

ARUM CYRENIACUM: INVESTIGATION OF VOLATILES AND HEAVY METALS REMOVING CAPACITY

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ABSTRACT

Arum cyreniacum is an endemic Libyan plant growing in Aljabal Al-Akhder region. Though *Arum* species have been used since antiquity in herbal medicine for a wide array of human disorders, information on the medicinally important *Arum cyreniacum* is very scarce. Hence this study was carried out to investigate the volatiles of this herb and to assess the whole plant as a heavy metal remover in artificial water. The unsaponifiable fraction and the fatty acid methyl esters were analyzed by GLC which revealed the presence of palmitic and linolenic acids as major components. The study of herb powder as an adsorbent for removal of chromium (II) and cadmium (II) from water samples proved that, the optimal conditions are : pH=4-6; shaking time 10 min. The limit of detection (3σ) was found to be 0.10, 0.12 and 0.19 $\mu\text{g/L}$ and preconcentration factor of 100 has been achieved for such elements.

KEYWORDS: *Arum cyreniacum*, *Araceae*, volatiles, heavy metals removal.

INTRODUCTION

The family *Araceae*, commonly known as aroids consists of 105 genera and more than 3300 species and is distributed worldwide. The family contains several well-known cultivated foliage and flowering plants like: *Philodendron*, *monster*, *Anthurium*, *Arum*,... etc. one of the important character of the family is the inflorescence structure, small flowers born on fleshy axis (spadix) subtended by a modified leaf (spathe).^[1]

The leaves and tubers of many species in this family are extensively used in folk medicine for the treatment of inflammation, arthritis, genitourinary tract disorders, wounds, cough, hemorrhoids, worms, CNS disorders and cancer. The tubers are potentially poisonous but used externally to treat breast mastitis and abscesses. Many plants in the family *Araceae* possess antifungal and antibacterial activity.^[2]

Studies of secondary metabolites of the *Araceae* family showed that, it has a simple profile of polyphenols and alkaloids, with flavone C-glycosides, flavanols, flavones, proanthocyanidins and polyhydroxy alkaloids as main classes.^[3]

The *Arum* genus was found to contain many classes of phytochemicals like volatile oils, terpenes, flavonoids

and alkaloids. The odours of many *Arum* species have been studied by Kite *et al.*^[4,5] where about 36 compounds have been identified. The volatile compounds emitted by the appendix are variable among the species, but in general their foul odour smells like decaying matter. Common compounds are butanoic acid esters, 1-decene, terpenes (citronellene and its derivatives), *p*-cresol, methyl salicylate, indole, 2-heptanone. Some species appear clearly different like *A. creticum* or *A. palaestinum*, whose rotten fruit odour is due to benzyl alcohol and ethyl acetate. *Arum rupicola* var. *rupicola* is also different because of a mixture of various sesquiterpenes even if *p*-cresol is abundant.^[6]

The ever increasing usage of heavy metals in industrial activities is reflected by a serious accumulation of these toxic metals in waste water. For example lead and cadmium accumulate in wastewater of industries such as electroplating, plastics and paints industries, mining, metallurgical and petrochemical processes.^[7,8]

In an attempt to remove such toxic, carcinogenic pollutants from waste water, the bio-adsorption process has been an area of extensive research. The most common and harmful heavy metals in waste water are lead, copper, nickel, chromium and zinc. They are stable elements that cannot be metabolized by the body and get

passed up in the food chain to human beings. When waste water is disposed into the environment, a further long-term hazard is encountered. There are possibly more problems from these metals, which interfere with normal bodily function, than have been considered in most medical circles.

A conventional method for removing metals from industrial effluents includes chemical precipitation, coagulation, solvent extraction, electrolysis, membrane separation, ion-exchange and adsorption. Most of these methods suffer high capital and regeneration costs of the materials.^[9]

Currently there is an urgent need for new, innovative and cost-effective methods for the removal of toxic substances from waste waters. Bio-sorption is an effective and versatile method and can be easily adopted in low cost to remove heavy metals from large amount of industrial waste waters. Recent studies have shown that heavy metals can be removed using plant materials such as palm pressed fibers and lignocellulosic - a substrate extracted from wheat bran and other plant materials.^[10,11]

By reviewing the most available literature, only few data is available on *Arum cyreniacum* which commonly grows in Aljabal Al-Akhder region-Libya. So, this study was carried out to investigate the volatiles of this plant and to assess its capacity as a heavy metal (Cr and Cd) remover from artificial aqueous solutions.

MATERIALS AND METHODS

Materials

Plant material

Arum cyrinaicum was collected from Aljabal Al-Akhder region- Libya during the flowering stage. The plant was kindly identified and authenticated by Dr. Naser Elshekhi, lecturer of taxonomy at Botany Dept, Faculty of Science, Binghazi University. A voucher specimen was deposited at the herbarium of faculty of science, Sirt university.

Phytochemical screening

Powdered whole plant of *Arum cyrenaicum* was screened for major secondary metabolites according to the method described by Harborne.^[12]

Extraction of lipid fraction *A. cyrenaicum*

About 250 g of the dried powdered plant material of *A. cyrenaicum* were extracted with n-hexane (BP. 40-60 °C) in a soxhlet apparatus. The combined n-hexane extract was passed through fuller's earth to remove the colored pigments, filtered, dried over anhydrous sodium sulphate and evaporated in *vacuo* at 40 °C till dryness to give a pale yellow residue (13.5 g). The n-hexane residue was dissolved in boiling acetone (200 ml) and left overnight at room temperature. An amorphous precipitate was filtered, washed with cold acetone and recrystallized from chloroform/methanol to give bright white crystals (1.15 g) of acetone insoluble fraction (fatty alcohols

mixture). The filtrate (acetone soluble fraction) was evaporated till dryness (10.99 g).

Saponification of the acetone-soluble fraction

The acetone-soluble fraction (10.99g) was saponified by refluxing with 100 ml N/2 alcoholic KOH. The alcoholic solution was concentrated to about 20 ml and diluted with cold distilled water. The unsaponifiable matter was extracted by shaking with successive portions of diethyl ether (3×100ml). The combined ether extract was washed with distilled water, dehydrated over anhydrous sodium sulphate and evaporated *in vacuo* till dryness to give a yellowish brown semi-solid residue of unsaponifiable matter (1.99 g). The unsaponifiable matter was subjected to GLC analysis under the following conditions.

Instrument	Agilent Technologies 6890N Network GC system
Column	Capillary column (ZB-5) ; (length 30m, i.d. 530µm, Film- thickness 50µm).
Oven temp. program	initial temp.: 80 °C, rate: 8°C/min., final temp.: 250°C, final time:50 min.
Inlet	270 °C
Mode	split
Split ratio	15:1
Detector	(FID) 300 °C
Carrier gas	N ₂ ;30 ml/min., Hydrogen : H ₂ 30 ml/min. and Air: 300 ml/min.

Extraction of the total fatty acids

The hydroalcoholic soap solution after saponification was rendered acidic (PH=1) with 5 % sulphuric acid. The liberated fatty acids were thoroughly extracted several times with diethylether. The combined ether extract was washed with distilled water till free from acidity and dehydrated over anhydrous sodium sulphate. The solvent was evaporated in *vacuo* at about 40°C till dryness (0.44 g).

Preparation of the fatty acid methyl esters

About 0.4 g of the total fatty acids was dissolved in 75 ml dry methanol containing 4-5 % dry HCl and refluxed on a boiling water bath for four hours. The solvent was concentrated by evaporation till 25 ml and diluted with 100 ml distilled water. The reaction mixture was extracted with successive portions of diethyl ether (3×100 ml). The combined ether extract was washed with distilled water till free from acidity, dried over anhydrous sodium sulphate, filtered and the solvent was evaporated *in vacuo* at 40 °C (0.3 g). GLC analysis of the fatty acid methyl esters was carried out using the following conditions.

Instrument	Hewlett Packar DHP-6890 series
Column	Capillary column HP-wax-bonded polyethylene glycol, (length : 60m, diameter : 320µm, Film thickness : 0.25µm),
Temperature program	70 °C for 2min., rate 4 °C/min, Final temp. 200°C, Final time, 30 min.
Detector temp.	(FID)275°C
Injector temp.	250°C
Carrier gas	N ₂ : 30 ml/min., H ₂ : 30 ml/min and Air : 350 ml/min

RESULTS AND DISCUSSION

Phytochemical screening

The whole plant of *Arum cyrenaicum* was screened for major secondary metabolites and the results are summarized in Table (1).

Table 1: phytochemical screening of *A.cyrenaicum*.

Constituents	Results
Volatil oils	+
Flavonoids	+
Carbohydrates and / or glycosides	+
Sterols and / or Triterpenoids	+
Tannins	-
Alkaloids	+
Saponins	-

Unaponifiable fraction of *A.cyrenaicum* flower

The unaponifiable fraction of flowers was analyzed by GLC. The total ion chromatogram is depicted in Fig.1, while the different constituents of this fraction are displayed in Table (2).The data of Table 3 suggests the presence of hydrocarbons, sterols and triterpenes. Major sterols are: stigmasterol (14.57%), cholesterol (7.63%) and campasterol (6.71%).

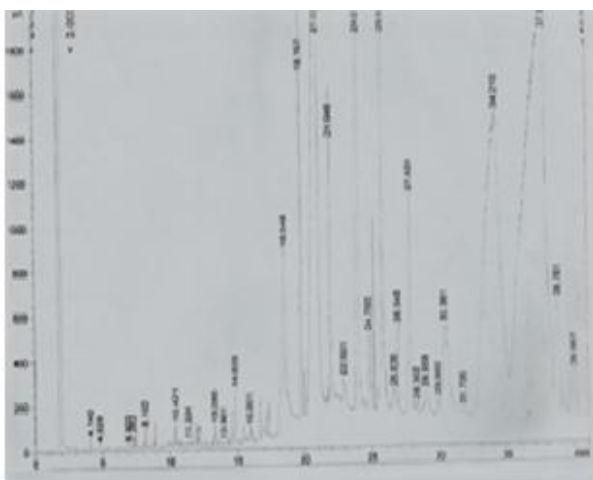


Fig.1:GLC Chromatograms of the unaponifiable fraction of *A.cyrenaicum* flower

Table 2: Constituents of unaponifiable fraction of *A.cyrenaicum* flower

Compound	RT(min).	Relative area%
n-C ₁₅	2.200	0.26
C ₁₆	13.309	0.30
C ₁₇	14.934	0.92
C ₁₈	15.996	0.43
C ₁₉	16.670	0.96
C ₂₀	18.270	3.94
C ₂₁	19.664	3.76
C ₂₂	20.384	12.90
C ₂₃	21.834	3.40
C ₂₄	22.814	4.40
C ₂₅	23.835	4.16

Table 2: Contd.

Compound	RT(min).	Relative area%
C ₂₆	24.734	5.27
C ₂₇	25.699	7.62
C ₂₈	26.850	4.67
C ₂₉	27.729	6.15
C ₃₀	28.718	4.39
Cholesterol	30.450	7.63
Campasterol	32.809	6.71
Stigmasterol	34.959	14.57
β-Sitosterol	36.636	2.48
α-Amyrine	38.029	5.26

GLC analysis of the unaponifiable fraction of *A. cyrenaicum* whole plant

The unaponifiable fraction of *A.cyrenaicum* whole plant was analyzed by GLC. The total ion chromatograms is shown in Fig.2, while the different constituents are displayed in Table 3.The results showed the presence of some hydrocarbons and sterols. Major sterols are: stigmasterol (13.02%) and β-sitosterol (7.36%)- (Table 3).

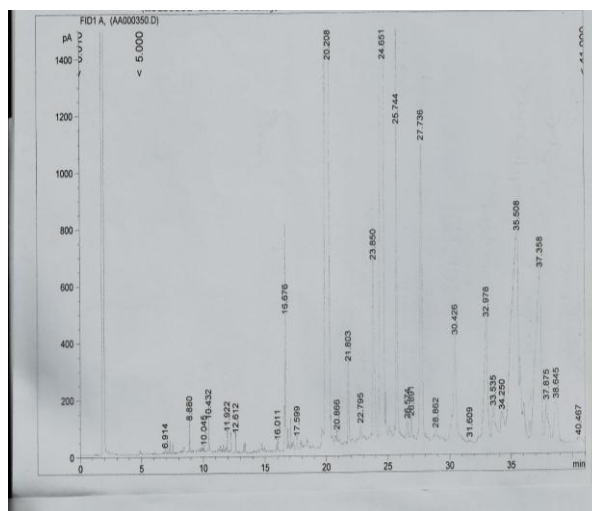


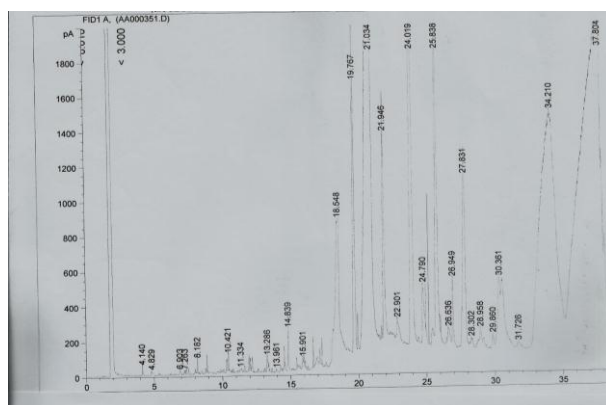
Fig. 2: GLC chromatograms of the unaponifiable fraction of *A. cyrenaicum* whole plant.

Table 3: Constituents of unsaponifiable fraction-whole plant.

Compound	RT(min).	Relative Area(%)	Compound	RT(min).	Relative area(%)
n-C ₁₃	8.880	0.46	C ₂₇	25.744	4.17
C ₁₄	10.432	0.27	C ₂₈	26.891	0.67
C ₁₅	11.922	0.32	C ₂₉	27.736	5.25
C ₁₆	12.612	0.29	C ₃₀	28.862	5.49
C ₁₉	16.676	1.66	Cholesterol	30.426	3.52
C ₂₂	20.208	27.89	Campasterol	32.978	3.87
C ₂₃	21.803	1.37	Stigmasterol	35.508	13.02
C ₂₄	22.795	1.08	β -Sitosterol	37.358	7.36
C ₂₅	23.850	3.16	α -Amyrine	38.645	2.05
C ₂₆	24.651	22.35			

GLC analysis of the unsaponifiable fraction of *A.cyrenaicum* tubers

The unsaponifiable fraction of *A.cyrenaicum* tubers was analyzed by GLC. The total ion chromatogram is shown in Fig.3, while the different constituents are shown in Table 4. The fraction contained some hydrocarbons and sterols. Major sterols are: β -sitosterol (31.73%) and stigmasterol(17.99%) - (Table 4).

Fig. 3: GLC Chromatogram of the unsaponifiable fraction of *A.cyrenaicum* tuber.Table 4: GLC analysis of unsaponifiable fraction of *A.cyrenaicum* tuber.

Compound	RT(min).	Relative area(%)	Compound	RT(min).	Relative area(%)
n-C ₁₁	7.263	0.05	C ₂₅	24.019	5.27
C ₁₂	8.182	0.21	C ₂₆	24.790	1.52
C ₁₄	10.421	0.24	C ₂₇	25.838	4.28
C ₁₅	11.334	0.13	C ₂₈	26.949	0.93
C ₁₆	13.286	0.38	C ₂₉	27.831	2.90
C ₁₇	14.839	0.58	C ₃₀	28.958	4.40
C ₁₈	15.901	0.28	Cholesterol	30.361	3.45
C ₂₀	18.548	4.51	Campasterol	31.726	0.87
C ₂₁	19.767	2.53	Stigmasterol	34.210	17.99
C ₂₂	21.034	13.83	β -Sitosterol	37.804	31.73
C ₂₃	21.946	3.20	α -Amyrine	39.967	2.11
C ₂₄	22.901	1.53			

RT: Retention time

Fatty acid methyl esters

The flowers, tubers and whole plant of *A.cyrenaicum* was analyzed by GLC for composition of fatty acids .Fig.4 shows the GLC chromatograms of the fatty acid methyl esters for flower. The different constituent are displayed in Table 5 . Major constituents are: palmitic acid (20.09%), arachidic acid (15.30%), stearic acid (13.4%) and erucic acid (13.03%). The fatty acid constituents of the tubers are shown in Table 6. The GLC chromatograms are depicted in Fig.5. Major constituents are: linoleic acid (46.36%), palmitic acid (22.53%) and

arachidic acid (10.25%). The fatty acid composition of whole plant is displayed in Table (7), while the GLC chromatograms are shown in Fig.6 .The following fatty acids esters appeared as dominant constituents: linolenic acid (31.89%), linoleic acid (21.3%) and palmitic acid (19.55%).

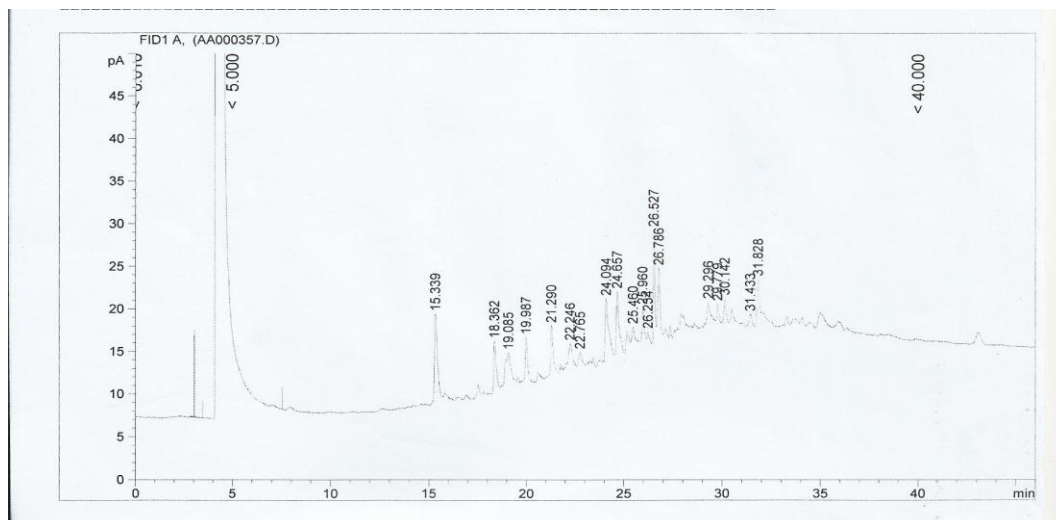


Fig. 4: GLC chromatograms of the fatty acid methyl esters for *A.cyrenaicum* flowers.

Table 5: GLC data of fatty acid methyl esters for *A.cyrenaicum* flowers.

Peak No.	Fatty acid	RT(min)	Relative area(%)
1	Palmitic (C _{16:0})	15.339	20.09
2	Stearic (C _{18:0})	19.085	13.48
3	Oleic (C _{18:1})	19.987	7.74
4	Linolenic (C _{18:3})	21.290	10.58
5	Arachidic (C _{20:0})	24.657	15.30
6	Behenic (C _{22:0})	26.254	2.53
7	Erucic (C _{22:1})	26.786	13.03
8	Lignoceric (C _{24:0})	29.296	7.07
9	(C _{26:0})	31.828	10.18

RT: Retention time

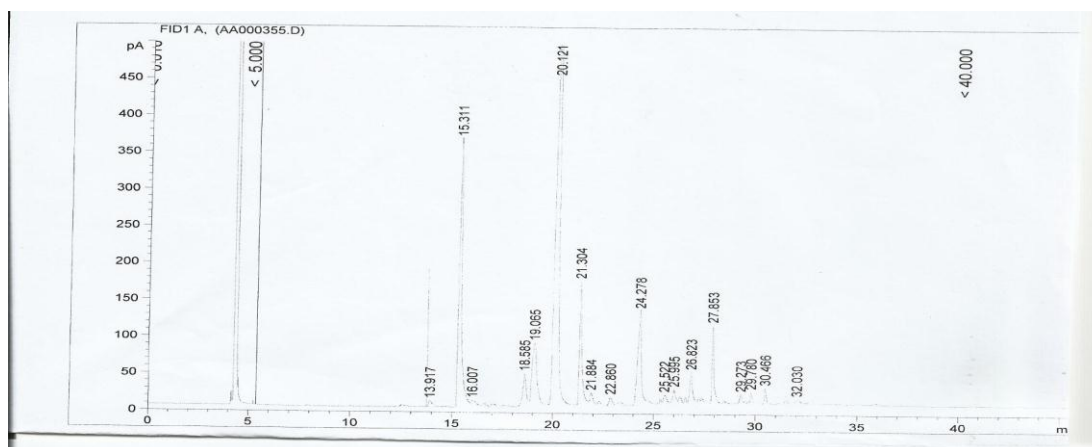


Fig. 5: GLC Chromatogram of the fatty acid methyl esters for tuber of *A.cyrenaicum*.

Table 6: GLC data of fatty acid methyl esters for tuber of *A.cyrenaicum*.

Peak No.	Fatty acid	RT(min)	Relative area(%)
1	Palmitic (C _{16:0})	15.311	22.53
2	Stearic (C _{18:0})	19.065	8.69
3	Linoleic (C _{18:2})	20.121	46.36
4	Linolenic (C _{18:3})	21.304	8.97
5	Arachidic (C _{20:4})	24.278	10.25
6	Behenic (C _{22:0})	26.823	2.56
7	(C _{26:})	32.030	0.64

RT: Retention time

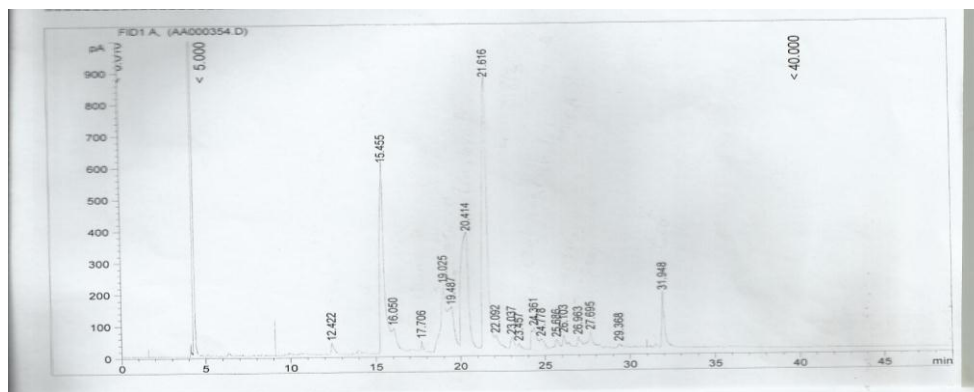


Fig. 6: GC Chromatogram of the fatty acid methyl ester for *A.cyrenaicum*-whole plant.

Table 7: GLC data of fatty acid methyl esters for *A.cyrenaicum*-whole plant.

Peak No.	Fatty acid	RT(min)	Relative area(%)
1	Myristic (C _{14:0})	12.422	0.86
2	Palmitic (C _{16:0})	15.455	19.55
3	Stearic (C _{18:0})	19.025	8.56
4	Oleic (C _{18:1})	19.487	7.18
5	Linoleic (C _{18:2})	20.414	21.83
6	Linolenic (C _{18:3})	21.616	31.89
7	Arachidic (C _{20:4})	24.361	3.14
8	Behenic (C _{22:0})	26.103	1.87
9	Erucic (C _{22:1})	26.963	1.29
10	(C _{26:})	31.948	3.84

Next the whole plant of *A.cyrenaicum* was assessed for its potential as a heavy metal adsorbent. The phytochemical screening of *A.cyrenaicum* revealed the presence of flavonoids. Flavonoids with a 4-keto,3-OH; 4-keto,5-OH and catechol systems are known to chelate with metals like Al(III) and Fe(III). Hence it seems challenging to investigate the flavonoid-containing *A.cyrenaicum* for heavy metal adsorbing capacity. Heavy metals like Cr(III) and Cd(III) are serious pollutants in industrial wastewater and they are known for their affinity for oxygen.

Instrumentation

A 1400 Microwave Plasma-Atomic (4100 MP-AES) Emission Spectrometer (Agilent-USA) was used for flame AAS determination of Cr and Cd. The operational conditions of FAAS measurements are depicted in **Table 8**. The pH measurements were carried out in a WTW720 pH meter model CT16 2AA (Dover Kent, UK) equipped with a combined glass electrode. Mechanical shaker, model EI75 (PA, U.S.A) was supplied by Burrell (USA).

Table 8: Operational conditions for flame AAS determination of Cr and Cd.

Property	Cd (II)	Cr (II)
HC lamp current, mA	5	12
Slit width (nm)	0.5	0.2
Wavelength (nm)	228.802	425.433
Fuel flow rate, L/min	0.8-1.11	0.9-1.2
Burner Height(nm)	213.9	279.5
Regression equation	Y=0.162X+0.02	Y=0.053X+0.0078
R	0.99795	0.99998

Batch method

The batch procedure was used to investigate the optimum conditions for sorption and desorption of each metal ion. Influence of sample pH, shaking time and interfering was examined. A 50 ml model solution, containing each Cr(II) and Cd(II) at concentration 0.1 µg ml⁻¹, was shaken for 1.0 h with 0.1g of *Arum* sorbent at room temperature. The sample pH was examined in the range 3-9. Shaking time was tested at 5, 10, 15, 25 and

30 min. After extraction, the retained elements were desorbed by shaking and determined by flame AAS.

Influence of solution pH

The careful optimization of solution p^H is a pivotal point. The influence of p^H on extraction was investigated in the range 2-9. The results are presented in Fig. 7. Maximum recovery for chromium and cadmium was achieved at p^H 6.0. The *Arum* sorbent has indicated high recovery of

chromium (100%) at pH₄ and cadmium (99.9%) at pH₆. A low recovery (50%) for chromium was observed at pH 2 but the recovery of cadmium was found to be 53.6%. In case of samples containing mixture of these elements, the optimum pH is 5.0.

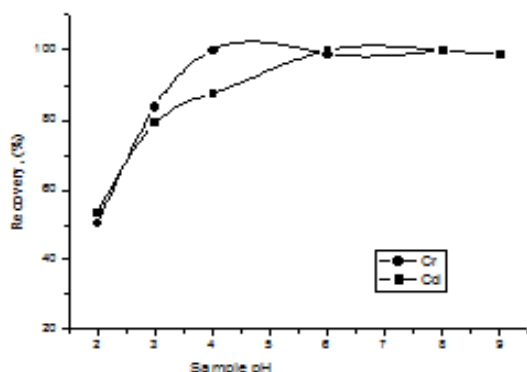


Fig. 7. Influence of sample pH on the recovery of metal ions (0.5 µg ml⁻¹, 10ml sample) and 0.1 Arum cyrinaicum sorbent.

Sorption kinetics

The effect of shaking time on the uptake of these metal ions by the proposed sorbent was investigated in the selected shaking intervals 5, 10, 15, 20, 25 and 30 min. This parameter is important to envisage the minimum period necessary to achieve maximum uptake. Results shown in Fig.8 indicated that, chromium and cadmium reached equilibrium after 10 min. This fast sorption is indicative of diffusion of metal ions throughout a hypothetical film or hydrodynamic boundary.^[13]

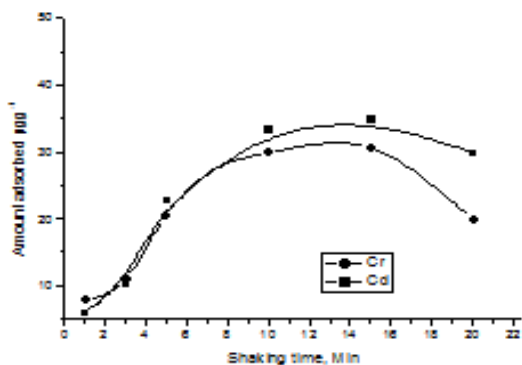


Fig.8 :Effect of shaking time on adsorption of metal ions by Arum cyrinaicum sorbent.

Analytical figures of merit

The analytical figures of merit of the present procedure with determination by FAAS were evaluated. Linear calibration graphs have been obtained that could be represented by the regression equations: A=0.19345C-0.01594 (R²=0.99972), and A=0.05431C+0.0178 (R²=0.99581) for Cr and Cd, respectively. The analytical range was found to be 0.1 –50 µg L⁻¹(Fig.9). The detection limit defined as (3σ) where σ is standard deviation of the blank determination was found to be

5.562, 5.883 µg L⁻¹ for Cd and Cr respectively, and the limit of quantification (10σ) was 18.538, 19.61397 µg L⁻¹, respectively. Obviously, the limit of detections are less than the concentration of the tested metal ions in the majority of real samples which enables quantification of these elements with sufficient accuracy.

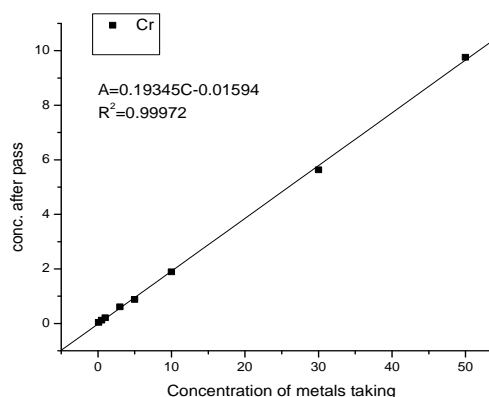
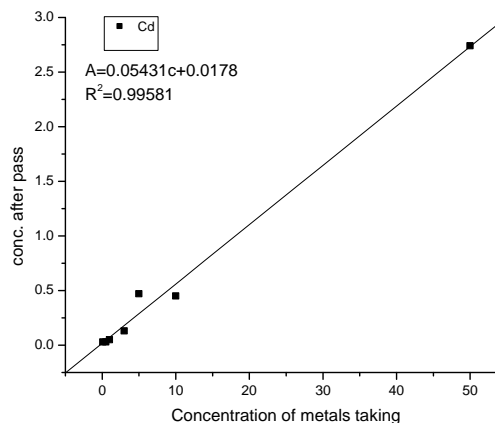


Fig.9 :Analytical figures of merit for Arum cyrinaicum sorbent.

Preconcentration

Sample volume is directly related to the value of preconcentration factor (CF). The higher the volume of the sample from which the metal ion can be quantitatively preconcentrated, the greater the value of CF. Therefore, the recovery of 10 µg of metal ions placed in solutions of varying volumes from 100 to 1000 ml is possible. The obtained results are summarized in Table 9. Quantitative recoveries (97-95.2%) were achieved at sample volumes in the range of 100-1000 ml. Above 1000 ml, the recoveries decreased and were not quantitative which might be attributed to incomplete retention of metal ions probably by the washing action of the column by the sample itself. Therefore, a sample volume ≤ 1000 ml is recommended for the simultaneous preconcentration of all metal ions in order to ensure good recovery. A preconcentration factor 100 was obtained by the ratio of the maximum sample volume / eluent volume.

Table 9: Preconcentration of metal ions on 1.0 g foam column.

Element	Initial volume (ml)	Concentration μgL^{-1}	Desorption Volume (ml)	Recovery (%)	CF
Cd(II)	750	10	10	97	100
Cr(II)	1000	10	10	95.2	100

CONCLUSION

The lipid constituents of *Arum cyreniacum* were identified for the first time. A simple and fast preparation of the plant as sorbent, showed fast equilibration with the studied elements and easy regeneration. Recycling the new sorbent is possible for more than 30 times and preconcentration factor 100 was achieved. This made the proposed procedure alternative to other reported solid phase extraction methods.

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