conclusion: The percentage of flavonoid compounds of vitex agnus fruits grown in Baghdad soil was found to be satisfactorily abundant and only slightly lower than humid region when compared to semi-arid regions like Baghdad.

Keywords: LC/MS, Fruits, Vitex Agnus-Castus

Introduction:

The use of medicinal plants to treat various illnesses is due to the wide-diversity of phytochemical compounds of herbal drugs, which exhibit a variety of biological activity. In addition, traditional medicine is preferred over that of synthetic because reduction in the efficacy of chemical drugs beside the rise of adverse reactions makes the need for a new effective drug with minimum side effects. This led to increase the utility of the naturally occurring drugs [(1-2)]. Vitex agnus-castus, also known Chaste Tree, Chasteberry and Monk's Pepper is a small tree or shrub that belongs to family Lamiacae. It's a deciduous plant native to European, Mediterranean and Central Asian countries [(3)]. Vitex agnus fruits especially contain a lot of compounds of interest and are known to treat many diseases [(4)]. The widespread uses of *Vitex agnus castu's fruits* in traditional medicine have resulted in considerable chemical analysis of the plant and its active principles. From the phytochemical point of view, the fruits were reported to contain: flavonoids, phenolic acids, diterpenes and glycosides [(5)]. Many properties of plant products are associated with the presence of flavonoids which play an important role in their defense mechanisms. Since flavonoids are the primary and most important group of polypeholic compounds with a wide availably of over 8000 flavonoid compounds, most of which have exerted multiple biological-activities

such as anti-microbial, anti-inflammatory, antioxidants, anticancer and cardio-vascular protection[6].

Figure(1): Fruits of *Vitex agnus* [7].

Materials and methods:

Plant material:

The fruits of Vitex Agnus were collected from shrubs spotted sporadically on the side of the road in Baghdad. Authentication of the plant was carried out at the herbarium of College of Science, University of Baghdad. The plant material was collected in September and dried at room temperature in the shade then grinded as powder and weighed.

Extraction of the plant material:

100 gram of powdered plant fruits extracted with 1000 mL of ethanol 95% after hexane extraction using soxhlet apparatus. The extract was filtered and then evaporated to dryness using rotary evaporator.

TLC analysis of ethanolic extract:

Equal quantities of ethanolic extract and ethyl acetate were mixed in a separatory funnel, with wise addition of H2O to increase the polarity, ethyl acetate fraction floated on the surface to ensure a higher quality separation of flavonoids as opposed to other ethanolic extract constitutes. Small amount of the ethyl acetate fraction was applied on TLC plate coated with silica gel and developed to investigate the presence of quercetin, luteolin, kamferol and apigenin by using their respective standards in the following mobile phases:

Toluene: ethyl acetate: formic acid (36:12:6)

Toluene: ethl acetate: methanol: formic acid (4:3:2:1)

Butanol: acetic acid: water (40:10:50) [8].

After drying, the developed spots were investigated by comparing their R_f values with the R_f values of their respective standards then examined under ultra violate light at 254 and 366 nm.

Liquid Chromatography/ Mass Spectrometry (LC/MS):

Liquid mass detection was performed using Bruker Daltonik (Bremen, Germany) Impact II ESI-Q-TOF System equipped with Bruker Dalotonik Elute UPLC system (Bremen, Germany) for screening various compounds of interest at the College of Pharmacy/Zarqa University in Jordan.

The instrument was operated using Ion Source Apollo II ion Funnel electrospray source. The capillary voltage was 2500 V, the nebulizer gas was 2.0 bar, the dry gas (nitrogen) flow was 8 L/min and the dry temperature was 200 ºC. The mass accuracy was < 1 ppm; the mass resolution was 50000 FSR (Full Sensitivity Resolution) and the TOF repetition rate was up to 20 kHz.

Standards for identification of m/z with high resolution Bruker TOF MS and stock solutions were prepared by dissolving of the appropriate amount of substance in Dimethyl sulfoxide-DMSO (analytical grade), then diluted with Acetonitrile then used for identification of exact MS and retention time. All the other reagents, Acetonitrile, methanol, water, and formic acid used were LC/MS grade.

Results and discussion:

1- Preliminary Chemical test (Alkaline Reagent Test) for flavanoids.

Alcoholic KOH (2-3mL) was added to 1 mL methanolic extract of the plant. A yellow color is detected if flavonoid compounds are present. The preliminary phytochemical screening of the plant extract indicated the presence of flavonoids in the ethanol extract. Accordingly, LC-MS was performed to confirm that and to determine which flavonoids are the major constituents of Iraqi *Vitex agnus.*

2- Characterization of flavonoids by TLC analysis:

Analytical TLC of ethyl acetate phase confirmed the presence of quercetin, luteolin and kamferol flavonoids by comparing their R_f values with the R_f values of their respective standards then examined under ultra violate light at 254 and 366 nm.

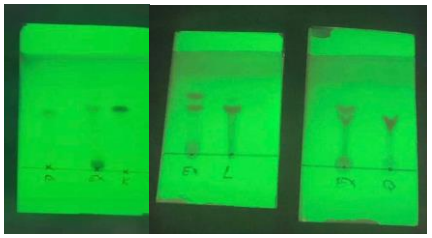


Fig. 2– TLC profiling of quercetin, luteolin, kampfeorl flavonoids Of Vitex agnus fruit

LC/MS characterization of active compounds:

Ethyl acetate extract was achieved by fractioning ethanolic extract with ethyl acetate solvent and water to increase polarity [9]. Liquid mass detection was performed using Bruker Daltonik (Bremen, Germany) Impact II ESI-Q-TOF System equipped with Bruker Dalotonik Elute UPLC system (Bremen, Germany) for screening various compounds of interest. Standards for identification of m/z with high resolution Bruker TOF MS and stock solutions were prepared by dissolving of the appropriate amount of substance in Dimethyl sulfoxide-DMSO (analytical grade), then diluted with Acetonitrile then used for identification of exact MS and retention time.

The LC-chromatogram obtained from the fruit of Vitex Agnus shown in Figure 3, peaks were labeled according to the order of their retention time. Structural characterization was performed using the retention time of standards, [M − H]− data, MS/MS fragmentations peaks and published data.

The retention time, m/z measures, M measures, [M − H]− peaks, molecular formula for each compound(1-15) are shown in table 1. Also, fragmentation pattern of some of the most significant compounds is shown in figure 5.

Judging from the highest peaks the major compounds belong to flavonoids(vitexin, quercetin, luteolin, taxifolin, eupatilin, iso-orientin and baicalien which is in sync with the research carried out on the plant that is cultivated in other countries [10]. Also, other phenolic compounds like caffieic acid and p-coumarin are present in considerable percentages while flavonoid glycosides example; Kaempferol-3-O-glucoside, Luteolin 7-O-glucoside, Apigenin-7-O-glucoside are present to a lesser extent [11]. The above results explain the main traditional and commercial uses of the plant as an antimicrobial and anti-inflammatory agent since polhyphenols and phenolic compounds have attracted the interest of researchers for their abundant existence, low toxicity and high potency against drug-resistant bacteria as exampled by Methicillin-Resistant-Staphylococcus Aureus (MRSA) [12].

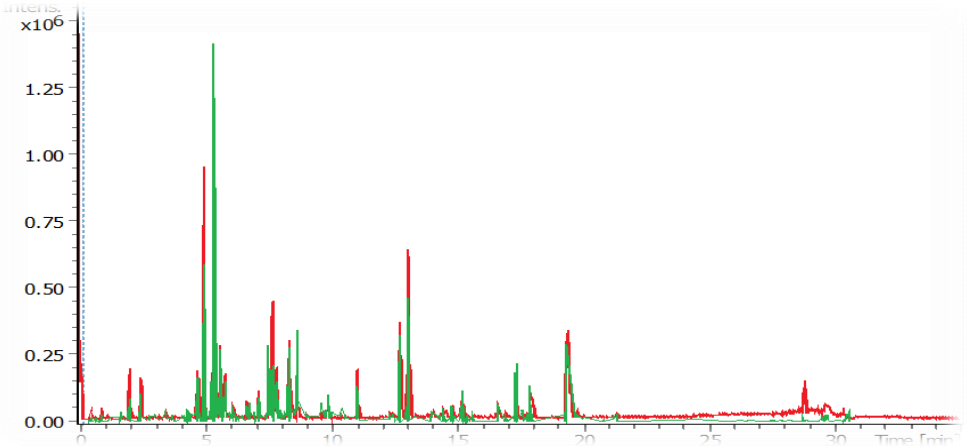


Figure 3-The LC-chromatogram obtained for the fruit of Vitex Agnus

Peak No	compound	RT(min)	m/z meas.	M meas.	Ions	Molecular formula
1	Caffiec acid	3.25	179.04	180.04	M-H	C9H8O4
2	P-cumaric acid	4.4	163.04	164.05	M-H	C9H8O3
3	ISO-Orientin	4.87	447.9	448.1	M-H	C21H20O11
4	Taxifolin	5.33	303.05	304.06	M-H, M-H-H2O	C15H12O7
5	Vitex	5.65	431.1	432.1	M-H	C21H20o10
6	Luteolin 7-O-glucoside	5.88	447.09	448.1	M-H,M+Cl	C21H20O11
7	Kaempferol-3-O-glucoside	6.54	447.09	448.1	M-H	C21H20O11
8	Apigenin-7-O-glucoside (Apigetrin)	6.76	431.1	432.11	M-H	C21H20O11
19	Eriodictyol	8.03	287.06	288.6	M-H	C15H12O6
10	Quercitin	8.49	301.04	302.04	M-H	C15H10O7
11	Leutolin	8.54	285.04	286.05	M-H	C15H10O6
12	3-O-Methyl Quercetin (Isorhamnetin)	9.13	315.05	316.06	M-H	C16H12O7
13	Baicalein	9.83	269.05	270.05	M-H	C15H105
14	Eupatilin	12.82	343.08	344.09	M-H	C18H1607

Table (1): the retention time, m/z measures, M measures, [M − H]− peaks, molecular formula for each compound(1-15)

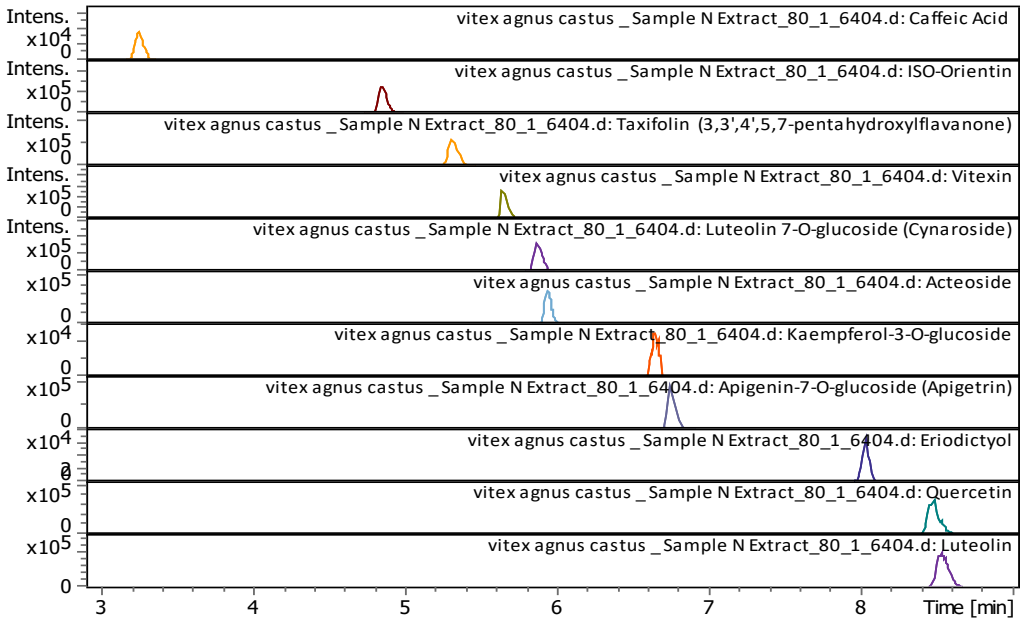
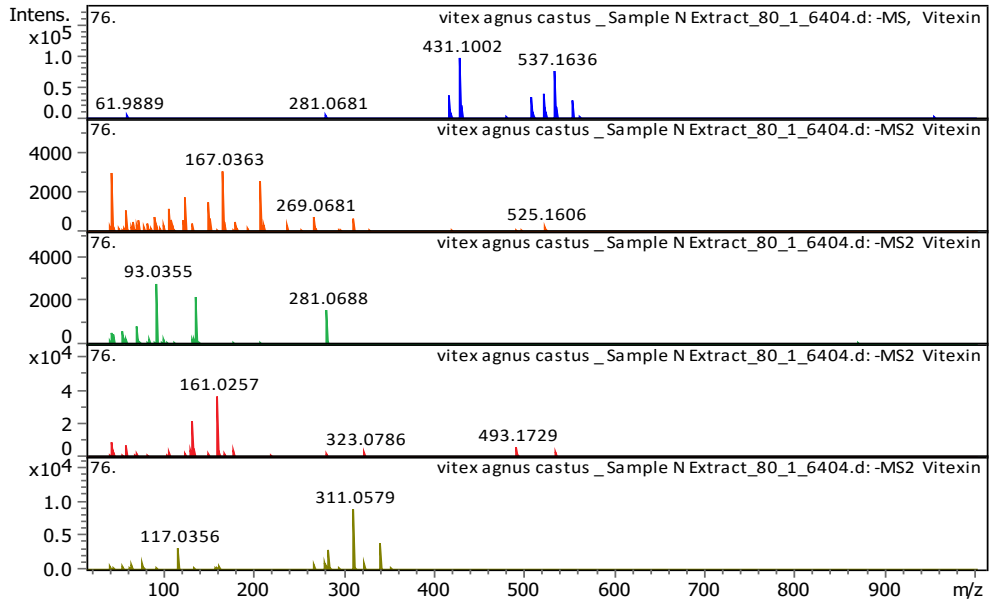
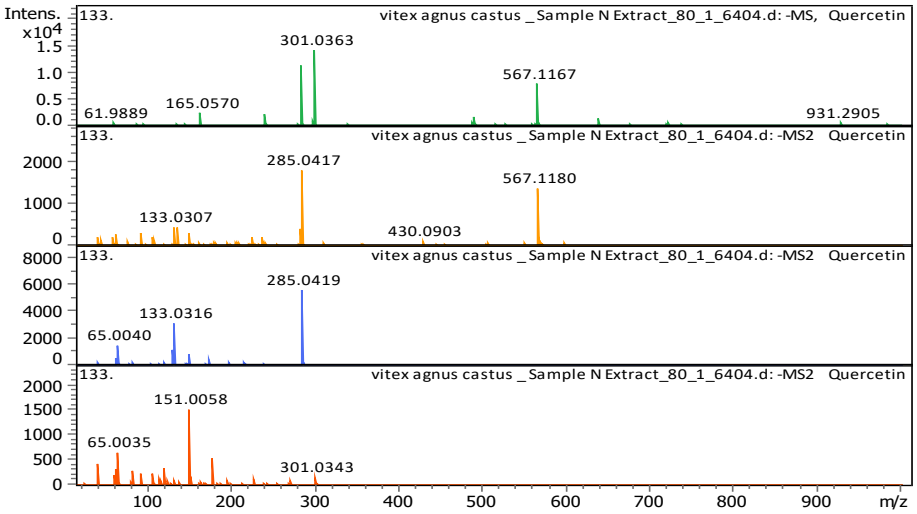


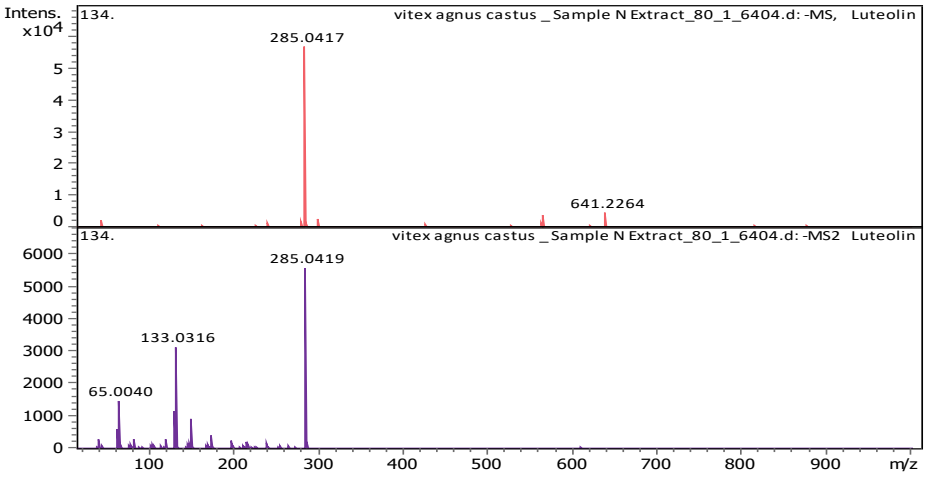
Figure 4-The LC-chromatogram of each individual peak of the main compounds obtained for the fruit of Vitex Agnus



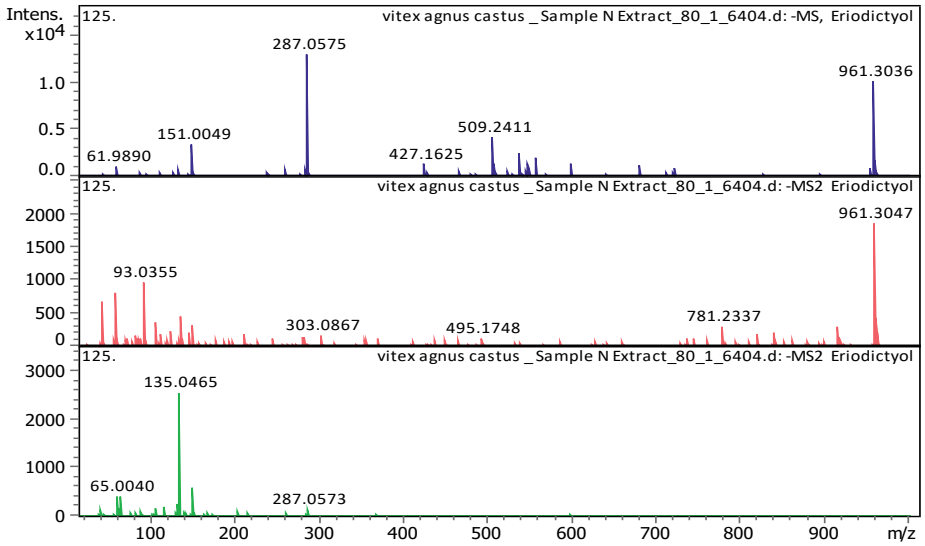
(A)


Intens.
x10^4
133. vitex agnus castus _Sample N Extract_80_1_6404.d: -MS, Quercetin
301.0363
567.1167
61.9889
165.0570
931.2905
133. vitex agnus castus _Sample N Extract_80_1_6404.d: -MS2 Quercetin
285.0417
567.1180
133.0307
430.0903
133. vitex agnus castus _Sample N Extract_80_1_6404.d: -MS2 Quercetin
285.0419
133.0316
65.0040
133. vitex agnus castus _Sample N Extract_80_1_6404.d: -MS2 Quercetin
151.0058
65.0035
301.0343
m/z

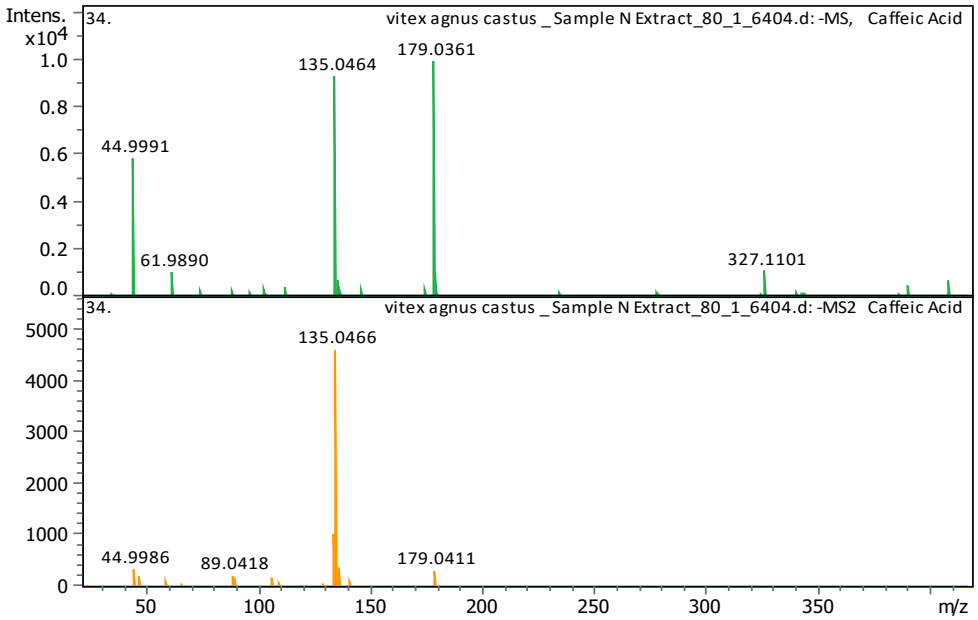
(B)


Intens.
x10^4
134. vitex agnus castus _Sample N Extract_80_1_6404.d: -MS, Luteolin
285.0417
641.2264
134. vitex agnus castus _Sample N Extract_80_1_6404.d: -MS2 Luteolin
285.0419
133.0316
65.0040
m/z

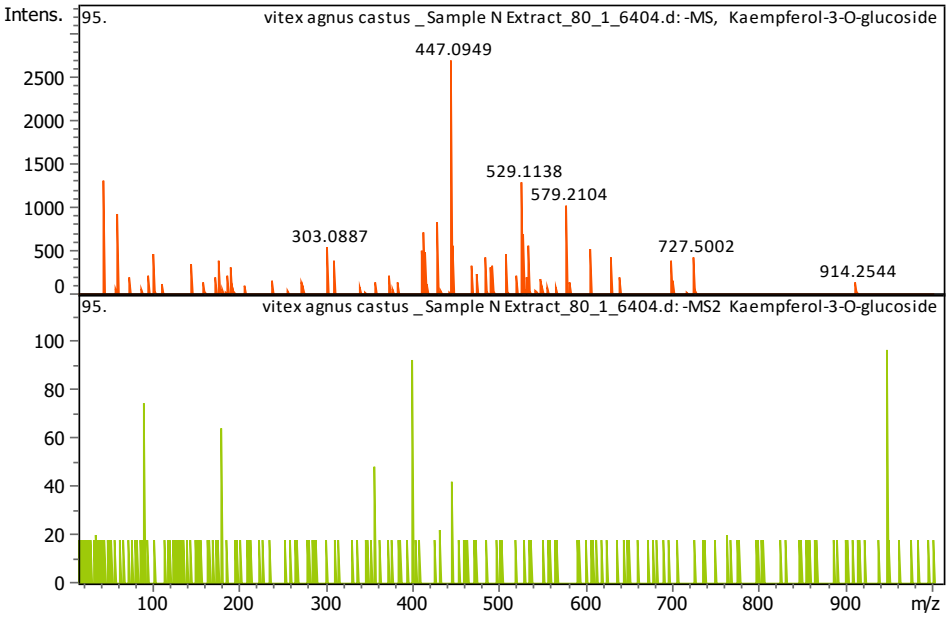
(C)


Intens.
x10^4
125. vitex agnus castus _Sample N Extract_80_1_6404.d: -MS, Eriodictyol
287.0575
961.3036
151.0049
61.9890
509.2411
427.1625
125. vitex agnus castus _Sample N Extract_80_1_6404.d: -MS2 Eriodictyol
961.3047
93.0355
303.0867
495.1748
781.2337
125. vitex agnus castus _Sample N Extract_80_1_6404.d: -MS2 Eriodictyol
135.0465
65.0040
287.0573
m/z

(D)



(E)



(F)

Figure5- fragmentation pattern of some of the most significant compounds: (A) Vitexin, (B) Quercetin, (C) Luteolin, (D) Eriodictyol, (E) Caffiec acid, (F) kaempferol-3-o-glucoside obtained for the fruit of Vitex Agnus.

References:

1. Tawfeeq, T. Jasim, G and Nasser, A. Isolation of umbelliferone from leaves of *Conocarpus erectus* L. cultivated in Iraq. Al Mustansiriyah Journal of Pharmaceutical Sciences, 2020, Vol. 20, No.4.
2. Eldalawy, R. Abdul kareem and W. Al-Ani, W. GC-MS analysis of Iraqi *Silybum marianum* Flowers, Leaves and Seeds Extracts. Al Mustansiriyah Journal of Pharmaceutical Sciences, 2020, Vol. 20, No.4.
3. Duke. J. A. 1985. Culinary Herbs. Trado –medic Books. New York, USA.
4. Stevens, F. Lamiales (Lamiaceae Family). Angiosperm Phylogeny. The Botanical Journal of the Linnean Society. Vol. 161:105–12.
5. Kaur S, Shri R. *Vitex agnus castus*. Ethnobotany, phytochemistry and pharmacology. Journal of pharmaceutical biology. 2015; 5(4): 267-277.
6. Xiao, J.; Kai, G. A review of dietary polyphenol-plasma protein interactions: characterization, influence on the bioactivity, and structure-affinity relationship. Critical reviews in food science and nutrition, 2012, 52(1), 85-101.
7. Zahid, H. Ghazala H and Ishaqe, H. Phytopharmacological Review on Vitex agnus-castus. A potential medicinal plant. *Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi.75270, Pakistan*
8. Havsteen, B.H., The biochemistry and medical significance of flavonoids, Pharmacol. Therapeut., 96, 67, 2002.
9. Sherma, J., Thin-layer chromatography in food and agricultural analysis, J. Chromatogr. A, 880, 129, 2000.
10. Middleton E J. Effect of plant flavonoids on immune and inflammatory cell function. Adv Exp Med Biol.1998; 439: 175–182.
11. Arceusz A, Wesolowski M, Konieczynski P. Methods for extraction and determination of phenolic acids in medicinal plants: a review. Nat Prod Commun .2013;8(12):1821–9.
12. Hatano, T.; Kusuda, M.; Inada, K.; Ogawa, T. O.; Shiota, S.; Tsuchiya, T.; Yoshida, T. Effects of tannins and related polyphenols on methicillin-resistant Staphylococcus aureus. Phytochemistry, 2005, 66(17), 2047-2055.
13. Koes R, Verweij W, Quattrocchio F. Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. Trends Plant Sci. 2005; 10(5): 236–242.