Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1314 - 1324
Received 05 May 2021; Accepted 01 June 2021.

Chemical Composition and Antioxidant Activity of Essential Oil from the Aerial Parts of Clematis Cirrhosa L. (Ranunculaceae) Growing in Algeria

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1. Abstract

Essential oil isolated from the aerial parts of Clematis cirrhosa L., growing in Algeria, was evaluated for its chemical composition and antioxidant activities for the first time, this species is used in Algerian folk medicine. The essential oil obtained by hydrodistillation was analyzed by GC/MS; the results indicated that the OE of C. cirrhosa contained 12 compounds, of which three predominant compounds are: Phytol (30.1%), Palmitic acid (29%), Juniper camphor (14.5%). The antioxidant activities were tested by using several methods (TAC, DPPH·, ABTS·+, FRAP and CUPRAC), the OE showed a high antioxidant activity in TAC, FRAP and ABTS tests with (291.36 ± 1.82 mg AAE/g, 119.71 ± 2.24 mg TE/g, 128.91± 0.40 µg TE/mg) respectively. Whereas a moderate antioxidant activity was obtained in CUPRAC and DPPH with (IC50 =5.10 ± 0.14 mg/ml). From our results the antioxidant activity of EO C. cirrhosa was certainly due to its chemical composition.

Keywords: Clematis; Ranunculaceae; Essential oil; Antioxidant

2. Introduction

Nature is considered, with its innumerable varieties of plants, an inexhaustible treasure of abundant and varied raw materials, and since ancient times man has been able to take advantage of this treasure, whether in nutrition, healing, or other applications in his daily life. These recorded uses of plants since ancient times also apply today in different parts of the world. Despite the great development in the field of chemical treatments, herbal medicine has seen a wide comeback, especially after the announcement of some side effects and adverse consequences of increased drug use [1] . Medicinal plants synthesize a huge variety of organic compounds that exhibit broad spectrum efficacy against several ailments. These bioactive substances named secondary metabolites including tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [2] .

The Essential oils are complex mixtures of lipophilic and highly volatile secondary metabolites formed by aromatic plant, characterized by a strong odour[3] .

Numerous studies have shown that essential oil has significant potential as antibacterial, antifungal, antioxidant, antidiabetic, anticancer, antimutagenic, antiviral, anti-inflammatory and antiprotozoal agents, and that these biological properties are due to their main constituents which are terpenoids and phenylpropanoids [4] . Through their various biological activities, essential oils find their use in many fields such as: food, pharmacy, perfumery, aromatherapy and others [5] .

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Several diseases, particularly cardiovascular, neurodegenerative, cancer, diabetes and asthma, result from biomolecular oxidation caused by free radicals and other reactive oxygen species. These species produce serious molecular alterations in particular: proteins, amino acids, unsaturated lipids and DNA[6] . Free radicals are almost present in all cells; they are highly reactive chemical species that contain unpaired electrons in their outer orbit. Free radicals contribute easily to a series of oxidative reactions with practically all cellular components resulting in tissue lesions [7]. In order to avoid these cellular damages, the organism equips a defense system to extinguish these species an imbalance between the production of free radicals and their elimination by the body's antioxidant system leads to a phenomenon known as oxidative stress. In this case, intake of exogenous antioxidants is essential to get the balance between free radicals and antioxidants [8] . In this context several studies have proven that plants are considered an important source of exogenous antioxidants, and a great interest is given to these antioxidants, especially after the discovery and isolation of ascorbic acid [9].

Algeria, with its particular geographical location and its diversified climate, has been characterized by a very diversified plant wealth which is distributed throughout the Algerian territory. Indeed, Algerian endemic plants constitute an important source of medicinal plants and therefore a source of active substances that can lead to the discovery of new antioxidant agents or molecules of scientific interest [10] .

Clematis cirrhosa a Mediterranean species belongs to Ranunculaceae family, an Algerian medicinal plant used in folk medicine, where the leaf infusion was used as diuretic agent by the north-eastern population (Edough) [11] . Also the leaf decoction was used by the populaion of the high lands (Bordj Bou Arreridj) to relieve rheumatic pains [12] .

Moreover our previous work on the extracts of C.cirrhosa showed good antioxidant activities. Therefore, we encouraged in this study to attest the antioxidant activities of the essential oil of this species and to identify its chemical composition for the first time.

3. Material and method

3.1. Plant material

The plant material was collected from Souk Ahras in Algeria; the harvest was made during the flowering period in December 2017. The identification of the plant and its voucher number were given by Dr. Tarek HAMEL.Voucher number of Clematis cirrhosa L., in the herbarium of Dr. Gérard DE BELAIR, is ph012_03. The aerial parts of the harvested plant were washed, cut into small pieces, and then dried in the dark at room temperature and then ground into a fine powder using a grinder.

3.2. Extraction of essential oil

The essential oil (EO) was obtained by hydrodisstillation; 600 g of powdered plant was subjected to distillation using a Clevenger apparatus for 6 hours. At the end of this period, the

essential oil obtained is diluted with hexane. Then, the essential oil sample was dried with sodium sulfate anhydrous (Na_2SO_4) and filtered with a 0.45 µm PTFE syringe filter. The EO yield was measured at the end of the process and the results were expressed in % (w/w). The OE is collected and stored at (+4°C) until analysis.

3.3.Gas chromatography/mass spectrometry

GC/MS analysis is performed using an Agilent GC/MS system. An HP-INNOWAX column (length 60 m, inner diameter 0.25 mm, and film thickness 0.25 µm) using He as the carrier gas. The

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temperature was applied to the column very slowly and the initial temperature was maintained at 60 °C for 10 minutes. Previously, it was increased by 4 °C per minute to reach 220 °C, and after being held at this temperature for 10 minutes, it was increased by 1 °C per minute up to 240 °C. In the last cycle, the analysis was completed by maintaining this temperature for 30 minutes. Thus, the total analysis time was 110 minutes. The temperature of the injector block was set to 240 °C and the mass spectra are recorded at 70 eV [13] .

The compounds of the EO were determined on the basis of retention index (RI) implemented by the injection of the samples and the injection of the reference of a homogeneous series of n-alkanes (C_8-C_{30}), in the same experimental conditions. Supplementary identifications were made by comparing the mass spectra obtained with those of NIST 05 and the Wiley 8th version and a home-made MS library constructed from pure materials and known components of essential oils, and also comparing their retention index with the values of literature [14] .

3.4. Antioxidant activities

3.4.1. Total antioxidant capacity

The total antioxidant capacity of EO is evaluated by the determination of phosphomolybdenum according to the method of Prieto et al [15]. First, a mixture consisting of three solutions is prepared and mixed: sodium phosphate (28 mM), ammonium molybdate (4 mM) and sulfuric acid (6 M). Next, 3 ml of this mixture are added to 100 µl of plant extract. A calibration curve is established with solutions of ascorbic acid prepared at different concentrations (1, 0.5, 0.250, 0.125 mg/ml). For the blank, only 300 µl of ethanol was used. All the prepared solutions are incubated at 95 °C for 90 min in the dark, the absorbance is read against a blank at 695 nm and the results are expressed in ascorbic acid equivalents (mg AAE/g).

3.4.2. Ferric ion reducing antioxidant power (FRAP)

This method consists of measuring the reducing antioxidant power of ferric ion, which uses a complex of ferric ions of tripyridyltriazine (TPTZ) as reactants. FRAP test is based on the reduction of the ferric ion complex Fe (III) -TPTZ to the ferrous ion complex Fe (II) -TPTZ by antioxidants. This reduction is monitored by measuring the variation in absorption at 593 nm[16] . The ferric ion reduction capacity of the EO was measured by the protocol described by Aktumsek et al [17]. A mixture of three solutions were prepared with a portion (10/1/1, v/v/v) 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) solution, FeCl3 solution and acetate buffer solution, respectively. Standard solutions of Trolox were prepared at different concentrations (25, 50, 75, 100, 150, 200 mg/ml). The absorbance of all samples is measured by a spectrophotometer at 593 nm, after 30 minutes of incubation at 30 °C.

3.4.3. Cupric ion reducing antioxidant capacity (CUPRAC assay)

With slight modifications brought to the method of Apak et al. [18] , the ability of OE to reduce the cupric ion was measured, initially three reagents were prepared (ammonium acetate, copper (II) chloride dihydrade and neocuproin) and mixed with 1 ml of the extract. butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) solutions were prepared at different concentrations (250, 125, 62.5, 31.25 mg / ml). Absorbance readings were taken by spectrophotometer at 450 nm after 30 min of incubation and compared to blank samples.

3.4.4. 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity (DPPH assay)

The DPPH free radical scavenging activity was tested using Sarikurkcu method [19] . Briefly1 ml of the ethanolic DPPH solution (0.4 mM) was mixed with 1 ml of sample, after 30 min incubation in dark at room temperature, the absorbance was taken at 517 nm against a blank and control. The

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inhibition ratio of DPPH free radicals (I %) was calculated (formula I), the IC_{50} value which is the inhibitory concentration of 50% of DPPH radicals was also determined.

$$I\ \% = 100 \times (A0-A1) / A0 \qquad (I)$$

A0: absorbance of the control.

A1: absorbance of the sample.

3.4.5. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid free radical scavenging activity (ABTS assay)

The ABTS radical cation scavenging activity was tested using the procedure described by Zengin et al. [20] . In which 2 ml of ABTS solution was mixed with 1 ml of sample, after 30 min incubation in obscurity, the absorbance was taken at 734 nm. The ABTS scavenging activity was expressed as Trolox equivalents (µg TE/mg).

4. Results and discussion

4.1. GC/MS

The EO of the aerial parts of C. cirrhosa was extracted by hydrodistillation, and the obtained distillate gave yellow oil with a characteristic pungent odour, the extraction yield was 0.016% (w/w).The EO was analyzed by GC/MS and the results are reported in **Table 1**. Analysis resulted in the identification of 12 compounds representing 96.2% of this oil.

The chemical composition of EO extracted from the plant of C. cirrhosa is characterized by the presence of three major compounds; alone represent 73.6% of the chemical composition of this essence (Phytol 30.1%, Palmitic acid 29%, Juniper camphor 14.5%). Hexahydrofarnesyl acetone was also determined, as a constituent of this oil, in appreciable quantity (8.1%). The other components identified are present in low content such as thymol (2.7%), octanol (2.6%), linoleic acid methyl ester (2.5%), ethyl ester of linolenic acid (2.1%), Myristicin ether oxide (1.8%), palmitic acid ethyl ester (1.4%), palmitic acid methyl ester (0.9%) and nonanol (0.5%).

In view of these results, we note that this essence consists of (30.1%) of oxygenated diterpenes (phytol), (29%) of acid (palmitic acid), (22.6%) of oxygenated sesquiterpenes (juniper camphor, hexahydrofarnesyl acetone), (6.9%) esters (methyl ester of palmitic acid, ethyl ester of palmitic acid, methyl ester of linoleic acid, ethyl ester of linolenic acid), (3.1%) alcohols (octanol, nonanol) followed by (2.7%) oxygenated monoterpenes (thymol), and finally (1.8%) phenylpropanoids (Myristicin) **Fig.1.**

The previous study carried out by Tao et al[21], identified the chemical composition of the essential oil of the species Clematis obscura, and GC/MS analysis revealed the presence of 37 compounds, among which the main compounds are hexadecanoic acid (palmitic acid), 11,14,17-eicosatrienoic acid methyl ester, tetradecanoic acid and hexadecanoic methyl ester (palmitic acid methyl ester).

A study carried out on the essential oil of the species Clematis hexapetala, showed that the main components are palmitic acid and 3-hydroxy-4-methoxyl benzaldehyde [22]. A study carried out on the essential oil of the species Clematis hexapetala, showed that the main components are palmitic acid and 3-hydroxy-4-methoxyl benzaldehyde . The chemical composition of the essential oil of Clematis apiifolia DC. is dominated by palmitic acid, octadecanoic acid, phytol attested by (Song et al.)[23] .

Zeng et al. carried out a comparative analysis of the volatile components of the Clematis species growing in China (C.serratifolia Rehder, C.brevicaudata, C.fusca, C.hexapetala Pall. and C. Chinensis Osbec.) the results have well showed that aliphatics were the major constituent group in

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the five samples of Clematis species ranging from 64.9 to 88.4%, which were characterized by their high content of n-hexadecanoic acid (palmitic acid) and (Z, Z) -9,12-octadecadienoic acid, the amount of the first varied from 26.3 to 39.5%, and the second from 34.6 to 55.1%. Monoterpenes were relatively poor, the total amount of which was 0.3 to 6.0 %, and it was 0.3 to 5.7 % in the oxygenated monoterpenes [24] .

To our knowledge, the volatile extract of C. cirrhosa has not been the subject of any prior chemical study. According to the literature, essential oils of the species of the genus Clematis have a great variety in chemical composition. Our results agree with those described in the work previously cited above; depending on which species of the genus Clematis contain predominantly palmitic acid as a major component. The difference in composition in many researches is likely due to various conditions including, environment, geographic origin, harvest time, location of drying, temperature and drying time, and method of extraction.

4.2. Total antioxidant capacity (TAC)

The total antioxidant capacity of C.cirrhosa EO was evaluated by the phosphomolybdenum method. This is the simplest and cheapest method, which allows the assessment of both hydro and lipo-soluble antioxidants. the principle of this method is based on the reduction of molybdenum Mo(VI) into a green complex of phosphate Mo(V) by the antioxidants of the extracts [25] .This method is adopted by a large group of researchers to estimate the total antioxidant capacity of plant extracts and is considered to be one of the most authentic methods used[26] .

The antioxidant capacity of our samples was determined based on the absorbance values of the solutions using the regression equation of the calibration curve of ascorbic acid obtained at different concentrations (50, 100, 200, 300, 400 and 500 µg/ml).The values of the total antioxidant capacity are expressed in mg ascorbic acid equivalents per g of EO and the results obtained are collated in Table 2. It is clear from the results that the EO of C. cirrhosa has a high total antioxidant activity (291.36 ± 1.82 mg AAE/g). It is evident that this activity results from the antioxidant effect of the compounds of this oil, as GC/MS analysis confirmed that the essential oil of C.cirrhosa contains compounds such as Phytol (30.1%), Juniper camphor (14.5%), Hexahydrofarnesyl acetone (8.1%), Thymol (2.7%), these compounds have might act a leading antioxidant agent as described in previous studies [27] , Moreover Lahlou et al. have attested that the activity of essential oils is often due to the activity of their main compounds, or of those likely to be active. However, minority compounds too can act synergistically[28] .

4.3. Ferric reducing antioxidant power

According to the results showed in **Table 2**, the EO of C. cirrhosa have an appreciable iron reducing capacity (119.71 ± 2.24 mg TE/g) these results indicating that certain EO compounds of this plant are donors of electrons and could react with free radicals to convert them into more stable products and to end free radical chain reactions. As we mentioned previously that the antioxidant activity of EO is linked to their chemical composition such as the major compounds. However, it is not only the latter that are responsible for this activity, but there may also be other minority compounds that can interact in a synergistic or antagonistic way to create an efficient system against free radicals, even for those present in small amounts [28]. Oxygenated terpenes have important and viable biological activities compared to non-oxygenated terpenes[29] . In this study, the majority of compounds are oxygenated terpenes (55.3%), which may explain the antioxidant activity of this EO.

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4.4. Cupric reducing antioxidant capacity

In this test, the antioxidants present in the sample will reduce the copper ions by a transfer of electrons which gives an orange-yellow complex of copper (I) neocuproine which is characterized by a maximum absorbance at 450 nm. Figure 2 shows the sample absorbance at different concentrations [30]. From the graph it is evident that with the increase in the concentration of the samples there is an increase in the absorbance, and consequently an increase in the reducing capacity of copper. Examination of these results, show that EO of C. cirrhosa showed a low copper reducing power. Despite the reducing activity recorded by EOs of C. cirrhosa, it remains far from being compared to that of BHA and BHT.

4.5. ABTS free radical scavenging capacity

The ability of C.cirrhosa EO to scavenge ABTS radicals was evaluated in this work, the radical cation ABTS•+ was reduced to ABTS+ by the antioxidants which serve to provide the H• which leads to a discoloration of the solution and consequently a decrease in absorbance measured at 734 nm. The recorded values were compared with the linear equation of the Trolox standard (25-200 µg/ml) and expressed in terms of Trolox equivalent (mg/g). The results obtained are shown in **Table 2.**

The results revealed that the samples exhibited good anti-free radical activity with (128.91± 0.40 µg TE/mg), which is an appreciable value due to the composition of EO characterized by the abundance of oxygenated sesquiterpenes (22.6%) (juniper camphor, hexahydrofarnesyl acetone) having in their structure free hydroxyl groups, which have been documented because of their important role in increasing the capacities antioxidants due to the high donation of hydrogen atoms[31]

Furthermore, it has been shown in the literature that the activity of an essential oil results from a complex interaction between its different constituents, capable of producing synergistic or antagonistic effects, even for those present in small quantities [32], In the same context, we can also link the antioxidant activity of our EO to the presence of minority components which have been shown to be effective in antioxidant activity, for example phytol and thymol [33] .

4.6 DPPH free radical scavenging capacity

The anti-free radical activity of C.cirrhosa EO was evaluated by the DPPH method, the most common method for determining the antioxidant power of plant extracts, due to its simplicity and lower cost. In this test, the reduction of free radicals by hydrogen donors present in plant extracts conduct to the decolorization of the solution and the decrease in absorbance which is measured at 517 nm [34] .

In this work the scavenging capacity of free radicals DPPH is evaluated by determining the value of the maximum inhibitory concentration (IC_{50}) of a sample while, a low IC50 refers to a high antioxidant capacity. The results of the DPPH free radical scavenging capacity of extracts and EO of C. cirrhosa are shown in **Table 2**.

The results showed that the essential oil of C. cirrhosa has a very low DPPH radical scavenging activity with (IC_{50} =5.10 ± 0.14 mg/ml), this is due, according to our estimate, to the reduced level of phenolic components present in this oil. Our results differ from those described by Mushtaq et al [35] according to which the EOs of Clematis graveolens leaves and stems show good anti-free radical activity with an IC_{50} value (31.93 ± 0.01 µg/ml) for the stems, and (15.83 ± 0.02 µg/ml) for the leaves.

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5. Conclusion

Volatile oils are compounds of medicinal plants with high biological potency used in several fields.

Compounds	RI*	Abundance (%)
Octanol	1556	2.6
Nonanol	1658	0.5
Hexahydrofarnesyl acetone	2134	8.1
Thymol	2196	2.7
Hexadecanoic acid methyl ester	2222	0.9
Hexadecanoic acid ethyl ester	2256	1.4
Juniper Camphor	2260	14.5
Myristicin ether oxyde	2291	1.8
9,12-Octadecadienoic acid ethyl ester	2534	2.5
9,12,15-Octadecatrienoic acid ethyl ester	2605	2.1
Phytol	2618	30.1
n-Hexadecanoic acid (Palmitic Acid)	2912	29.0
Total		**96.2**

Therefore, and in view of the results obtained in our previous carried out on the medicinal plant Clematis cirrhosa L., we decided on this work to study the chemical composition of volatile oils of this species and to evaluate their antioxidant activity. The results of this work proved that the volatile oil of the studied plant have high antioxidant activity and this is due to its chemical composition. This justifies the use of this plant in traditional medicine. Further studies should be done on this plant in order to find its application on several fields.

Table 1.

Chemical composition of the essential oil from *Clematis cirrhosa* L.

*RI : Kovats retention index (calculated on the basis of n-alkanes)

Table 2.

Antioxidant activities of the essential oil from Clematis cirrhosa L.

Sample	TAC (mg AAE/g)	FRAP (mg TE/g)	ABTS (µg TE/mg)	DPPH IC_{50} (mg/ml)
Essential oils	291.36±1.82	119.71±2.24	128.91±0.40	5.10±0.14

All values are expressed mean ± SD.

AAE: Ascorbic acid equivalents, TE: Trolox equivalents.

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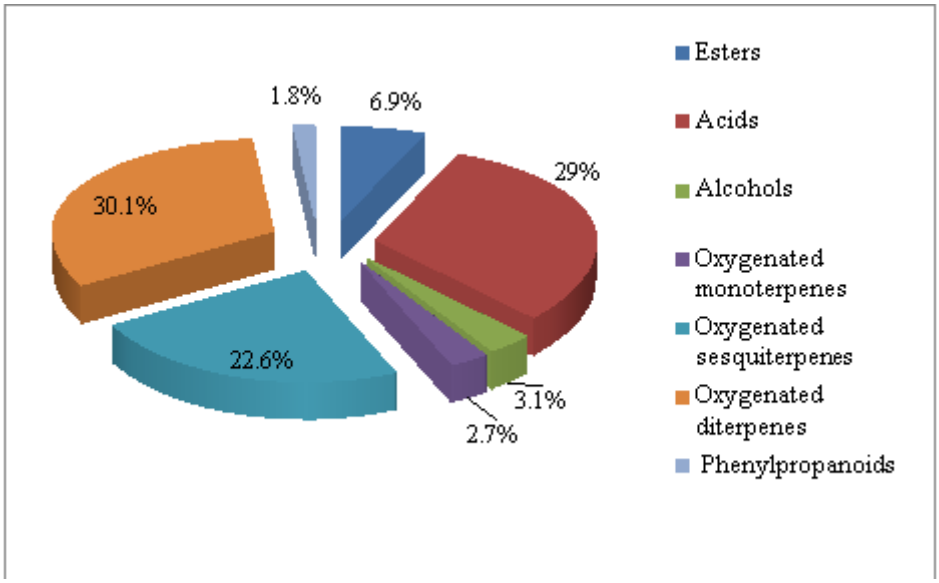

Fig.1. Distribution of essential oil from Clematis cirrhosa L.

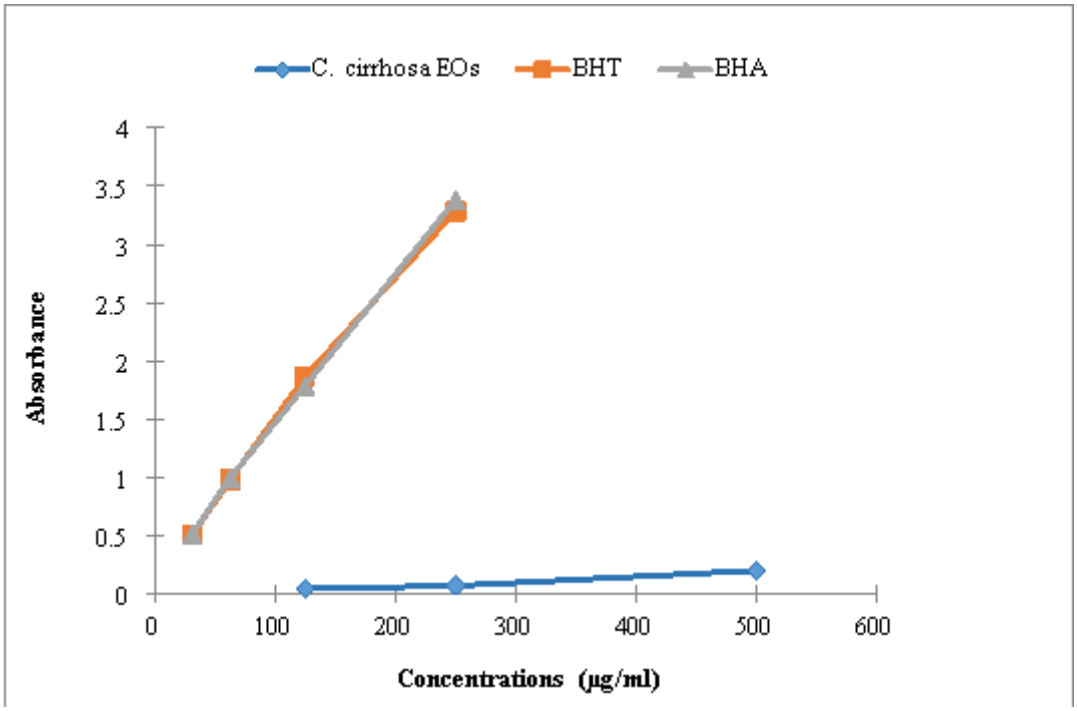

Fig.2. Cupric ion reducing power of the essential oils from Clematis cirrhosa L.

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