

Biochemical reactions for the removal of heavy metals from aqueous solutions

Abstract

In a previous study, lead (Pb) and cadmium (Cd) have been removed successfully from aqueous solutions using naturally occurring saponin isolated from *Olea europaea* and *Citrus aurantium* leaves. It was shown that biochemical removal of heavy metals was affected greatly by setting time, the concentration of heavy metal, concentration of saponin extract, and pH value. The process can easily remove heavy metals biochemically via coordinated complex formations between a central heavy metal and saponin as a natural bio-ligand. Accordingly, the effect of chemical factors is well-known but, the effect of physical factors is not. Our present work is focuses on the effect of some physical operational variables on the performance of saponin extraction for the removal of heavy metals. Temperature, agitation, and the synergism between them were the main physical factors that influence the biochemical reaction under investigation. Also, density, moisture content, and organic/inorganic contents have been determined for *Olea europaea* and *Citrus aurantium* leaves.

Keywords: biochemical removal, lead, cadmium, saponin extract, *olea europaea*, *citrus aurantium*

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Introduction

Olea europaea and *Citrus aurantium* are wild plants distributed in the Mediterranean region and grown since ancient time. Leaves of *Olea europaea* and *Citrus aurantium* contain so many biologically active ingredients such as fats/lipids, saponin, flavonoids, terpenoids etc. Occasionally, saponin was considered as a biological naturally occurring plant glycoside, characterized by their strong foaming properties in an aqueous solution that can be used as a ligand for decontamination of aqueous solutions due to its ability to form chemical complexes with heavy metals.^{1,2} Also, saponin has been applied as a surfactant having the complex-forming ability for determination of iron spectrophotometrically.³ The surfactant properties of saponin have been utilized as an important agent for heavy metal removal from aqueous wastes and remediation of contaminated sites. According to modern literature, saponins have served as a natural chelating agent to eliminate problems due to several heavy metals such as Cr, Cd, Cu, Pb, Zn, and As from soil and wastewater.⁴⁻⁷ It has been shown that heavy metals were retained from its original solution due to some form of complexation with a carboxyl group (-COOH) in saponin.⁸ From a chemical point of view, the biochemical reaction between heavy metals and saponin represents a modern approach to (1) the synthesis of new organometallic complexes; (2) decontamination of industrial and radioactive effluents; (3) a non-traditional method for isolation of saponin from the plant kingdom. Our previous studies¹ prove the presence of an oleanolic acid or hederagenin as an aglycone of triterpenoid saponin in *Olea europaea* and *Citrus aurantium* leaves as shown as in Figure 1. This means that there are at least two active centers (-OH and -COOH groups) coordinated directly with heavy metal atoms forming a complex. The concentration of the heavy metal, the concentration of saponin extract and pH were found the chemical operational variables affecting the biochemical reaction between saponin and heavy metal containing aqueous solutions. The present work aimed firstly to evaluate the biochemical reactions between saponin extracted from *Olea europaea* and *Citrus aurantium* leaves and lead/cadmium aqueous solutions under different physical

environments. The effects of temperature, agitation, and synergism between them are the main operating factors under investigation affecting the reaction. Secondly, physicochemical characteristics of *Olea europaea* and *Citrus aurantium* leaves were determined to ensure the presence of a structure-behavior relationship.

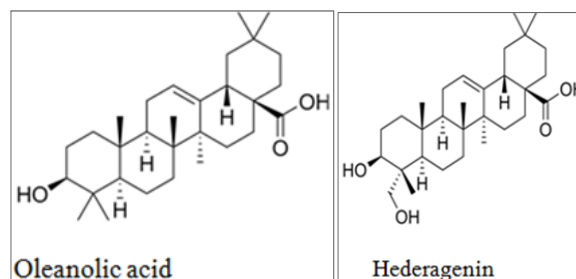


Figure 1 Chemical structure of Oleanolic acid and Hederagenin.

Experimental

Materials and chemicals

The leaves of *Olea europaea* (*Oleaceae*), and *Citrus aurantium* (*Rutaceae*) were collected during May 2017 from Tripoli region, Libya. The leaves of the two plants were cleaned with tap water followed by deionized water to remove dust and dried in shade at about 25±2°C and then cut into small pieces before extraction. Deionized water was used in all experimental investigations. Cadmium (II) nitrate tetrahydrate Cd(NO₃)₂·4H₂O was obtained from LANCASTER, ENGLAND. Lead (II) acetate (CH₃COO)₂Pb is from SURE CHEM PRODUCTS LTD, ENGLAND. Petroleum ether (60-80), n-hexane, methanol and absolute ethyl alcohol are from MERCK.

Characterization of leaves

The density of dry clean *Olea europaea* and *Citrus aurantium* leaves was determined by measuring the volume of a definite weight

of three samples by water displacement method with the density calculated using the following equation:

$$\text{Density}(\rho) = m(g) / \Delta v(\text{cm}^3) \dots\dots\dots [1]$$

The moisture content was determined by water loss method using a drying oven at 105°C for three samples of leaves and, % water content was calculated using equation [2] where, ΔW is the weight difference before and after complete dryness and, W_i are the initial weight of leaves.

$$\% \text{Moisture Content (MC)} = \frac{\Delta W}{W_i} \times 100 \dots\dots\dots [2]$$

The bio-organic content of non-moisturized leaves was determined by direct open combustion method using the non-luminous flame of benzene-burner for a sufficient time. After complete combustion, the weights of residues were measured and % bioorganic content was calculated using the following equation:

$$\% \text{Bioorganic Content (BOC)} = \frac{\Delta W_{BO}}{W_i} \times 100 \dots\dots\dots [3]$$

Where, ΔW_{BO} is the weight difference before and after complete combustion of leave's material and, W_i are the initial weight of leaves.

During combustion, bioorganic compounds are completely converted to CO_2 and water vapor. After removing of moisture by evaporation and bioorganic compounds by combustion, the remaining residue is completely inorganic minerals which can be calculated from the following equations where, W_t is the total weight of sample:

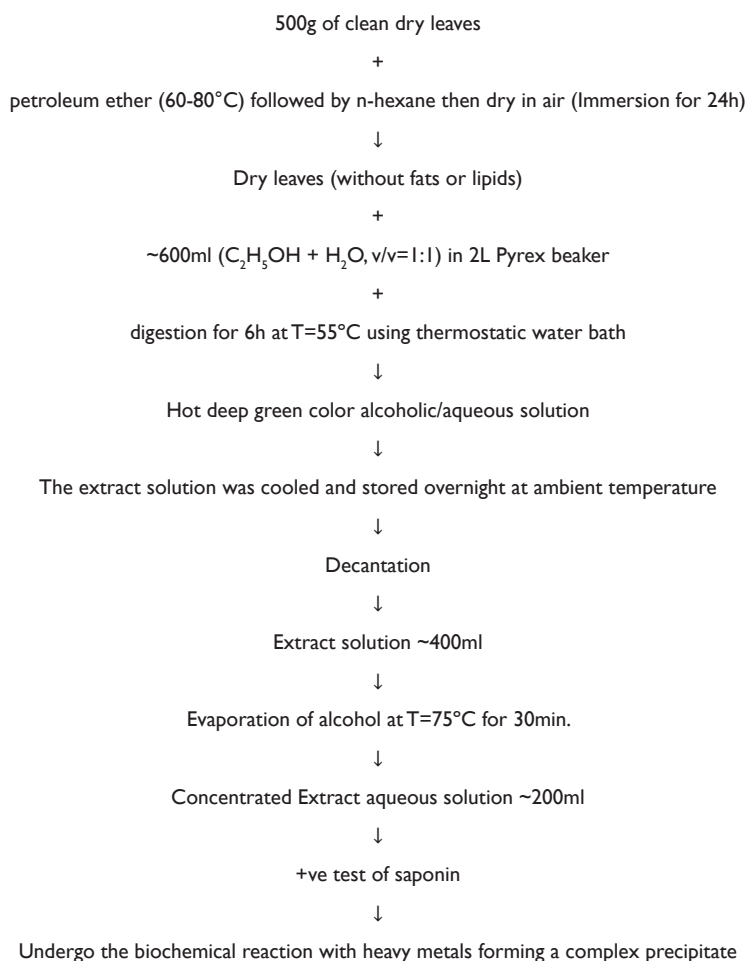
$$\text{Weight of Inorganic Minerals (} W_{inorg.} \text{)} = W_t - [W_{mois.} + W_{BO}] \dots\dots\dots [4]$$

$$\% \text{Inorganic Content (IOC)} = \frac{W_{inorg.}}{W_t} \times 100 \dots\dots\dots [5]$$

Physical parameters

Preparation of crude extracts and heavy metal saponin complexes were carried out according to reference.¹ Figure 2 summarizes the preparation steps of saponin extract solution from *Olea europaea* and *Citrus aurantium* leaves. The effect of temperature on the biochemical reaction between saponin extract and heavy metal aqueous solutions was carried out at different reaction temperatures (15, 40, 60, 80°C), then the volume and weight of wet and dried saponin complex were measured respectively. The effect of agitation was examined at different agitation time (1, 3, 5min) under optimum conditions. Finally, synergism between temperature and agitation was tested for the reaction between lead (Pb) and the saponin extract of *Olea europaea*.

Figure 2 Flowchart for preparation of saponin extract.



Results and discussions

Physicochemical characteristics of leaves

In this part of the present study, density (ρ), percentage water content or moisture content (%MC), percentage bioorganic content (%BOC) and percentage inorganic content (%IOC) for *Olea europaea* and *Citrus aurantium* leaves have been determined experimentally and the obtained results are presented in Table 1. From this data we found that the density for both *Olea europaea* and *Citrus aurantium* leaves is nearly the same having the density of pure natural water at 25°C. For all samples, the density was found to be $\sim 1\text{g/cm}^3$. The above result may reflect the behavior of leaves when contacted with water where leaves neither floated nor immersed in water. This property decreases the chance for the leaves to directly remove heavy metals. Besides, the amount or concentration of free saponin is not enough in leaves. So, the use of a saponin extract is more favorable than the application of leaves for the removal of heavy metals from aqueous solutions. For the two last reasons, direct usage of plant leaves was excluded and not recommended for decontamination of aqueous solutions from heavy metals.

The percentage moisture content (%MC) for *Olea europaea* (triplicate) and *Citrus aurantium* (1 sample) leaves were determined practically. The weight of the samples was determined over 40 minutes to obtain a constant weight. The weight of investigating samples is 10, 15, and 20g for *Olea europaea* and the weight of the *Citrus Aurantium* sample is 10g. The behavior of decreasing the weight of samples during heating at 105°C for 40min. is shown in Figure 3. From which it can conclude that for all samples, the weight was decreased along the examined time period (40min). The rate of moisture losses during heating were determined to be 82.5, 130, 165mg/min for 10, 15, 20g of *Olea europaea* samples and 70mg/min. for 10g of *Citrus aurantium* sample respectively. As the weight of sample increased from 10 to 20g, the rate of moisture loss was increased from 82.5 to 165mg/

min. It was found that for samples of the same weight (10g) of both plants, the rate of loss of moisture from *Olea europaea* is more than that occurring from *Citrus aurantium*, and may be affordable to the biological, morphological and biochemical identity of individual plants. According to the percentage (%) moisture content values in Table 1, the leaves of *Olea europaea* can be described as more moisturized than the leaves of *Citrus aurantium*. The main values of % water content are 33.5 and 28.0% for *Olea europaea* and *Citrus aurantium* leaves respectively.

Similarly through moisture content, bioorganic and inorganic contents could be evaluated in terms of percentage or gram weight. Complete chemical analysis of both *Olea europaea* leaves and *Citrus aurantium* leaves was summarized in Table 1. We noticed that for all investigated samples, the amount of water stored in leaves, the amount of organic compounds, and the amount of inorganic minerals are nearly the same for both *Olea europaea* leaves and *Citrus aurantium* leaves. The minor differences between the two plants may reflect some kind of experimental, manipulation, or detection errors or may be attributed to the differences in biochemical nature of plants.

Olea europaea can be described as moisture and organic rich plant while, inorganic poor one compared to *Citrus aurantium*. The similarity in biochemical compositions of the two plants was confirmed by the obtained density data. The more similarity in biochemical composition, the nearest density will be and vice versa. Recall that saponin will be present as a bioorganic molecule in plant leaves, and hence the more bioorganic rich plant the high saponin content will be.⁹ Saponin containing organic compounds are easily oxidized by oxygen during direct combustion process, and it has been completely converted into carbon dioxide and water vapor. After complete mineralization of bioorganic compounds, dry inorganic residue/ash will be present in the porcelain crucible. In general, inorganic minerals are not greatly affected by elevated temperature due to its high thermal stability or resistance.¹⁰

Table 1 Chemical analysis of *Olea Europaea* and *Citrus Aurantium* leaves

Item	<i>Olea europaea</i> leaves		<i>Citrus aurantium</i> leaves	
	Weight(g)	Percentage (%)	Weight(g)	Percentage (%)
Moisture	3.3	33.6	2.8	28
Bio-organics	6	52.8	5	50
Inorganics	0.7	13.6	2.2	22
Summation	10	100	10	100
Density	1.00 g.cm ⁻³		1.05 g.cm ⁻³	
Test of saponin	++ ve		+++ve	

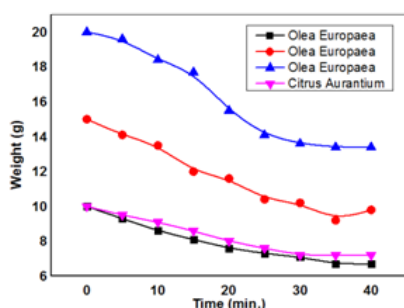


Figure 3 Decreasing behavior of weight of samples during heating at 105°C for 40min.

Previous study

Recently, previous work¹ was published by our research group. The study detailed the effect of some chemical operational variables on performance of saponin with a variety of heavy metals. It has been shown that the chemical reaction between saponin and heavy metals was affected greatly by the following influences:

- The type of heavy metal
- The concentration of heavy metals
- The concentration of saponin extract
- pH value of the medium
- Setting time

Aqueous and/or ethanolic extracts of *Olea europaea* and *Citrus aurantium* have been investigated as a test of saponin. According to Frothing test, *Olea europaea* and *Citrus aurantium* gave a positive response to the stable foam test, forming an emulsion with an ethanolic solution.

Ultraviolet/visible (UV/Vis.) analysis of different heavy metal saponin complexes exhibit major absorption peaks in the narrow wavelength range of ~320 to 400nm with absorbance values in the range of 0.2 to 1.2. Most of saponin itself has a normal absorption peak in the range of ~250 to 350nm, and hence the peaks of the prepared complexes were shifted towards longer wavelength (red shift), and this result may be due to a complex formation between saponin and heavy metals. The above mentioned results prove that saponin can easily separate and complex with heavy metals through the direct combination method. Fortunately, from an environmental point of view, heavy metals can easily be eliminated from its aqueous effluents by a natural saponin and may be lead to a more developed treatment method for so many hazardous waste streams. From the chemical point of view, a new class of organo-metallic complexes has been prepared successfully by the direct combination/chelation between heavy metal and natural saponin.

The obtained results show that the medium of the crude extract solution is weakly acidic (pH=5.16 and 6.35) possibly due to the triterpenoid saponin containing a carboxyl group (-COOH) at carbon number 28. This supports the presence of triterpenoid saponin such as Oleanolic acid and Hederagenin. Six heavy elements (Pb, Cd, Mn, Co, Ni, and Zn) participated in the biochemical reaction with saponin but Hg and Ag failed along a wide/broad range of pH values (5.16-10.5) for both plants. The final volume of wet complexes after adequate setting times varied from the minimum volume ~7mL (for Mn & Co) to the maximum volume ~23mL (for Ni). The final setting time varied from 80 to 135min.

The effect of temperature, agitation and synergism

Our present studies were focused on the effect of temperature and agitation on the biochemical reaction between lead/cadmium and saponin as a natural bio-ligand. Also a synergism between the two affecting variables was observed to ensure that the reaction will proceed *via* optimum conditions. The effect of temperature on the biochemical reaction has been studied and the obtained results were shown in Figure 4.

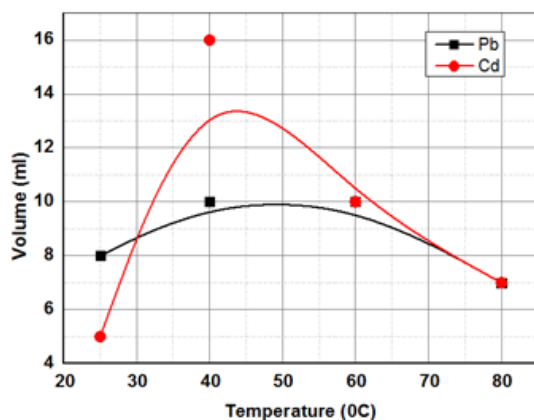


Figure 4 Temperature dependent of the reaction between Pb/Cd and saponin.

As shown from this figure, the reaction was markedly improved within the temperature range of 40 to 50°C to reach a normal maximum at the middle center of the curve. This enhancement may be attributed to the increase in molecular effective collisions between the main reactants with elevated temperature at a microscopic level.¹¹ Inhibition of the reaction starts near 50°C to reach the minimum steady state at 80°C. According to the above mentioned results, it is highly recommended that the reaction should proceed at temperatures less than 50°C. The reaction with cadmium seems to be more sensitive than lead towards temperature. In general the reaction is slightly affected by the temperature for lead.

The effect of agitation for 5min. on the biochemical reaction between heavy metal and saponin was studied and presented in Figure 5. This figure summarizes the obtained scientific fact that agitation was considered as the powerful operating factor affecting the reaction, where the reaction was enhanced continually as the agitation time increased, particularly for lead. For example, with lead the volume of the precipitate was increased from 8mL without agitation (zero time) to about 33mL at 5min. agitation period. The rate of improvement of biochemical reaction was equal to about ~5ml.min⁻¹. The effectiveness of the reaction with cadmium is less than that with lead. This may be due to the effect of side reactions of cadmium with bioorganic compounds other than saponin. The amount of the products of the reaction was observed to be: *Pb-saponin* > *Cd-saponin complex* at the same agitation time.

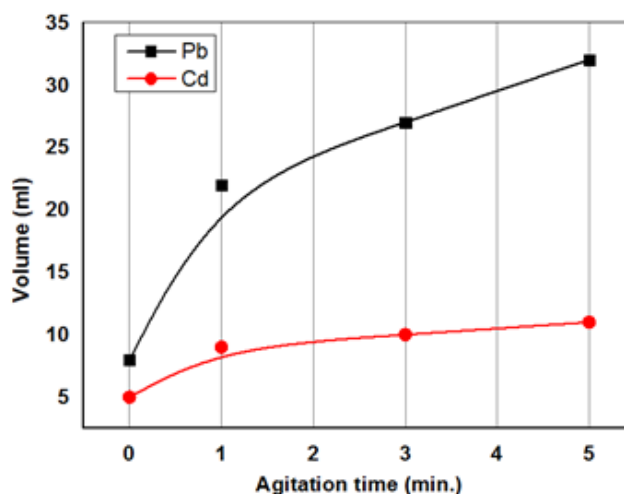


Figure 5 Agitation as a powerful operational variable.

A synergism effect may be defined as a simultaneously doubling of effects (at the same time) on the counter reaction. In a separate experiment, a simultaneously doubly effect of temperature and agitation was studied to know the optimum reaction conditions. The results of synergism effect on the reaction between lead and saponin to produce a complex were displayed as three dimensions (3D) in Figure 6. From which we found that the reaction was slightly affected by synergism between temperature and agitation. This result may be explained by the thermal decomposition of the product during agitation process and the rate of formation of the product is not equal to the rate of decomposition leading to slightly synergistic effect.

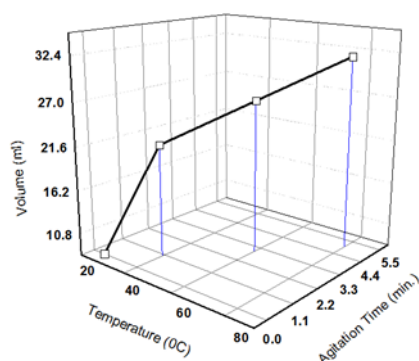


Figure 6 3D presentation of synergism effect.

Conclusion

The removal of heavy metal from aqueous solutions by saponin extract has been established using biochemical reaction between them. The reaction under investigation was affected by means of physical factors such as temperature and agitation as well-as chemical factors such as concentrations, pH, and setting time. It is highly recommended to apply water-saponin extract instead of leaves for the removal of lead and cadmium below 50°C. A huge enhancement of the biochemical reaction can be obtained using agitation for 5mins. Temperature, agitation can achieve the reaction separately but, the synergism between them leads to slight enhancements due to thermal decomposition of the product. The removal of cadmium (Cd) using saponin is facilitated by temperature while, agitation facilitates the removal of lead (Pb).

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Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this manuscript.

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