

Determination of Some Heavy Metals in *Cistus Salviifolius*  
Traditional Drugs

<http://www.doi.org/10.62341/istj-vol38-2-mi54>

Received	2026/05/30	تم استلام الورقة العلمية في
Accepted	2026/06/23	تم قبول الورقة العلمية في
Published	2026/06/24	تم نشر الورقة العلمية في

**Determination of Some Heavy Metals in *Cistus Salviifolius* Traditional Drugs**

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**Abstract**

This research is focused on enhancing our plant heritage, emphasizing the need to exploit it effectively. Specifically chose the medicinal plant *Cistus salviifolius* for our study, where we determined the concentration of seven heavy metals (Zinc, Copper, iron, Lead, nickel, manganese, and cobalt) using flame atomic absorption spectrometry (FAAS). The results from our phytochemical tests revealed a rich presence of secondary compounds, such as flavonoids, tannins, saponins, alkaloids, phytosterols, terpenoids, and phenolic compounds. Heavy metal analysis indicated that *Cistus salviifolius* contained certain heavy metals detected in traditional prescription extracts, including nickel, copper, iron, manganese, and zinc 3.03, 13.15, 387.33, 1.52 and 5.43  $\mu\text{g/g}$ , all of which fell within the safe limits established by WHO/FAO (20-99)  $\mu\text{g/g}$ . In contrast, heavy metal analysis of the acid digest extract of *Cistus salviifolius* showed levels of copper, zinc, nickel, manganese 6.52  $\mu\text{g/g}$  and cobalt 1.13  $\mu\text{g/g}$  that were

Determination of Some Heavy Metals in *Cistus Salviifolius*  
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within the safe limits set by WHO/FAO. However, it is important to note that lead and iron were found to exceed the safe limits, (Iron 476 µg/g) (Lead 7.86 µg/g) raising concerns about potential health risks associated with these contaminants in this medicinal plant. These findings highlight both the therapeutic potential of *Cistus salviifolius* as well as the necessity for careful monitoring of heavy metal contamination to ensure safety in traditional medicinal applications. This study Objectives to determine the concentrations of selected heavy metals in *Cistus salviifolius* using Flame Atomic Absorption Spectrometry (FAAS) through two main sample preparation approaches.

**Keywords:** Heavy metals, Phytochemicals, *Cistus salviifolius*, Traditional drugs.

تقدير بعض العناصر الثقيلة في الدواء التقليدي البريش (سيتوس  
سالفيفوليوس)

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الملخص

يركز هذا البحث على تعزيز تراثنا النباتي، مؤكداً على ضرورة استغلاله بفعالية. وقد اخترنا تحديداً نبات سيتوس سالفيفوليوس الطبي لدراستنا، حيث قمنا بتحديد تركيز سبعة معادن ثقيلة (الزنك، والنحاس، والحديد، والرصاص، والنيكل، والمنغنيز، والكوبالت) باستخدام مطياف الامتصاص الذري باللهب (FAAS). وكشفت نتائج اختبارنا الكيميائية

Determination of Some Heavy Metals in *Cistus Salviifolius*  
Traditional Drugs

<http://www.doi.org/10.62341/istj-vol38-2-mi54>

النباتية عن وجود غني بالمركبات الثانوية، مثل الفلافونويدات، والتانينات، والصابونينات، والقلويدات، والفيتوستيرولات، والترينويدات، والمركبات الفينولية. أظهر تحليل المعادن الثقيلة أن نبات سيتوس سالفيفوليوس يحتوي على بعض المعادن الثقيلة التي تم الكشف عنها في مستخلصات الوصفات الطبية التقليدية، بما في ذلك النيكل والنحاس والحديد والمنغنيز والزنك بتركيز 3.03 و 13.15 و 387.33 و 1.52 و 5.43 ميكروغرام/غرام على التوالي، وجميعها تقع ضمن الحدود الآمنة التي حددتها منظمة الصحة العالمية ومنظمة الأغذية والزراعة (20-99 ميكروغرام/غرام). في المقابل، أظهر تحليل المعادن الثقيلة لمستخلص الهضم الحمضي لنبات سيتوس سالفيفوليوس مستويات من النحاس والزنك والنيكل والمنغنيز (6.52 ميكروغرام/غرام) والكوبالت (1.13 ميكروغرام/غرام) تقع ضمن الحدود الآمنة التي حددتها منظمة الصحة العالمية ومنظمة الأغذية والزراعة. مع ذلك، تجدر الإشارة إلى أن تركيزي الرصاص والحديد تجاوزا الحدود الآمنة (476 ميكروغرام/غرام للحديد و7.86 ميكروغرام/غرام للرصاص)، مما يثير مخاوف بشأن المخاطر الصحية المحتملة المرتبطة بهذه الملوثات في هذا النبات الطبي. تُبرز هذه النتائج الإمكانيات العلاجية لنبات سيتوس سالفيفوليوس، فضلاً عن ضرورة المراقبة الدقيقة لتلوث المعادن الثقيلة لضمان سلامة استخدامه في التطبيقات الطبية التقليدية. تهدف هذه الدراسة إلى تحديد تركيزات معادن ثقيلة مختارة في نبات سيتوس سالفيفوليوس باستخدام مطيافية الامتصاص الذري باللهب (FAAS) من خلال طريقتين رئيسيتين لتحضير العينات.

**الكلمات المفتاحية:** المعادن الثقيلة، المواد الكيميائية النباتية، نبات سيتوس سالفيفوليوس، الأدوية التقليدية.

## 1. Introduction

Until the advent of iatrochemistry in the 16<sup>th</sup> century, plants were the primary source for treating and preventing ailments. However, the decreasing effectiveness of synthetic drugs and the rising number of associated contraindications have brought natural remedies back into focus. (Abdullah et al., 2023). The use of plants as herbal or natural health products has surged worldwide,

Determination of Some Heavy Metals in *Cistus Salviifolius*  
Traditional Drugs

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particularly in developed countries. (Ozyigit et al., 2022). Traditional and complementary medicine represents a significant yet often underestimated health resource with numerous applications, especially in the prevention and management of chronic diseases and in addressing the health needs of aging populations. (Mesert et al., 2020).

Traditional drug includes herbs, herbal materials, herbal preparations, and finished herbal products, whether wild or cultivated. Although some widely used traditional drug show promising potential, they are often not monitored for potential adverse effects due to their frequent use, making it challenging to determine the safest and most effective treatments. (Al kertawy et al., 2023). While the use of plants for medicinal purposes in traditional treatments has diminished due to technological advancements, the demand for medicinal plants is increasing, primarily because of the chemical side effects of modern drugs. It is estimated that 80% of the world's population relies on medicinal plants for healthcare, with over 80,000 plant species utilized for medicinal purposes. (Karahan, 2022).

Environmental pollution caused by heavy metals and its impact on food safety has become a significant global issue in recent times. When heavy metals accumulate in concentrations exceeding the body's requirements, they can lead to serious health risks for all living organisms, particularly humans. (Gebeyehu & Bayissa, 2020) Secondary metabolites are small organic molecules that arise from primary metabolites during the metabolic processes of plants. The chemical characteristics and composition of these metabolites vary among different plant species. The distinction between primary and secondary metabolites can be ambiguous, since many of the metabolites found in the natural products of plants are secondary in nature. (Twajj & Nazmul Hasan, 2022).

*Cistus salviifolius* (Figure 1), native to the Libyan flora. This shrub typically grows to a height of 60 cm, featuring ovate-elliptic leaves and white flowers, with a flowering period that extends from March to May. (Alamami et al., 2021). *Cistus salviifolius*, also known as Sage-leaved Rock-rose, blooms in sheltered coastal areas and is easily recognized by its beautiful white flowers with an orange

Determination of Some Heavy Metals in *Cistus Salviifolius*  
Traditional Drugs

<http://www.doi.org/10.62341/istj-vol38-2-mi54>

center and widely spread smooth petals among embossed leaves. This xerophilous and thermophilous shrub prefers sunny places with calcareous or nutrient-poor soils. It is cultivated as an ornamental plant and is an important feed source for cattle. Bees frequently visit it for pollination. The plant's adaptation to extreme environmental conditions is mainly due to the efficiency of its secondary metabolites. (Boubekeur et al., 2022).

*Cistus salviifolius* has been traditionally used to treat rheumatism, inflammatory diseases, and intestinal pain. It is also used as an analgesic, an expectorant in bronchitis, and for its antimicrobial and antidiarrheal properties. Despite its medicinal potential, *Cistus salviifolius* remains relatively under-investigated. (Boubekeur et al., 2022). Specifically, *Cistus salviifolius* produce high amounts of natural metabolites, including flavonoid compounds, tannins, and terpenoids, known for their health benefits. (Sayah et al., 2017).



Figure 1. *Cistus salviifolius* (Birbish).

This study Objectives to determine the concentrations of selected heavy metals in *Cistus salviifolius* using Flame Atomic Absorption Spectrometry (FAAS) through two main sample preparation approaches. The first approach involves acid digestion of plant

Determination of Some Heavy Metals in *Cistus Salviifolius*  
Traditional Drugs

<http://www.doi.org/10.62341/istj-vol38-2-mi54>

samples; the second approach consists of aqueous extraction, which simulates the traditional use of medicinal plants.

Assessment of the risk associated with usage of the studied plant as a drug.

## Materials and Method

### 1. Sample location and collection

The *Cistus salviifolius* have been collected from Al-Hmda; Al-Hamda area is located 70 km east of Benghazi city-Libya, it is a mountainous agricultural area.

The samples were cleaned, washed with tap water then with distilled water, dried at room temperature in the shade; in order to preserve the maximum integrity of the molecules targeted by our study, which used 2g for extrac.

### 2. Chemicals and Reagents

All chemicals and reagents with analytical grades were used in this study. Concentrated nitric acid and 30% hydrogen peroxide were used for plant sample digestion. Distilled water was used throughout the experiment for sample preparation, dilution, and rinsing.

### 3. Sample preparation for analysis

#### • Extract as used in the traditional prescription

The traditional method of drug hot drink prescription has been applied. After weighing 2 g of *Cistus salviifolius* powder into a flask, and then add 50 ml of deionized water and place on the heater until boiling for 5-7 minutes (Figure 2) and the final step is filtered through Whatman No. 41 filter paper then finally analysed by using FAAS.

#### • Acid-digested extract (extraction according to EPA protocol)

Digestion method of (EPA-3050b, 1996) has been applied for the digestion of plant samples. After weighing 2 grams of the *Cistus salviifolius* ground, 10 milliliters of highly pure concentrated nitric acid were added before reaching the boiling point. The mixture was then heated for 15 minutes and allowed to cool. (Figure 3)

Determination of Some Heavy Metals in Cistus Salviifolius  
Traditional Drugs

<http://www.doi.org/10.62341/istj-vol38-2-mi54>

- **Extraction by methanol for qualitative phytochemical analysis**

The dried samples were gently ground by using acid washed porcelain pestle and mortar, then 6.5g of the Cistus salviifolius has been transferred into a conical flask, after that methanol was added (the level of methanol must be above the sample), and the flask has been shaken vigorously without heat and kept at the room temperature for about 7-10 days. Then solution used for phytochemical screening after filtration. (Figure 3).



Figure 2 Samples preparation for analysis

Determination of Some Heavy Metals in *Cistus Salviifolius*  
Traditional Drugs

<http://www.doi.org/10.62341/istj-vol38-2-mi54>

### Instruments (FAAS)

FAAS is one of the most conventional techniques for the determination of trace metal ions because of the relative simplicity and inexpensiveness of equipment. In this technique, a sample is introduced into a flame where it is dissociated into constituent atoms.

### Results and Discussion

The following results include numerous calculations that provide a comprehensive understanding of the overall mechanism of *Cistus salviifolius*.

#### 1. FAAS analysis results of heavy metals in *Cistus salviifolius*

The obtained results of the metal concentrations in *Cistus salviifolius* are illustrated in Table 1.

**Table 1 FAAS results for heavy metals in *Cistus salviifolius* ( $\mu\text{g/g}$ )  
traditional prescription and acid extract**

Element analyzed	Acid digest average	Traditional prescription
Pb	$7.86 \pm 0.379$	Below LOD of FAAS
Ni	$3.93 \pm 0.28$	$3.03 \pm 0.143$
Co	$1.13 \pm 0.18$	Below LOD of FAAS
Mn	$6.52 \pm 0.19$	$1.52 \pm 0.19$
Fe	$476 \pm 17.97$	$387.33 \pm 13.68$
Cu	$15.03 \pm 0.14$	$13.15 \pm 0.75$
Zn	$6.53 \pm 0.26$	$5.43 \pm 0.29$

Pb: Lead, Ni: Nickel, Co: Cobalt, Mn: Manganese, Fe: Iron, Cu: Copper, Zn: Zinc, and LOD: Limit of detection.

As shown in Table 1 in the case of nickel, the amounts are approximately similar in both traditional prescription ( $3.28 \mu\text{g/g}$ ) and acid digest ( $3.98 \mu\text{g/g}$ ) extract. In the case of Manganese, the highest amount was found in acid-digested extract ( $6.95 \mu\text{g/g}$ ) while the lowest amount was found in traditional prescription ( $1.28 \mu\text{g/g}$ ). Lead was not found in traditional prescription extract but found in acid-digested extract equal to ( $7.80 \mu\text{g/g}$ ). Cobalt was also not found in traditional prescription extract but was found in acid-digested

Determination of Some Heavy Metals in *Cistus Salviifolius*  
Traditional Drugs

<http://www.doi.org/10.62341/istj-vol38-2-mi54>

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extract and equal to (1.20  $\mu\text{g/g}$ ). In the case of Iron, the highest amount was found in acid digested extract which is equal to (476  $\mu\text{g/g}$ ) while the lowest amount was found in traditional prescription extract equal to (391.33  $\mu\text{g/g}$ ). In the case of copper, the highest amount was found in acid digested extract (15.58  $\mu\text{g/g}$ ), and the lowest one was found in traditional prescription extract (13.05  $\mu\text{g/g}$ ). Zinc was found in high amounts in acid digested extract (6.70  $\mu\text{g/g}$ ) but the least amount was found in traditional prescription extract (5.57  $\mu\text{g/g}$ ).

As the demand for and consumption of plants continues to rise globally, it is important to note that both commercial and residential plant cultivation often occurs in urban areas. These locations are frequently exposed to anthropogenic contamination from various sources, such as urban and industrial waste, mining, smelting, and the metallurgical industry. Consequently, food safety concerns and potential health risks are emerging as major public health issues worldwide, positioning heavy metal pollution as one of the most pressing environmental challenges. (Gebeyehu & Bayissa, 2020) This is one of the reasons for the difference in the results.

As illustrated in Figure 3 the highest total concentration of metal presented in *Cistus salviifolius* acid-digested extract is iron (476  $\mu\text{g/g}$ ), followed by copper (15.03  $\mu\text{g/g}$ ), and lead (7.86  $\mu\text{g/g}$ ), and manganese (6.52  $\mu\text{g/g}$ ), and zinc (6.53  $\mu\text{g/g}$ ), then nickel (3.93  $\mu\text{g/g}$ ) and, the lowest concentrations presented from cobalt (1.13  $\mu\text{g/g}$ ).

Determination of Some Heavy Metals in *Cistus Salviifolius*  
Traditional Drugs

<http://www.doi.org/10.62341/istj-vol38-2-mi54>

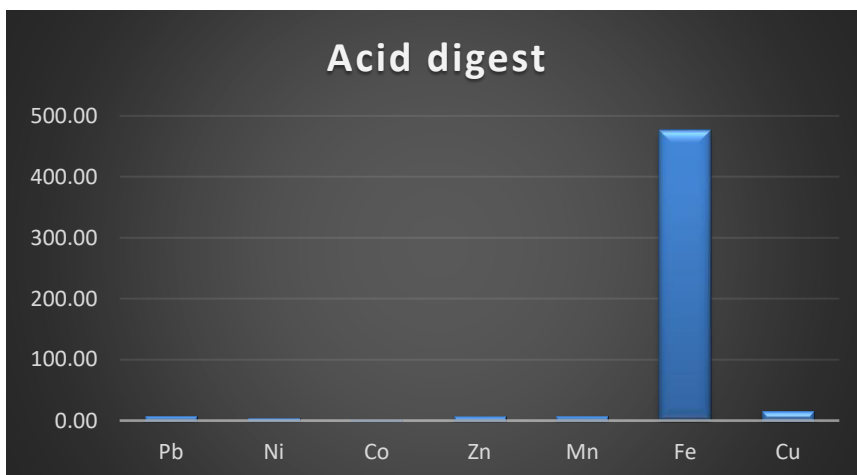


Figure3: Average concentrations of metals in *Cistus salviifolius* acid digested extract

As illustrated in Figure 4 the highest concentration of metal presented in *Cistus salviifolius* traditional prescription is iron (387 µg/g), followed by copper (13.15 µg/g) and zinc (5.43 µg/g) then the lowest concentrations presented from manganese (1.52 µg/g) and least one was nickel (3.03 µg/g), while lead, and cobalt, have not been detected.

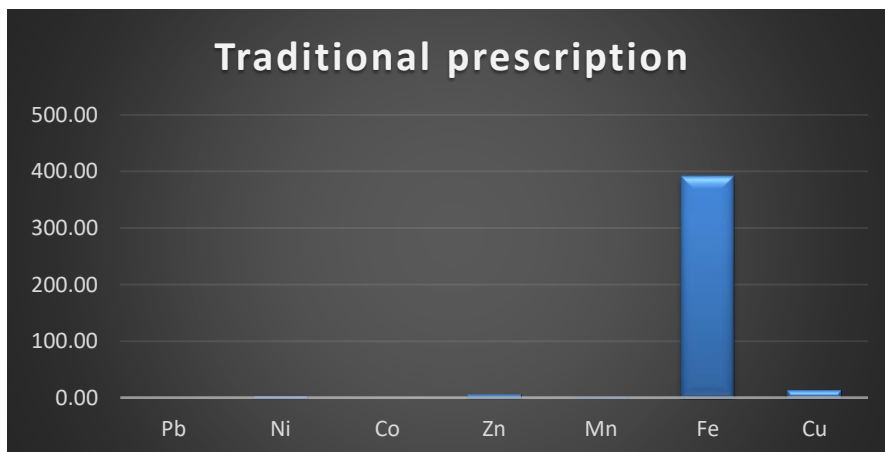


Figure4: Average concentrations of metals in *Cistus salviifolius* traditional prescription extract

Determination of Some Heavy Metals in *Cistus Salviifolius*  
Traditional Drugs

<http://www.doi.org/10.62341/istj-vol38-2-mi54>

The detection limits of elements in Flame Atomic Absorption Spectroscopy (FAAS) can vary depending on factors like the instrument used, the specific analytical conditions (such as the flame type and matrix), and the method calibration. Here are the approximate detection limits for the elements you listed based on typical conditions in FAAS.

## 2. Qualitative Chemical Analysis of phytochemical in *Cistus salviifolius*

The results of chemical analyzes and assays of alcoholic leaf extracts from *Cistus salviifolius* have revealed the presence of active compounds, including phenols, flavonoids, tannins, alkaloids, saponins and steroids. The results of the various phytochemical analysis obtained during the experiment are shown in (Table 2).

**Table 2. Qualitative phytochemicals result in *Cistus salviifolius* plant**

Phytochemical	Result
Flavonoids	++
Terpenoids	++
Steroids	+
Phenolic	+++
Saponins	++
Tannin	++
Alkaloids	+++
+: low quantity, ++: medium quantity, +++high quantity.	

The results were estimated as: (+) presence of the phytochemical, (+++) high concentration, (++) Medium, (+) Trace, (-) shows absence of the phytochemical.

Alkaloids and Phenolic are found in the highest amount, but flavonoids, terpenoids saponins and tannin are approximately found in the middle range, while steroids and amounts are approximately the lower. The qualitative phytochemical analysis of *Cistus salviifolius* presented in Table 2 indicates the presence of several bioactive compounds with varying amounts.

Determination of Some Heavy Metals in *Cistus Salviifolius*  
Traditional Drugs

<http://www.doi.org/10.62341/istj-vol38-2-mi54>

**3. Comparison of heavy metals concentrations in *Cistus salviifolius* with World Health Organization safe limits**

According to the FAO/WHO, (Mensah et al., 2009) the maximum safe limit for nickel in medicinal plants is 67.9 µg/g, while the permissible limit for iron is 425.5 µg/g. The limit manganese at 20-40 µg/g, and the safe limit for copper is 73.3 µg/g. additionally, the acceptable safe limit for zinc is 99.4 µg/g, and the safe limit for lead is 0.3 µg/g.

**Table 3. The FAO/WHO maximum safe limit of heavy metals in medicinal plants. (Mensah et al., 2009)**

Sample	Standards	Ni	Cd	Mn	Fe	Cu	Zn	Pb
Plant µg/g	WHO/FAO (2009)	67.9	0.02	20-40	425.5	73.3	99.4	0.3

According to results of Tables 1 and 3, the concentrations of lead, and iron in *Cistus salviifolius* were above the safe limits of the WHO/FAO while manganese, nickel, zinc and copper were within the safe limits in *Cistus salviifolius* extract. (Figure 5)

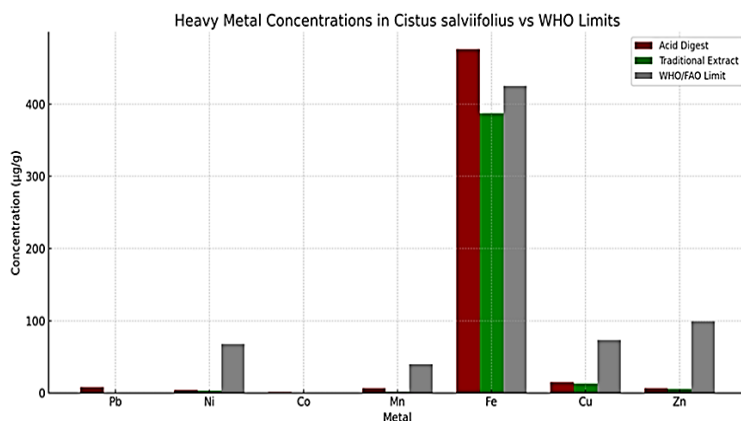


Figure5: Comparison of heavy metals concentrations with WHO safe limits.

To discuss the phytochemical results in *Cistus salviifolius* in the light of heavy metal presence (Pb, Ni, Co, Mn, Fe, Cu, and Zn), we need to consider how the bioactive compounds in the plant interact with or might be affected by the presence of these heavy metals.

Determination of Some Heavy Metals in *Cistus Salviifolius*  
Traditional Drugs

<http://www.doi.org/10.62341/istj-vol38-2-mi54>

Both the phytochemical profile and the potential contamination by heavy metals could influence the plant's medicinal value and its safety for human consumption.

### Conclusion:

In this research, focused on *Cistus salviifolius*, a plant traditionally used for its therapeutic properties, including antimicrobial, antiviral, anti-tumor, and cytotoxic effects. It has been determined concentration of heavy metals (Fe, Ni, Mn, Co, Pb, Zn, and Cu) in this plant by using FAAS. The phytochemical analysis revealed the presence of flavonoids, tannins, saponins, alkaloids, phytosterols, terpenoids, and phenolic compounds in the studied sample, which potential sources for new drugs development. The chemical extraction of heavy metals through acid digestion indicated a lead contamination in *Cistus salviifolius*, which raises significant concerns regarding contamination within the food chain. In contrast, other metals such as manganese, cobalt, nickel, copper, and zinc were found to be within acceptable limits, and extraction of heavy metals through water extract as used in the traditional prescription were found to be within acceptable limits. This noteworthy finding in this species and contributes valuable information to the field. Ultimately, this study is positioned within a multidisciplinary framework, integrating analytical chemistry, and opens new avenues for enhancing our understanding of local flora.

### Recommendations for Future Studies

1. Phytochemical Quantification: Future studies must identify, isolate, and precisely quantify the specific bioactive phytochemicals (e.g., polyphenols, flavonoids, terpenes) responsible for the plant's therapeutic effects. This will help standardize its preparations.
2. Essential Clinical Trials: Clinical trials on humans are absolutely essential to move from traditional use to proven application.
3. Investigating the source of lead contamination in the plant's growing environment.

Determination of Some Heavy Metals in *Cistus Salviifolius*  
Traditional Drugs

<http://www.doi.org/10.62341/istj-vol38-2-mi54>

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4. Developing cultivation or processing methods to reduce heavy metal content without compromising its beneficial compounds.

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Determination of Some Heavy Metals in *Cistus Salviifolius*  
Traditional Drugs

<http://www.doi.org/10.62341/istj-vol38-2-mi54>

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