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DETERMINATION OF PHYTOCHEMICAL COMPOSITION AND TEN ELEMENTS CONTENT (CD, CA, CR, CO, FE, PB, MG, MN, NI AND ZN) OF*CARDARIA DRABA* BY GC-MS, FT-IR AND AAS TECHNIQUES

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ABSTRACT

The research objectives were to investigate the phytochemical composition of the methanolic leaves extract of *Cardaria draba* and its elements content. GC-MS analysis revealed the existence of the8-Hydroxy-2-octanone, Phosphorothioic acid , S-ester with trimethylenediiminodipropa, Pyrrolidine , 1,1'-methylenebis,N[4Aminobutyl]aziridine,Cycloheptane,1,2dimethoxy,trans,N[3[NAziridyl]propylidene]tetrahydrofurfuralamine, o-Cymene, 1,13-tetradecadien-3-one, 5-Octadecenal, 2,6-Nonadienal , (E,Z), Piperidine , 1-(1-propenyl), Imidazole , 2-amino-5-[(2-carboxy)vinyl], S-Benzyl-l-cysteinyl-S-tetrahydropyranyl-l-cysteinylhydrazide,β-D-Glucopyranose,1-thio-,1-[Nhydroxy5(methylthio)pentar,9Oxabicyclo[3.3.1]nonane-2,6-diol, Formamide ,N-methyl-N-4-[1-(pyrrolidinyl)-2-butynyl], Naphthalene,1,2-dihydro-2,5,8-trimethyl, Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester),Cis,-5,8,11,14,17-Eicosapentaenoic acid, Durohydroquinone, N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid], Phenol ,2,4-dichloro-6-[(1H-indazol-7-ylamino)methyl],(3,3-Dimethyl-5methylthio3,4(2H)dihydropyrrol2ylidene)ace,Corymbolone, Folic acid, Oxiraneundecanoic acid , 3-pentyl-, methyl ester , cis, Benzeneethanamine ,2-isothiocyanato-N-methyl-N-[2.trans, Paromomycin, Cyclodeca[b]furan-2(3H)-one , 9-(acetyloxy)-3a,4,5,8,9,11a-h, 13-Heptadecyn-1-ol, Hexadecanoic acid , 1-(hydroxymethyl)-1,2-ethanediyl ester, l-(+)-Ascorbic acid 2,6-dihexadecanoate, 2,6-Bis[2-[2-S-thiosulfuroethylamino]ethoxy]pyrazine, Dasycarpidan-1-methanol , acetate (ester), 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyrid, 9,12,15-Octadecatrienoic acid , (Z,Z,Z), and Ethyl iso –allocholate. The FTIR analysis of *Cardaria draba* leaves proved the presence of Alkenes, Alcohols, Ethers, Carboxlic acids, Esters, Alkanes, Hydrogen bonded Alcohols and Phenols. Several metals were determined in *Cardaria draba*medicinal plant by applying Atomic Absorption Spectrophotometer. The obtained results showed that (Cadmium, Calcium, Chromium, Cobalt, Iron, Lead, Magnesium, Manganese, Nickel and Zinc) content in tested plant were (0.998±0.07, 4.020±0.58, 7.350±2.09, 0.660±0.09, 40.000±1.20, 3.000±0.26, 27.300±0.98, 15.022±0.61, 5.990±0.27 and 7.190±0.30) respectively.

KEYWORDS: ***Cardaria draba*, Bioactive compounds, FT-IR, GC/MS**.

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INTRODUCTION

Heavy metal availability can also be directly affected by plant itself [1] and must be controlled[2].Heavy metals (cadmium, lead, nickel,..) are considered as the most hazardous environmental contaminants[3,4].Some of medicinal plants are also used for prophylactic purposes[5-10]. Diseases that have been managed traditionally using medicinal plant include malaria, epilepsy, infantile convulsion, diarrhea, and dysentery, fungal and bacterial infections[11-14]. Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compound as antimicrobial agent.*Cardaria draba* (Brassicaceae; syn. *Lepidium draba* (L)is a perennial herb that multiply by seed and by horizontal creeping roots[15,16]. *C. draba* is native to Eastern Europe and western Asia, including Iran, and is an invasive species in North America. *Cardaria draba* commonly known as hoary cress, is a perennial herb that multiply by seed and by horizontal creeping roots[17,18]. *Cardaria draba* (Brassicaceae; syn. *Lepidium draba* (L) Link), commonly known as whitetop or hoary cress, is a perennial herb that reproduces by seed and by horizontal creeping roots.*Lepidium draba* L.has been shown that this plant like the other Brassicaceae genus contains glucosinolates, a unique group of secondary metabolites containing sulfur and nitrogen. So far, more than 100 kinds of these metabolites have been identified[19,24]. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients valuable in the management of many intractable diseases[25,26]. Numerous studies have identified compounds within herbal plants, which are effective antibiotics[26-30]. Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics[31,32]

MATERIALS AND METHODS

Plant material and extraction

The leaves wastes were collected from the local market then washed it with distilled water and cut it into small pieces.The leaves of *C. draba* were dried at room temperature, and the powdered plant was size reduced with a sieve. About twenty grams of the plant sample powdered were soaked in 200 mL methanol. The filtrates (crude extract of *C. draba*) obtained were concentrated in rotary evaporator keeping the water bath at 50 Cº. Then all the extracts were preserved in separate containers at 5 Cº for further experimentations.

Calibration curves and elementary analysis (heavy metals) of C. draba samples

Calibration curves were prepared using five concentrations, and the linear correlation coefficients had been determined. Know weight of the *C. draba* leaves was mixed with few drops of Conc. trioxonitriate acid until a slurry is formed. Concentration of elements present were determined using atomic absorption spectrophotometer.

Gas chromatography – Mass Spectrum analysis

Identification of compounds interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NIST, USA). The database consists of more than 62,000 patterns of known compounds. The spectrum of the extract was matched with the spectrum of the known components stored in the NIST library.*C. draba* GC–MS analysis were carried out in a GC system (Agilent 7890Aseries, USA). The flow rate of the carrier gas, helium (He) was set to beat 1 mL min−1, split ratio was 1:50. The injector temperature was adjusted at 250◦C, while the detector temperature was fixed to280◦C. The column temperature was kept at 40◦C for 1 min fol-lowed by linear programming to raise the temperature from 40◦to 120◦C (at 4 Cº min−1with 2 min hold time), 120 Cº to 170 Cº (at 6 Cº min−1with 1 min hold time) and 170 Cº to 200 Cº (at10◦C min−1with 1 min hold time). The transfer line was heated at 280 Cº. Two microliter of FAME sample was injected for analysis. Mass spectra were acquired in scan mode (70 eV); in the range of 50–550 m/z.

Statistical analysis

Results of the study were based on SPSS version 16.0 and differences among the means were determined for significance at $P < 0.05$.

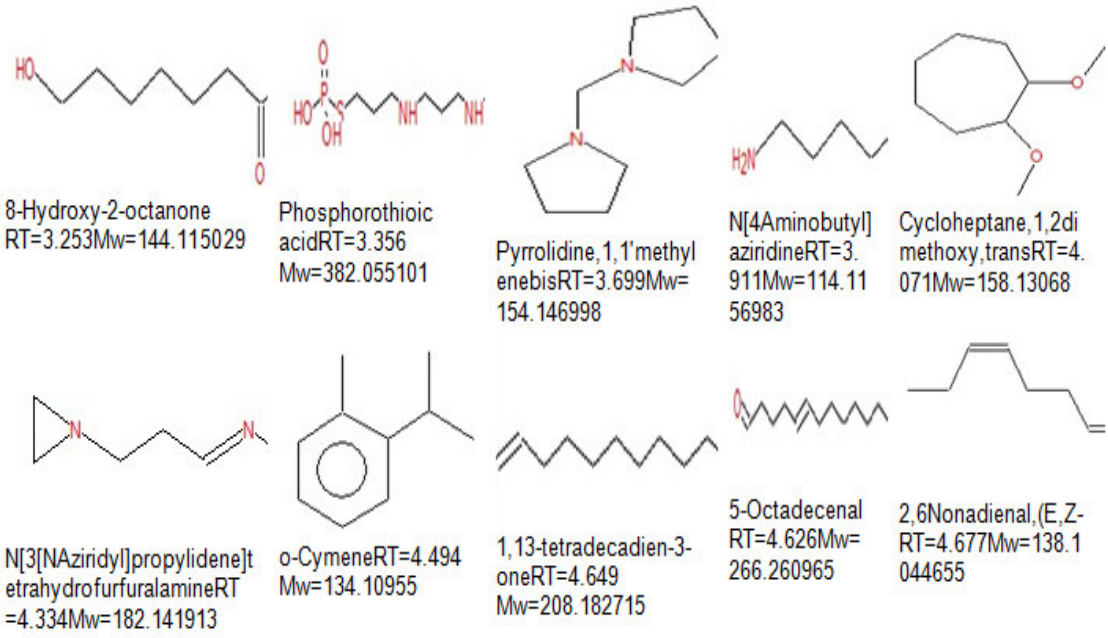
RESULTS AND DISCUSSION

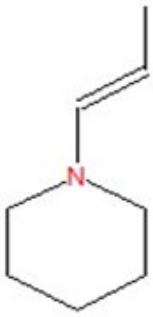
Heavy metals concentrations in herbs must be controlled[33]. Heavy metal toxicity (cadmium, lead, nickel, ..) are considered as the most hazardous environmental contaminants[34]. Heavy metal availability can also be directly affected by plant itself [35]. Most of these metal ions (Cd, Cu, Zn, Hg, As, Ag, Cr, and Fe) can be released from the industries in simple cationic forms[36]. Metallic levels in the analyzed samples are shown in Table3. In this study Cadmium content found (0.998±0.07 ppm) which is less than WHO and Germany limitations[37-39]. Calcium is essential material of structural parts of human and animals such as bones, teeth, egg shell, and necessary element in cellular processes with daily intake (350-1100) mg/day. It is also used in several chemical applications (reducing, deoxidizer) [40]. Its content in tested herb was (4.020±0.58) ppm. Chromium is required for human approximately (0.03 ppm) where its accumulation causes reducing glucose level in blood, gastrointestinal disorder, cardiovascular shock, etc…[41-43]. Results of this study showed that chromium recorded (7.350±2.09) ppm that may be a result of its low solubility water and plant uptake. Fe content was (40.000±1.20 ppm) and higher content compare with (WHO, 2007). Iron is an essential element for plant growth and human life. Lead Pb content (3.000±0.26ppm) was less than the WHO. In our study, (Cobalt) required for several biological actions in human body. Magnesium content was (27.300±0.98 ppm). Mg is essential metal and major biological compound in DNA and ATP[44]. Manganese content was (15.022±0.61 ppm). Mn is a trace element necessary for plant, animal, and human as enzyme cofactor[45]. Nickel is an essential element for animal nutrition[46]. In this study Nickel recorded 5.990±0.27. In *C. draba* leaves zinc metal reach 7.190±0.30 ppm.Medicinal plants are the richest bio-resources of drugs of traditional medicinal systems, modern medi-cines, nutraceuticals, food supplements, folk medicines, pharmaceuticals,

intermediate and chemical entitled for synthetic drugs[47-49].Recently the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants[50-52].Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic leaves extract of *Cardaria draba,* shown in Table 1. The GC-MS chromatogram of the 46 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Cardaria draba* showed the presence of forty six major peaks and the components corresponding to the peaks were determined as follows. The next peaks considered to be, 8-Hydroxy-2-octanone, Phosphorothioic acid , S-ester, Pyrrolidine , 1,1'-methylenebis, N-[4-Aminobutyl]aziridine, Cycloheptane , 1,2-dimethoxy-,trans, N-[3-[N-Aziridyl]propylidene] tetrahydrofurfuralamine, o-Cymene, 1,13-tetradecadien-3-one, 5-Octadecenal, 2,6-Nonadienal , (E,Z), Piperidine , 1-(1-propenyl), Imidazole , 2-amino-5-[(2-carboxy)vinyl], S-Benzyl-l-cysteinyl-S-tetrahydropyranyl-l-cysteinyl hydrazide, β-D-Glucopyranose , 1-thio-,1-[N-hydroxy-5-(methylthio)pentar,9Oxabicyclo[3.3.1]nonane-2,6-diol, Formamide ,N-methyl-N-4-[1-(pyrrolidinyl)-2-butynyl], Naphthalene ,1,2-dihydro-2,5,8-trimethyl, Pyrrolizin-1,7-dione-6-carboxylic acid , methyl (ester), Cis ,-5,8,11,14,17-Eicosapentaenoic acid, Durohydroquinone, N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid], Phenol ,2,4-dichloro-6-[(1H-indazol-7-ylamino)methyl], (3,3-Dimethyl-5-methylthio3,4(2H)dihydropyrrol2ylidene)ace,Corymbolone, Folic acid, Oxiraneundecanoic acid , 3-pentyl-, methyl ester , cis, Benzeneethanamine ,2-isothiocyanato-N-methyl-N-[2.trans, Paromomycin, Cyclodeca[b]furan-2(3H)one,9(acetyloxy)3a,4,5,8,9,11a-h, 13-Heptadecyn-1-ol, Hexadecanoic acid , 1-(hydroxymethyl)-1,2-ethanediyl ester, l-(+)-Ascorbic acid 2,6dihexadecanoate,2,6Bis[2[2Sthiosulfuroethylamino]ethoxy]pyrazine, Dasycarpidan-1-methanol , acetate (ester),2,7Diphenyl1,6dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyrid, 9,12,15-Octadecatrienoic acid , (Z,Z,Z), and Ethyl iso –allocholate (Table1).The FTIR analysis of *C. draba* proved the presence of Alkenes, Alcohols, Ethers, Carboxlic acids, Esters, Alkanes, Hydrogen bonded Alcohols and Phenols which shows major peaks at 667.37, 898.83, 920.05, 1031.92, 1068.56, 1145.72, 1151.50, 1234.44, 1261.45, 1313.52, 1392.61, 1454.33, 1519.91, 1531.78, 1631.78, 1743.65, 2854.65, 2926.01, 2956.87, 3080.32 and 3271.27.(Table 2; Figure 2). The search of biologically active component of plants has always been great interest to scientists looking for new sources of practical for herb-based medicines, food supplements, pharmaceuticals and health products[53-55]. The most therapeutic effect of this isothiocyanate is antioxidant properties[56], anti-bacterial effects on *Helicobacter pylori*[57,58], apoptosis induction in cancer cells[59-70], anti-metastatic[71] and anti-angiogenesis properties[72] .

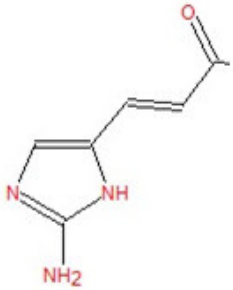
Table 1

Bioactive chemical compounds identified in methanolic extract of Cardaria draba.

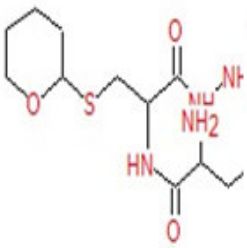




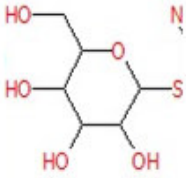
Piperidine,1-(1-propenyl)-RT=4.780Mw=125.1204495 Mw=



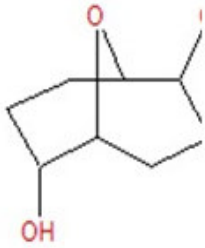
Imidazole,2-amino-5-[(2-carboxy)vinyl]-RT=4.941Mw=153.053826



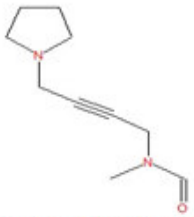
S-Benzyl-l-cysteinylS-tetrahydropyranyl-lcysteinyl hydrazide RT=5.313Mw=412.160282



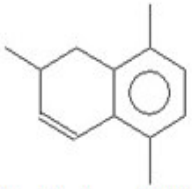
βDGlucopyranose,1thio,1[Nhydroxy5(methylthio)pentarRT=5.971Mw=341.09668



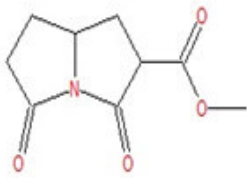
9Oxabicyclo[3.3.1]nonane2,6diolRT=6.154Mw=158.094295



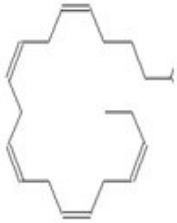
Formamide,N-methyl-N-4-[1-(pyrrolidinyl)-2-butynyl]-RT=6.417Mw=180.126264



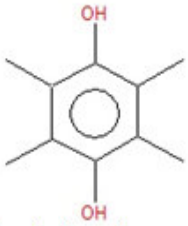
Naphthalene,1,2dihydro-2,5,8-trimethyl-RT=7.447Mw=172.1252



Pyrrolizin-1,7-dione-6-carboxylicacid ,methyl (ester)RT=8.191 Mw=197.068808

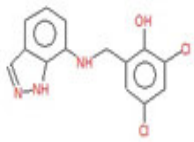


Cis,5,8,11,14,17Eicosapentaoic acidRT=9.455 Mw=302.22458

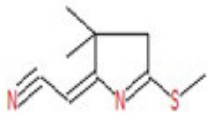


Durohydroquinone RT=9.215Mw=166.09938

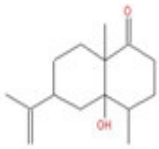
N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid]RT=9.427Mw=410.067371



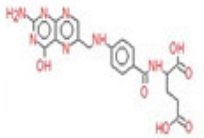
Phenol,2,4-dichloro-6[(1Hindazol7ylamino)methyl]-RT=9.558 Mw=307.027918



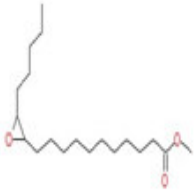
(3,3Dimethyl5methylthio3,4(2H)dihydropyrrol 2ylidene)aceRT=9.747Mw=180.07212



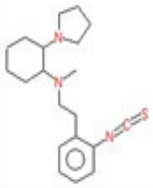
CorymboloneRT=10.829Mw=236.17763



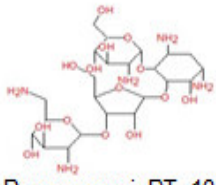
Folic acidRT=11.120 Mw=441.13968



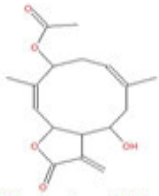
Oxiraneundecanoic acid , 3-pentyl- methyl ester, cis-RT=11.223Mw=312.266445



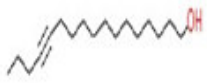
Benzeneethanamine ,2-isothiocyanato-N-methyl-N-[2.trans-[RT=11.567Mw=343.20822



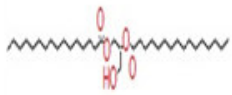
ParomomycinRT=12.648Mw=615.296303



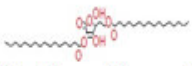
Cyclodeca[b]furan-2(3H)-one , 9-(acetyloxy)-3a,4,5,8,9,11a-hRT=12.517 Mw=306.146725



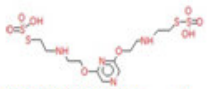
13-Heptadecyn-1-ol RT=12.837 Mw=252.245316



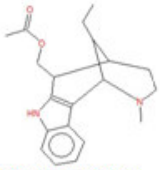
Hexadecanoicacid,1(hydroxymethyl)-1,2-ethanediyl esterRT=13.072Mw=568.506676



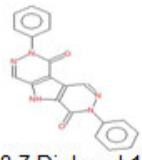
l-(+)-Ascorbic acid 2,6dihexadecanoate RT=13.838Mw=652.49142



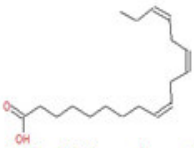
2,6Bis[2[2Sthiosulfuroethylamino]ethoxy]pyrazineRT=14.416Mw=478..032047



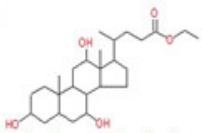
Dasycarpidan-1methano,acetate(ester)RT=14.445Mw=326.199429



2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'd]pyridRT=15.212 Mw=355.106924



9,12,15-Octadecatrienoic acid , (Z,Z,Z)-RT=15.658 Mw=278.22458



Ethyliso –allocholate RT=22.416Mw=436.318874

Table 2
FT-IR peak values of Cardaria draba.

No.	Peak (Wave number cm-1)	Intensity	Bond	Functional group assignment	Group frequency
1.	667.37	57.466	C-H	Alkenes	675-995
2.	898.83	76.787	C-H	Alkenes	675-995
3.	920.05	76.478	C-F stretch	Alkenes	675-995
4.	1031.92	63.118	NO2	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
5.	1068.56	61.559	NO2	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
6.	1145.72	67.288	NO2	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
7.	1151.50	67.207	NO2	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
8.	1234.44	69.641	NO2	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
9.	1261.45	72.985	NO2	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
10.	1313.52	74.042	-	Unknown	-
11.	1392.61	70.978	-	Unknown	-
12.	1454.33	72.287	-	Unknown	-
13.	1519.91	61.746	-	Unknown	-
14.	1531.78	61.557	-	Unknown	-
15.	1631.78	56.566	-	Unknown	-
16.	1743.65	80.486	-	Unknown	-
17.	2854.65	83.221	C-H	Alkanes	2850-2970
18.	2926.01	76.517	C-H	Alkanes	2850-2970
19.	2956.87	82.767	C-H	Alkanes	2850-2970
20.	3080.32	86.576	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600
21.	3271.27	78.018	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600

Table 3
Total Cardaria draba elementary content.

No.	Elements	Concentration range (ppm)
1.	**Cadmium (Cd)**	0.998±0.07
2.	**Calcium (Ca)**	4.020±0.58
3.	**Chromium (Cr)**	7.350±2.09
4.	**Cobalt (Co)**	0.660±0.09
5.	**Iron (Fe)**	40.000±1.20
6.	**Lead (Pb)**	3.000±0.26
7.	**Magnesium (Mg)**	27.300±0.98
8.	**Manganese (Mn)**	15.022±0.61
9.	**Nickel (Ni)**	5.990±0.27
10.	**Zinc (Zn)**	7.190±0.30

Figure 1
GC-MS chromatogram of methanolic leaves extract of Cardaria draba.

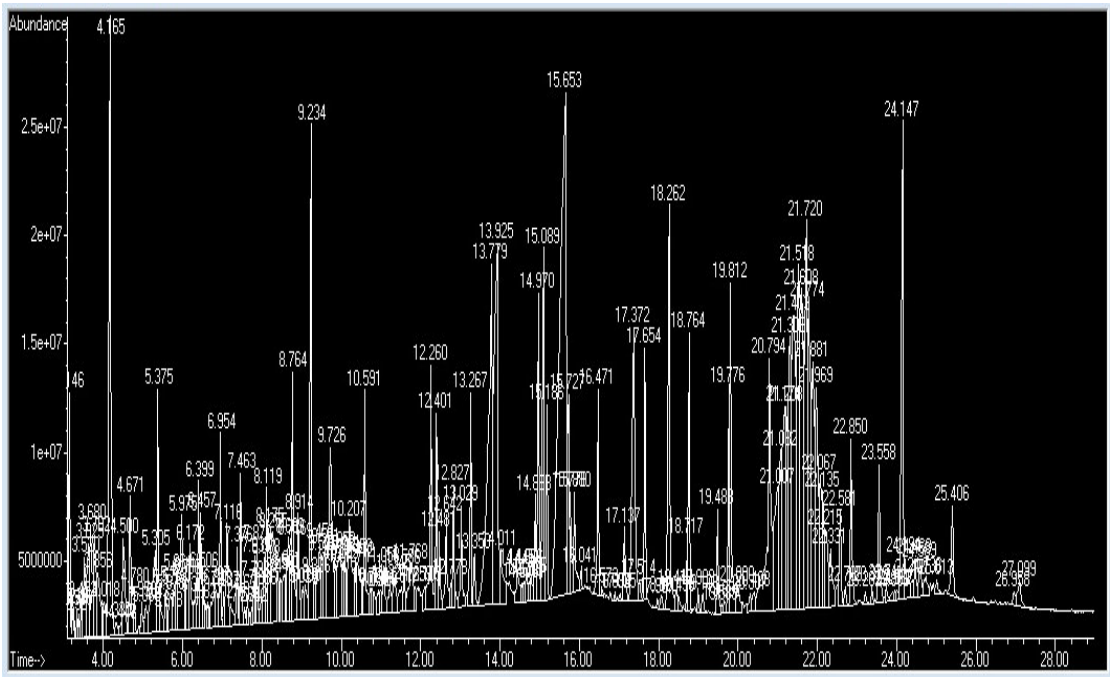
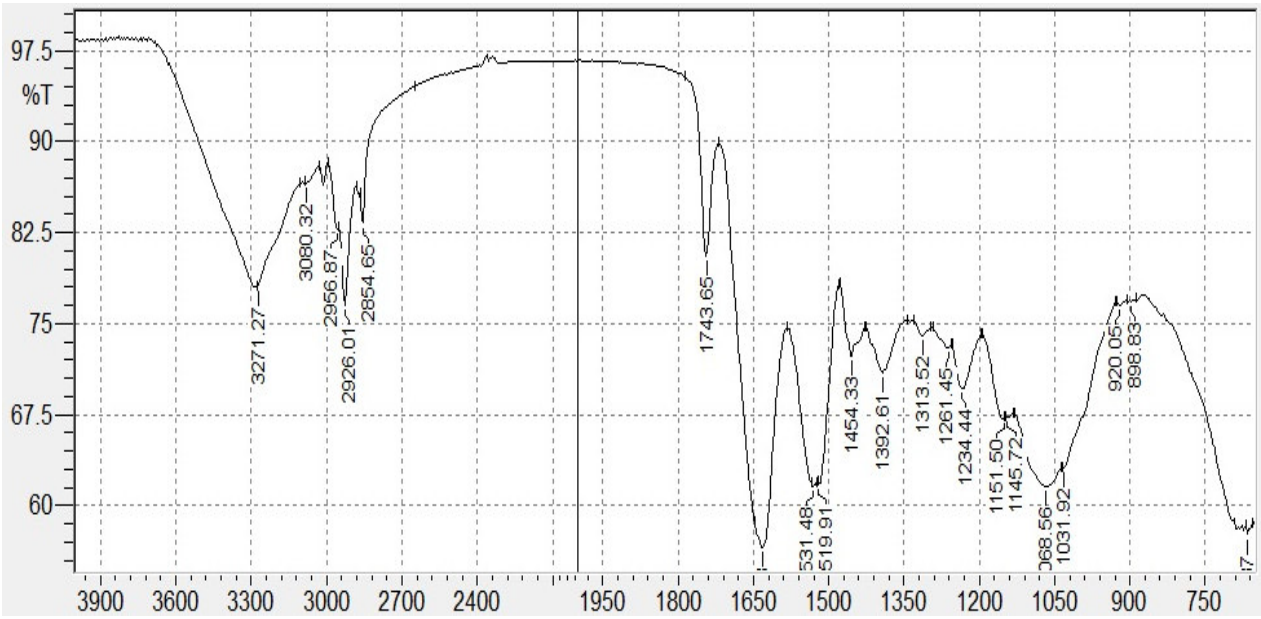


Figure 2
FT-IR profile of Cardaria draba



CONCLUSION

Cardaria draba leaves are rich source of essential bioactive chemical compounds, fibers and minerals. *C. draba* is native plant of Iraq. In this study that forty six phyto-constituents were identified from methanolic leaves extract of *C. draba* by gas chromatogram and mass spectrometry (GC-MS) analysis. *C. draba* leaves can be used as a promising multipurpose medicinal source whereas further clinical trial is required to prove its efficacy.

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