Chemical comparison in stem and leaves of *Cupressussempervirens* L. of Cuban origin

Eva Salas[1], Chavelys López[2], Bárbara González[3]
Flavio José Hernández Bonilla[4] and JhuniorAbrahan Marcía Fuentes*[5]

[1]InstituteofPharmacy and Food, UniversityofHavana, Cuba.
[2]Pharmaceutical Laboratories, Biocubafarma, Havana, Cuba.
[3]Central Laboratory of Pharmacology. Faculty of Medical Sciences Salvador Allende, Cuba.
[4]Faculty of Sciences, National University ofAgriculture, Olancho, Honduras.
[5]Faculty of Technological Sciences, National University of Agriculture, Olancho, Honduras.

.

ABSTRACT

The Cupressussempervirens Linnaeus (Cypress) species, belonging to the botanical family of the Cupressaceae, is an arboreal species native to the eastern Mediterranean and is of great importance for presenting metabolites with great therapeutic potential. In Cuba, it is uncommon, and this is the reason why this research took place to compare the chemical characterization of the stem and leaves of the ¨Ciprés¨ of Cuban origin. The present research is the first one in Cuba for the species which underwent a pharmacognostic study of the crude drug and the extracts of the plant parts. To be carried out, quality parameters of the leaves and stem were evaluated where the results obtained met with the specifications described in the official monographs. The Maceration Method was applied using 95% ethanol for the extraction of its chemical constituents. These were qualitatively determined by the Phytochemical Screening Techniques; expressing with greater incidence essential oils, fats, alkaloids, triterpenoids and steroids, resins, reducing sugars, saponins, flavonoids, phenolic compounds, mucilages, and bitter principles. High-Resolution Liquid Chromatography suggests that the compounds that showed retention time on the stem were 15.2 and 15.9 minutes and the retention times on leaves were 15.2 and 16.0 minutes.

Keywords: Cupressussempervirens, chemical composition, Cypress, Mediterranean.

1. INTRODUCTION

Medicinal plants have several beneficial conditions for humanity, one of them is their medicinal contribution due to the presence of phytochemicals and antioxidants, characterized by these bioactive compounds as the main source of nutraceuticals [1]. Furthermore, they are considered a source of vitamins and minerals [2]; therefore, they can be used in the food industry for the elaboration of functional foods [3] contributing to the development of new products, with a positive economic and social impact [4].

C. sempervirens L is a tree of European origin (figure 1) [5] [6]; with medicinal properties and potential pharmacological interest, it is known in the world as cypress and cypress, its cultivation is ornamental, it maintains a height between 10 and 20 m in height, it has smooth bark, grayish color, it has a long and narrow crown, fusiform, with upright branches and small leaves, empirically the fruits and bark have been used to control stomach problems, it has also been used in the treatment of parasites, as an anti-abortion and as an insect repellent, in Cuba they are grown in addition to the Mediterranean cypress, other species among which *C. arizonica* Greene, *C. macrocarpa*Martweg, *C. lusitanica* Mill, *C. funebris*Lindlstand out,*C. torulosaa* Don, *C. Benthami*Endl and*C. glabra*Sudwort [7].

Figure 1.*C. sempervirens*L. of Cuban origin.

2. MATERIAL AND METHODS

The experimentation work was carried out in the Synthesis Laboratories of the Pharmacy Department at the Institute of Pharmacy and Food (IFAL), University of Havana. The plant material of *C. sempervirens*L. (Cypress) used in this research is made up of fresh stems and leaves. The material was collected at the Novartis Laboratories Commercial House in Vedado, Plaza de la Revolution Municipality in the province of Havana, Cuba, with herbarium number HAC43083.

2.1 Washing and disinfection of the drug

The washing was carried out with abundant demineralized water, immersed in 1% sodium hypochlorite for five minutes, and then washed for ten minutes [8].

2.2 Macroscopic evaluation of the drug

The macroscopic evaluation of the plant was performed by determining the quality control parameters [8]. For the characterization of the leaves, the following parameters were determined: size, color, odor, shape, condition, and surface characteristics.

2.3 Preparation of samples and extracts

Fresh samples were micronized at a particle size between 0.8 to 2 mm in a FUMAR brand knife mill (Germany). The extracts of the leaves and stems of the species *C. sempervirens*L. (Cypress) were prepared with samples of 50 g by the method of maceration with 95% ethanol (Class A) for 7 to 14 days at room temperature and shaking in a shaker for qualitative characterization in HPLC equipment. In all cases, reagent grade solvents and the solutions corresponding to each test were used.

2.4 Phytochemical screening

Phytochemical screening was carried out through identification reactions through a color change, flavor identifier, the formation of precipitates [8], from the evaluation of essential oils and fats, saponins, polysaccharides, resins, alkaloids, lactones and coumarins, cardiotonic glycosides, phenols, triterpenes, and steroids, reducing compounds, amino acids and amines, quinones, flavonoids, and anthocyanidins.

2.5 High-Resolution Liquid Chromatography (HPLC) Chromatographic Separation

Samples of stem and leaves of the species *C. sempervirens*L. (Cypress) were analyzed by HPLC using a KNAUER Chromatograph (Germany) with a C-18 column and dilution of the sample by eight times. The HPLC chromatographic separation, with a 280 nm UV detector, consisting of an Azura P6.1L low-pressure quaternary gradient pump, an Azura UVD 2.1L UV-Vis detector, an Azura CT 2.1 Thermostat, and a manual injector. The identification and integration of peaks were performed using a computer compatible with the HPLC Control Program for the acquisition and manipulation of chromatographic data, ClarityChrom version 6.1.0.130 (KNAUER, Germany). Chromatographic separation was performed using a Eurospher C-18 reverse phase column, 60 A, 5 µm (d.i), 250 x 4 mm, of German manufacture. HPLC chromatographic analysis was carried out using water as eluent A and acetonitrile and a gradient from 15 to 85% B as eluent B, for forty minutes at 1mLmin-1 followed by maintenance of the gradient, increasing A by 50%, for ten sustained minutes, reverting to 0% B for five minutes, and rebalancing for five minutes at a temperature of 25 0C. In the analyzes carried out, we used extracts made with fresh cypress plants at concentrations of 95% (v / v) in ethanol.

3. RESULTS AND DISCUSSION

3.1Phytochemical Screening Results

The "screening" techniques allow to qualitatively detect the presence of certain groups of compounds, which rely on microchemistry to show these groups of constituents through the formation of precipitates, colorations, flavor, among others. The screening was carried out

on two parts of the fresh plant of the same species *C. sempervirens*L.(Cypress), one with the leaves (Table 1) of intense green color and the other with the stem (Table 2) to observe if the results of both samples from the same plant were similar in terms of qualitative chemical composition or differed.

The results of the two phytochemical screenings of *C. sempervirens*L. (Cypress) show the presence of bioactive compounds that make the plant of interest to the pharmaceutical industry, these results, as well as the tests carried out are shown in Tables 1 and 2. experimentally found that the most significant results in both screenings were those of Dragendorff, Libermann-Burchard, Ferric Chloride, and Shinoda. Both phytochemical screenings arise the presence of fatty compounds, essential oils, alkaloids, triterpenes and/or steroids, reducing sugars, saponins, phenolic compounds, flavonoids, anthocyanins, and mucilages. Therefore, both screenings show the presence of some metabolites common in both parts of the plant and others not, except for saponins that are reported in the leaves and not in the stem.

111 **Table 1.Results of phytochemical screening in leaves of *C. sempervirens*L**

Tests	*Metabolites*	*Results*		
		Etheral	*Alcoholic*	*Aqueous*
Sudan	Fatty compounds	+++		
Dragendorff	Alkaloids	+++	-	+++
Baljet	Lactonic grouping	-	-	
Libermanm.B	Triterpenes / steroids	+++	+++	
Catequins	Catechins		-	
Resins	Resins		+++	
Fehling	Reducing sugars		+++	+++
Foam	Saponins		+++	+++
Chloride Fe	Phenolic compounds		+++	-
Ninhydrin	Free amino acids		-	
Borntrager	Benzoquinquinones		-	
Shinoda	Flavonoids		+++	+++
Kedde	Cardiotonic glycosides		-	
Anthocyanidin	Anthocyanins		-	
Mucilages	Mucilages			+++
Beginnings A	Beginnings A			+++
Essentialoils	Essential oils	+++		

112

113

Table 2.Results of the phytochemical screening in stems of *C. sempervirens*L

Tests	*Metabolites*	*Results*		
		Etheral	*Alcoholic*	*Aqueous*
Sudan	Fatty compounds	+++		
Dragendorff	Alkaloids	+++	-	+++
Baljet	Lactonic grouping	-	-	
Libermanm.B	Triterpenes / steroids	+++	+++	
Catequins	Catechins		-	
Resins	Resins		-	
Fehling	Reducing sugars		+++	+++
Foam	Saponins		-	-
Chloride Fe	Phenolic compounds		+++	-
Ninhydrin	Free amino acids		-	
Borntrager	Benzoquinquinones		-	
Shinoda	Flavonoids		+++	-
Kedde	Cardiotonic glycosides		-	
Anthocyanidin	Anthocyanins		-	
Mucilages	Mucilages			+++
Beginnings A	Beginnings A			+++
Essentialoils	Essential oils	+++		

3.2 Analysis of the Chromatogram of the Ethanolic Extract of the Stem of *C.sempervirens*L. (Cypress)

Through the analysis of the two extracts by high-performance liquid chromatography (HPLC), it was observed that in the case of the stem extract, a simple chromatogram was obtained with the isocratic water / ACN elution system with the C-18 column and 8-fold dilution of the sample, detecting 44 components, with the presence of two very significant chromatographic peaks between 15,167 and 15,883 minutes, obtaining the highest percentage of relative area for the peaks that eluted between 9,500 and 15,883 minutes (Figure 2). The major compounds, or those that present the greatest interest in the chromatographic profile of the hydroalcoholic extracts of the stems, are those that elute at 10.4; 11.0; 11.3; 11.7; 12.4; 13.3; 15.2; 15.9; 16.4; 17.9; 20.5; 25.6; 27.6; 29.9 and 36.6 minutes, respectively. The two major compounds showed a retention time of 15.2 and 15.9 minutes, respectively.

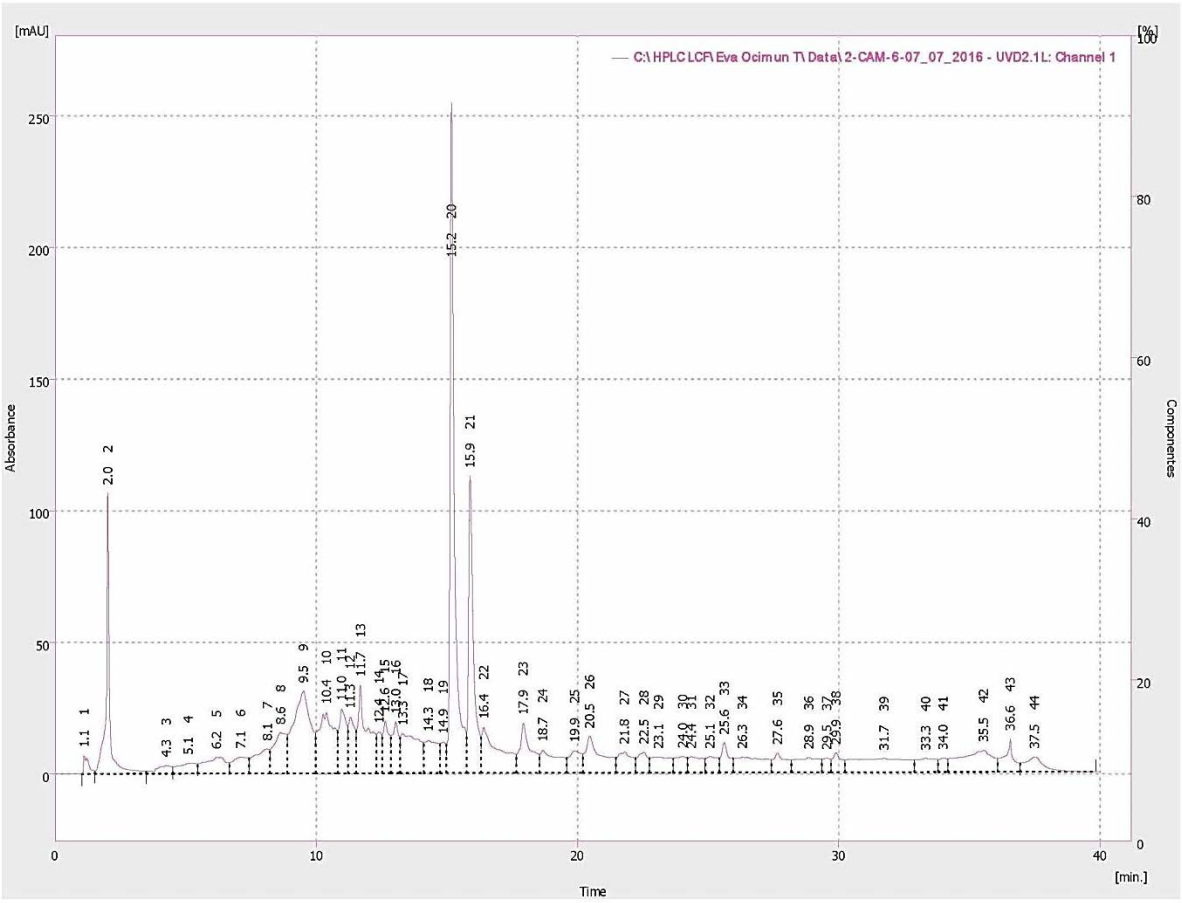

Figure *2*.Chromatograms of the ethanolic extract of the stem of *C. sempervirens L. from Cuba*

3.3 Analysis of the Chromatogram of the Ethanolic Extract of the Leaves of *C.sempervirens*L. (Cypress)

In the case of the leaves, a simple appearance was also shown, although visualizing the increase in complexity in them, nine significant chromatographic peaks were shown, obtaining the highest percentage of the relative area (40.2%) for the peak that eluted at 11.8 minutes. The compounds of greatest interest in the chromatographic profile of the leaves of the plant are those that eluted at 10.1; 11.1; 11.8; 12.7; 15.2; 16.0; 18.0; 20.4 and 36.6 minutes, respectively. In this case, the majority compounds are those that eluted at 11.8; 15.2 and 16.0 minutes, respectively (figure 3).

It is suggested that possibly the compounds that presented a stem retention time of 15.2 and 15.9 minutes and those with a leaf retention time of 15.2 and 16.0 minutes, respectively, are two compounds that represent the same type of metabolite, in each case, produced by the plant in each part of the plant that has been analyzed in this study. It also shows compounds present in leaves that do not appear on stems and vice versa.

It is inferred, therefore, that the ethanolic extract of the leaves is the one that contains the greatest amount of compounds (47 compounds) according to the results achieved under the test conditions used, compared to the stem that contains (44 compounds).

When comparing the two extracts of the two parts of the plant material, leaves and stem, differences were observed in the High-Resolution Liquid Chromatography, with the stem extract being the one with the most complex chromatograms.

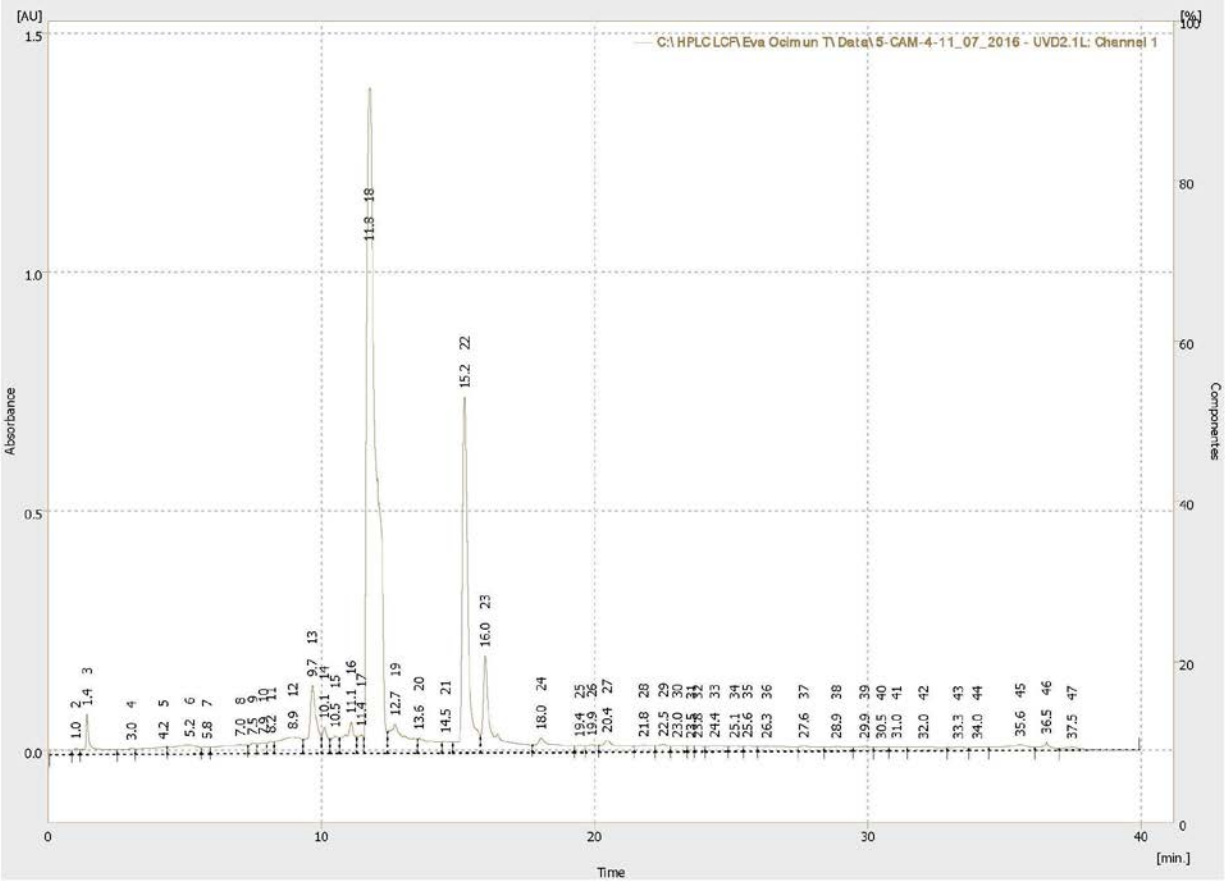

Figure 3. Chromatograms of the ethanolic extract of the leaves of *C. sempervirens*L. from Cuba

3.4. Discussion of the Pharmacological Importance of some of the metabolites determined experimentally in *C. sempervirens*L. (Cypress) of Cuban origin

Alkaloids

The alkaloids expressed in leaves and stem of Cuban *C. sempervirens*L. have pharmacological properties depending on the molecular configuration, such as: for sympatholytics and antispasmodics, local anesthetics and sources of drug addiction, they act as anticholinergics, that is, in gastric motility disorders and Muscle spasm, especially in some ulcer patients and as central depressants of motor activity, with an anthelmintic effect,

a very positive result for the present evaluation. It has vasoconstrictive effects due to its action on the sympathetic ganglia and to promote the release of vasopressin and adrenaline. In high doses, they can become toxic) [9, 10, 11]

Simple phenols

In the results in leaves and stem of *C. sempervirens*L. of the test with ferric chloride, the presence of an intense green coloration was observed, for which the possible presence of condensed tannins can be inferred.

The presence of phenols is because these compounds are closely related to the activity of repellency by plants to insect populations and since leaves are the most common target, it is logical that they are used in chemical defense. Due to their antioxidant properties, these compounds have shown beneficial effects on human health as anti-inflammatory, anti-sclerotic, and antiviral [12].

Flavonoids

The flavonoid result was positive in the hydroalcoholic extract of leaves and stem of *C. sempervirens* L. showing a phase separation with a brown color, which is indicative of flavonoids of flavones and flavonols. These metabolites have been shown to exhibit a wide range or spectrum of pharmacological and biochemical actions such as antimicrobial, antithrombotic, antimutagenic, and anti-carcinogenic activities [13, 14]. The activity of flavonoids as antioxidants depends on the redox properties of their hydroxyphenolic groups and the structural relationship between the different parts of the chemical structure. Flavonoids, in particular, exhibit a wide range of biological effects, including antiviral, anti-inflammatory, antiallergic, antioxidant, and vasodilator activity [11, 15,16].

The *C. sempervirens* L. (cypress), is another species that has great variability in the chemical composition, reported with indifference but that in summary coincide with the global

results described in the present work, which for the first time differentiates two parts (leaves-stem) of the same variety for the present analysis; both parts (stem-leaves) of the evaluated plant material have chemically active properties for the pharmaceutical use of the plant species *Cupressussempervirens,* L. ¨ciprés¨ of Cuban origin [13, 17,18].

4. CONCLUSION

*C. sempervirens*L. of Cuban origin is an antiseptic, antirheumatic, antispasmodic, astringent, anti-sudorific, diuretic, neuronal restorer, taking into account the presence of important and dissimilar chemical constituents of great pharmaceutical value. It could be used in baths, showers, massages, cosmetics, inhalations, compresses, evaporators, among others, given by the varied constituent chemical composition; both the leaves and the stem. Besides, these results serve as a guide and orientation on which secondary metabolites may be responsible for the biological effects suggested for this species and the extraction method that should be applied to them where they are expressed.

REFERENCES

1. Marcía Fuentes, J.; Montero Fernández, I.; Zumbado, H.; Lozano Sánchez, J.; Santos Alemán, R.; Navarro Alarcón, M., Bórras Linares, I.; Saravia, S. (2020).Quantification of BioactiveMolecules, Minerals and BromatologicalAnalysis in Carao (*Cassia grandis*). *Journal of Agricultural Science;*12 (3); 88-94.

2. Scherer, R.;Rybka, ACP.;Ballus, CA.;Meinhart, AD.;Filho, JT.; Godoy, HT. (2012). Validation of a HPLC method for simultaneous determination of main organic acids in fruits and juices. *Food Chemistry;* 135:150-154.

3. Ayala J.F.; Vega, V.; Rosas, C.; Palafox, C.H.; Villa, C.;Wasim, M.; Dávila, J.E. González, G.A. (2011). Agro-industrial potential of exotic fruit byproducts as a source of food additives. *FoodResearch International;* 44:1866-1874.

4. Montero Fernández, I.; Saravia, S.; Perpetua, V.; Santos, R.; Marcía Fuentes, J. &Filho, A. (2020). Chemical characterization of seeds of Amazon fruits as nutritional contribution with functional medicinal potential.*African Journal of Pharmacy and Pharmacology;* 4(14); 67-76.

5. Roig, JT. Plantas medicinales, aromáticas o venenosas de Cuba. Ed. Científico-Técnica. La Habana, Cuba. 1988.

6. Tapondjou AL, Adler C, Fontem DA, Bouda H, Reichmuth C (2005). Bioactivities of cymol and essential oils of *Cupressussempervirens* and *Eucalyptus saligna* against *Sitophiluszeamaismotschulsky* and *Triboliumconfusum* du Val. *J. Stored Prod. Res*. 41: 91-102

7. Mazari, K.; Bendimerad, N.; Bekhechi, C. Fernández, X. (2010). Chemical composition and antimicrobial activity of essential oils isolated from *Algerian Juniperusphoenicea*L. *and Cupressussempervirens*L. *Journal of Medicinal Plants Research*; 4(10); 959-964.

8. Miranda, M. & Cuéllar, A. Farmacognosia y Productos Naturales. Libro de texto. Edit. Félix Varela. La Habana, Cuba. 2001.

9. Arango, G. J. Alcaloides y compuestos nitrogenados. Tesis Doctorado en Química, Facultad de Química Farmacéutica, Universidad de Antioquia, Medellín, Colombia. 2008.

10. Hih-Hsien C. (1990). Flavonoids, coumarins and acridone alkaloids from the root bark of Citrus limonia. *Phytochemistry*, (29); 351-353

11. Islam, S. K. N.; Gray, A. Y.; Waterman, P. G.; Ahasan, M. (2002). Screening of eight alkaloids and ten flavonoids isolated from four species of the genus Boronia (*Rutaceae*)

for antimicrobial activities against seventeen clinical microbial strains. *Phytotherapy, Res*., (39); 672-674.

12. Khokhar, S.; Apenten, R. K. O. (2003). Iron binding characteristics of phenolic compounds: some tentative-structure activity relations. *Food Chem;* (81); 133- 140.

13. Montes de Oca, Y. Compuestos bíoactivos con interés farmacéutico. Tesis Máster en Química Farmacéutica, Instituto de Farmacia y Alimentos, Universidad de La Habana, Cuba. 2015.

14. Harborne, J.B., T.J. Mabry, & H. Mabry. (1975). The Flavonoids. *Chapman and Hall*; 47-61.

15. Janet, A.M., &Duthie, G. (2006).Flavonois in foods. Chemistry, Biochemistry and applications. CRC press: Boca Raton. 219-262.

16. Al-Snafi, A. (2016). Medical importance of *Cupressussempervirens* - A review. *IOSR Journal Of Pharmacy; 6* (2);66-76.

17. De Luca A, Graziani E, Anticoli S, Simeoni S, Terzano C and Mannino F. (1997). Respiratory allergy to *Cupressussempervirens* in Rome. *AllergolImmunopathol*; 25(5): 229-232.

18. Koriem, K. (2009). Lead toxicity and the protective role of *Cupressussempervirens* seeds growing in Egypt. Rev LatinoamerQuím; 37(3): 230-242.