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Antioxidant Potential (Peroxynitrite scavenging, Singlet oxygen scavenging, and Hypochlorous acid scavenging) and Anti-Inflammatory Effect of *Apium graveolens* and Investigation of Its Bioactive Natural Compound Using FTIR Analytical Technique

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Abstract:- The objective of our study is to conduct a chemical analysis of Apium graveolens and to assess the anti-inflammatory and antioxidant capabilities of several extracts, including methanol, ethyl acetate, ethanol, hexan, and water. To get ready for FTIR analysis, a tiny quantity of powdered samples was dissolved in KBr and then compressed into a thin film. A wave number range spanning from 4000 cm−1 to 500 cm −1 was used to gather data on the transmittance of infrared light. The albino rats used in the studies ranged in weight from 175 to 225 gram. The animal breeding house was the source for the animals. The animals in group I were utilized as a control group and were given maize oil as a vehicle. 100 mg/kg Di-(2- ethylhexyl) phthalate were the positive controls in all trials involving Apium graveolens methanol fraction. Group 1 consisted of Di-(2- ethylhexyl) phthalate at a concentration of 100 mg/kg, Groups 2, and 3 of fractions of Apium graveolens at concentrations of 0.50 mL/kg, and 0.75 mL/kg, respectively. Di(2-ethylhexyl)phthalate causes chronic hepatotoxicity in rats. Hence, we find that taking di(2-ethylhexyl) phthalate orally (1000 mg/kg body weight/day) as laboratory tested for 4 weeks in rats resulted in a noticeable and significant increase in the levels of serum enzymes such as (SGOT), (SGPT), and (ALP). Hence, we found that taking A. graveolens seed extract (250 mg/kg.w./day) for 4 weeks leads to a significant and significant recovery of these biochemical parameters towards the normal stable state compared to di(2-ethylhexyl) phthalate-treated rats and their control. The results of this study lend credence to the traditional medicinal usage of Apium graveolens, which has been found to have anti-inflammatory and antioxidant properties. .

Keyword:- Peroxynitrite, Singlet oxygen, Hypochlorous acid scavenging, Apium graveolens, Natural Compound, FTIR Technique.

Supplementary information The online version of this article (https://doi.org/xx.xxx/xxx.xx) contains supplementary material,which is available to authorized users.

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Introduction

From ancient times, people have employed medicinal plants to treat common ailments. Different components of the plants were used for public health purposes [1, 2]. Natural remedies have a lower price tag. Plants have long been recognised for their analgesic and analgesic-like effects; however, modern research has been primarily focused on their healing characteristics and their potential to treat a wide range of ailments. Herbs and medicinal plants have been demonstrated in

numerous trials to alleviate a variety of medical conditions, including infertility, hormonal imbalances, liver problems, anaemia, kidney illness, and neurological and psychological disorders [3]. The antioxidant effects of flavonoids and other phenolic chemicals, which are abundant in plants, have been the subject of extensive research into a variety of medical conditions, including cancer, coronary heart disease, diabetes, and cardiovascular disease [4]. The main reason herbal medications are utilised instead of chemical ones nowadays is because they have less adverse effects. The Apium graveolens L. family counts celery, an annual or perennial plant native to Asia, Europe, and the tropics and subtropics of both Africa and Asia, among its most important members [5]. Celery, as it is known, is the most widely used medicinal plant in traditional medicine because it contains many well-known and important substances, including limonene, selenine, frucoumarin glycosides, flavonoids, and vitamins A and C. There are many heart diseases, liver disease, gout, and urinary tract obstruction, so this celery can help you avoid them. Rat studies have shown that ethanol extracts of celery leaves enhance fertility and spermatogenesis. One way celery can help the heart is by lowering blood sugar, cholesterol, and hypertension. Scientific investigations have demonstrated that celery possesses anti-inflammatory and antifungal characteristics. The antimicrobial properties of its essential oils are an added bonus [6]. The seeds of this plant have medicinal uses in the treatment of a variety of conditions, including bronchitis, asthma, chronic skin illnesses (such as psoriasis), nausea, vomiting, fever, and tumours.

A diuretic, celery root is a common remedy for infant colic. The natural active compounds found in plants can differ in their action mechanisms and biological characteristics, making them a rich source of diversity. Polyphenols, which include flavonoids, phenolic acids, and tansipropanoids, are among the several phytochemical substances that plants use as antioxidants and for collecting free radicals [7]. Polyphenols impact living things. Free radicals and peroxidation can be inhibited by these effects, particularly the antioxidant actions. The chemical characteristics of polyphenols are often quite similar; this means that a number of the phenolic groups can neutralise free radicals by reacting with hydrogen donors. Research on the antioxidant properties of celery is extensive [8]. Numerous researchers have examined the phenolic and antioxidant components found in celery. As a protective measure, celery can neutralise free radicals such OH and DPPH (2,2-diphenyl-1-picrylhydrazyl), and it can also lessen the severity of liposomal peroxidation.

Therefore, this study set out to use FTIR analysis to determine which Apium graveolens extracts had the greatest anti-inflammatory effect in vivo and which had the best antioxidant properties in vitro, as well as to discover whether there was a correlation between the antioxidant activity and the identified constituents.

Materials and Methods

Extract Preparation and Plant Collecting

The city of Al-Hillah in the Babil Governorate of Iraq was the site of the Apium graveolens collection. After a 30-hour period of drying in the shade, they were dissolved in methanol and used in a soxhlet extraction. Under controlled conditions of temperature (40°C) and lowered pressure, the filtrate was further concentrated until it was dry. The resulting brown viscous material was dissolved in distilled water and utilised in the experiment.

FTIR analysis of *Apium graveolens* methanol fraction

To acquire the FTIR spectra of native and defatted GLVs, a Bruker, Germany-based FTIR equipment (Model/Make: IFS 25, Bruker, Germany) was used, with data processed using PC-based software. A little amount of powdered leaf samples was dissolved in KBr and compacted into a thin film in preparation for FTIR analysis. A wave number range spanning from 4000 cm−1 to 500 cm−1 was used to gather data on the transmittance of infrared light. Untreated KBr pellets served as a control, and all samples were subjected to three separate analyses [9]. The functional groups found in the sample were determined by comparing the spectral data to a reference.

Animals and drug administration

The albino rats used in the studies ranged in weight from 175 to 225 grammes. The animal breeding house was the source for the animals. The rats were kept in cages with good ventilation and kept in a typical environment with a temperature range of 23±3°C, relative humidity of 55-70%, and a 12-hour light/dark cycle. They were also given regular rat food. Twelve rats were distributed among three groups of four. The animals in group I were utilised as a control group and were given maize oil as a vehicle. In all assays including the Apium graveolens methanol fraction, 100 mg/kg of Di-(2-

ethylhexyl) phthalate served as the positive control. Di-(2- ethylhexyl) phthalate (100 mg/kg) was in Group 1, while fractions of Apium graveolens (0.5 mL/kg) and 0.75 mL/kg, respectively, made up Groups 2 and 3.

In *vitro* antioxidant assay

Peroxynitrite scavenging

While placed on an ice bath for 1 second, 5 millilitres of 0.6 M KNO_2 and 5 millilitres of ice-cold water were added to 5 millilitres of 0.7 M H 2 O2 acidic solution. 12.2 M NaOH was introduced. We eliminated the surplus H2O2 after treating the reaction mixture with granular MnO2 that had been prewashed with 1.2 M NaOH. After that, it was allowed to sit at -20°C for the night. Spectrophotometry was used to measure the concentration at 302 nm ($\varepsilon = 1670\ M^{-1}\ cm^{-1}$) following the recovery of the peroxynitrite solution from the surface of the frozen mixture. The peroxynitrite scavenging activity was evaluated using an Evans Blue bleaching assay. A significantly modified version of a common method was used to conduct the assay [8]. In a final volume of 1 ml, the reaction mixture included the following components: 50 mM phosphate buffer (pH 7.4), 0.1 mM DTPA, 90 mM NaCl, 5 mM KCl, 12.5 µM Evans Blue, dosages ranging from 0 to 200 µg/ml of plant extract, and 1 mM peroxynitrite. In practice, a reading of 611 nm was taken after a calculated time of approximately 30 minutes of incubation at a temperature of 25 degrees Celsius. After we compared the results of this test and the laboratory blank sample, we were actually able to calculate the percentage of ONOO scavenged laboratory. Each reality test was run three times. At the same time, the material that was used practically as a reference was gallic acid.

Singlet oxygen scavenging

In the laboratory, the previously published spectroscopic approach was experimentally used by [9]. The single oxygen (O2) generation process was already evaluated by monitoring the N,N-dimethyl-4-nitrosoaniline (RNO) bleaching process. RNO bleaching was observed at 440 nm, and at the same time singlet oxygen was produced through a validated process involving NaOCl and H2O2. There were 45 mM phosphate buffer (pH 7.1), 50 mM NaOCl, 50 mM H2O2, 50 mM histidine, 10 µM RNO, and various known concentrations of sample (0-200 µg/ml) were present in the final volume. Of 2 ml of the reaction mixture used. Incubation for 40 min at 30 °C was followed by 440 nm measurement of decreased RNO absorption [10]. As a reference laboratory chemical, lipoic acid has already been used to compare scavenging activity within a single sample. Each test was run four times.

Hypochlorous acid scavenging

Here in the laboratory the pH of a 10% (v/v) solution of NaOCl has already been adjusted to 6.2 with 0.6 M H 2 SO4 immediately before the laboratory experiment in order to make hypochlorous acid (HOCl). The reduction in catalase absorbance at 404 nm was used to measure the scavenging activity. In a final volume of 1 ml, the reaction mixture included 50 mM phosphate buffer (pH 6.8), 7.2 µM catalase, 8.4 mM HOCl, and varying amounts of plant extract (0-100 µg/ml) [11]. After 20 minutes of incubation at 25°C, the absorbance was measured by comparing it to a suitable blank. Each test was run six times. The reference material utilised was ascorbic acid, which is an effective HOCl scavenger.

Statistical analysis

The statistical analysis was carried out using the Windows version of Graph Pad Prism, which is 5.01. We used one-way analysis of variance (ANOVA) followed by Dunnet's test to analyse all of the in vitro results, which were expressed as mean±standard deviation. The mean±SEM of six experiments was used to present the pharmacological results. We used paired Student's t-test to compare the values. A p-value of less than 0.05 was deemed statistically significant across the board.

Results and Discussion:

The most crucial factors, measured in wave number cm^{-1}, were the FT-IR peak values: 667.37 (alkyl halides), 873.75 (Alkenes), 921.97 (Alkenes), 1026.13 (alkyl halides), 1139.93 (alkyl halides), 1234.44 (alkyl halides), 1317.38 (alkyl halides), 1379.10 (alkyl halides), 1415.75 (Aromatic), 1519.91 (Aromatic), 1598.99 (Aromatic), 1740.72 (Aldehyde), 2852.72 (Alkane), 2922.16 (Alkane), 3223.05 (Amide), and 3265.49 (Amide). Figures 2, 3, and 4 display the free radical scavenging activity, namely peroxynitrite, singlet oxygen, and hypochlorous acid, of several Apium graveolens extracts. This activity demonstrates the antioxidant potential of the Apium graveolens methanol fractions in vitro.

A variety of extract types were documented, including crude, ethyl acetate fraction, ethanol fraction, hexane fraction, water fraction, and standard recorded 745.87 ± 29.21, 755.99 ± 30.00, 755.99 ± 30.00, 749.03 ± 29.34, 710.29 ± 27.12 and Gallic acid (standard) 876.24 ± 34.81 respectively Potential for scavenging peroxynitrite. A much higher proportion of peroxynitrite scavenging activity ($P<0.05$) is inhibited by crude and other fractions when compared to standard, normal Gallic acid, according to peroxynitrite scavenging activity Figure 2. While recorded 53.18 ± 4.39, 52.70 ± 4.28, 54.16 ± 4.38, 50.76 ± 4.364, 53.68 ± 4.39, and Lipoic acid (standard) 41.26 ± 1.13 respectively Singlet oxygen scavenging potential. The results for the standard Lipoic acid were considerably lower ($P<0.05$) compared to the percentage inhibitions of crude and other fractions against Superoxide radical scavenging activities, as shown in Figure 3. At the same time record 109.43 ± 5.37, 116.94 ± 6.01, 114.32 ± 5.90, 129.93 ± 5.38, 137.05 ± 6.21 and Ascorbic acid (standard) 210.06 ± 8.07 respectively Hypochlorous acid scavenging potential. In comparison to the standard Ascorbic acid, the crude and other fractions showed significantly larger percentage inhibitions ($P<0.05$) against Hypochlorous acid scavenging activities, as shown in Figure 4.

The Impact of Apium graveolens extract on rat serum enzymes when given orally

Experiments were conducted in a controlled environment to determine the effects of oral administration of Apium graveolens extract on serum enzymes SGPT, SGOT, and ALP in rats. The results showed that SGPT recorded 68.82±3.74, SGOT recorded 81.61±3.37, and ALP recorded 15.78±1.03 for Apium graveolens extract, respectively. While 120.69±5.83, 146.73±7.34 and 28.22±1.45 were recorded respectively for using Di-(2- ethylhexyl) phthalate and 43.42± 3.66, 62.93± 2.33 and 12.60±0.93 were recorded to Control (vehicle) (0.5 ml/kg Corn oil). After 4 weeks of oral administration of an extract from A. graveolens seeds (250 mg/kgb.wt./day), these biochemical parameters show a marked improvement towards normalcy when compared to the control group and rats treated with Di-(2- ethylhexyl) phthalate.

There was a phytosterol in celery that had anti-inflammatory properties, but researchers eventually determined that unknown polar compounds were responsible for the herb's main anti-inflammatory impact [12, 13]. Celery also contains mannitol, but unlike the anti-inflammatory celery fraction, this molecule had no effect on carrageenan-induced oedema in rats. There is evidence that mannitol can alleviate inflammation in rats with adjuvant-induced arthritis [14]. The study's findings support celery stem's reputation as a therapeutic remedy for rheumatic disease, suggesting that it may have anti-inflammatory qualities. The abundance of phenolic chemicals in the Apium graveolens extracts was the primary reason for their strong antioxidant activity. Since these polyphenols include hydroxyl groups and conjugated ring structures, they may be able to scavenge free radicals [15], lipid peroxy radicals, and stabilise free radicals that are implicated in these oxidative processes in vitro. Previous research has shown that these secondary metabolites can lower blood pressure through multiple mechanisms, including endothelium-dependent vasodilation (ECD) production, inhibition of angiotensin converting enzyme (ACE) activity, and a decreased oxidative state due to their antioxidant properties [16, 17]. However, polyphenols' antihypertensive effect remains a mystery for the time being. Natural therapies using polyphenols for blood pressure lowering impact can be developed with a solid grasp of these mechanisms.

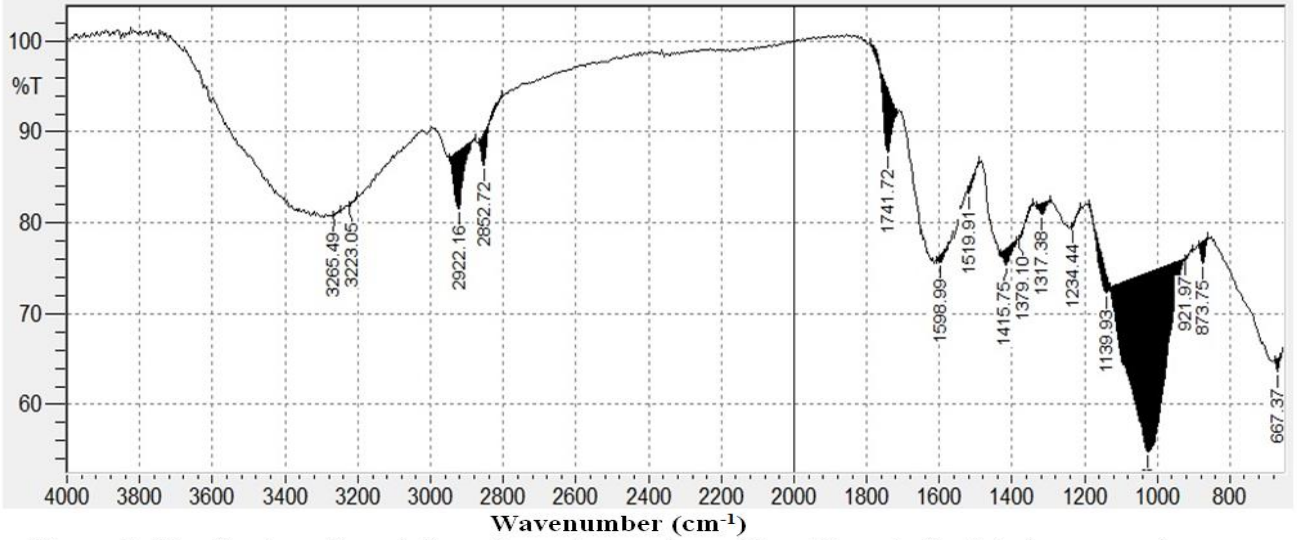

Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Apium graveolens*

Table 1. FT-IR peak values of solid analysis of methanolic extract of *Apium graveolens*.

No.	Peak (Wave number cm-1)	Intensity	Corr. Intensity	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	667.37	63.602	1.458	2.947	0.064	Strong	C-Cl	Stretch	alkyl halides	600–800
2.	873.75	75.464	2.486	2.864	0.161	Strong	=C–H	Bending	Alkenes	650-1000
3.	921.97	76.037	0.115	2.459	0.006	Strong	=C–H	Bending	Alkenes	650-1000
4.	1026.13	54.832	19.670	40.600	14.196	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1139.93	72.360	1.874	6.633	0.381	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1234.44	79.518	0.208	2.346	0.015	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1317.38	80.927	1.224	3.234	0.116	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1379.10	78.194	0.230	3.677	0.026	Strong	C-F	Stretch	alkyl halides	1000-1400
9.	1415.75	75.384	1.784	4.948	0.223	Medium	C=C	Stretch	Aromatic	1400-1600
10.	1519.91	83.275	0.580	2.191	0.065	Medium	C=C	Stretch	Aromatic	1400-1600
11.	1598.99	75.718	0.635	3.642	0.085	Medium	C=C	Stretch	Aromatic	1400-1600
12.	1740.72	87.747	6.874	2.000	0.743	Strong	C=O	Stretch	Aldehyde	1720-1740
13.	2852.72	86.395	3.584	2.649	0.244	Strong	C-H	Stretch	Alkane	2850-3000
14.	2922.16	81.542	6.382	4.668	0.893	Strong	C-H	Stretch	Alkane	2850-3000
15.	3223.05	81.889	0.120	1.955	0.016	Bending	N-H	Stretch	Amide	3100-3500
16.	3265.49	80.746	0.173	1.934	0.010	Bending	N-H	Stretch	Amide	3100-3500

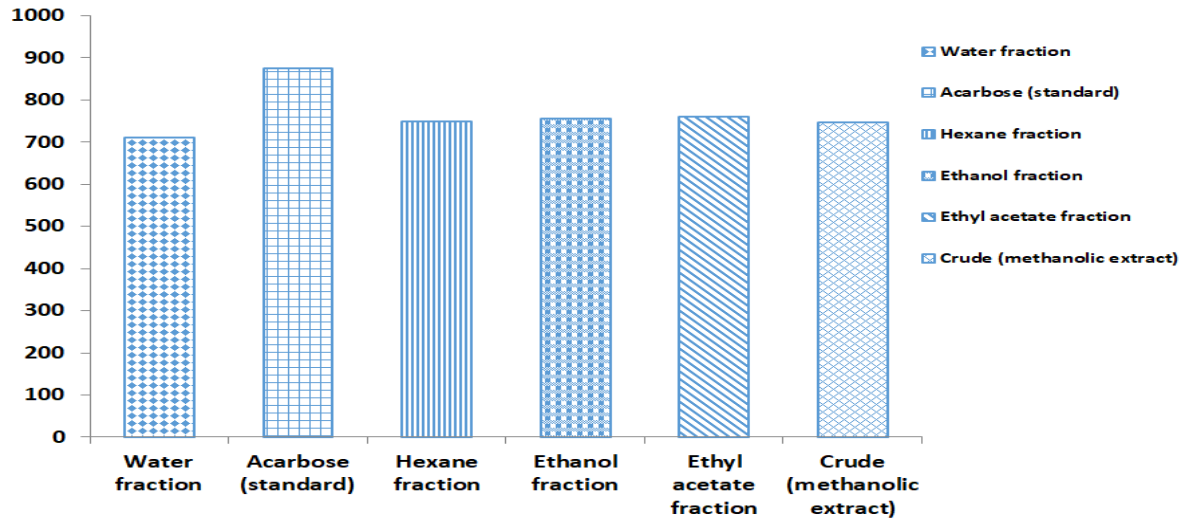

Figure 2. Radical scavenging activities of Peroxynitrite scavenging of *Apium graveolens* crude extract and fractions compared with Gallic acid (Standard)

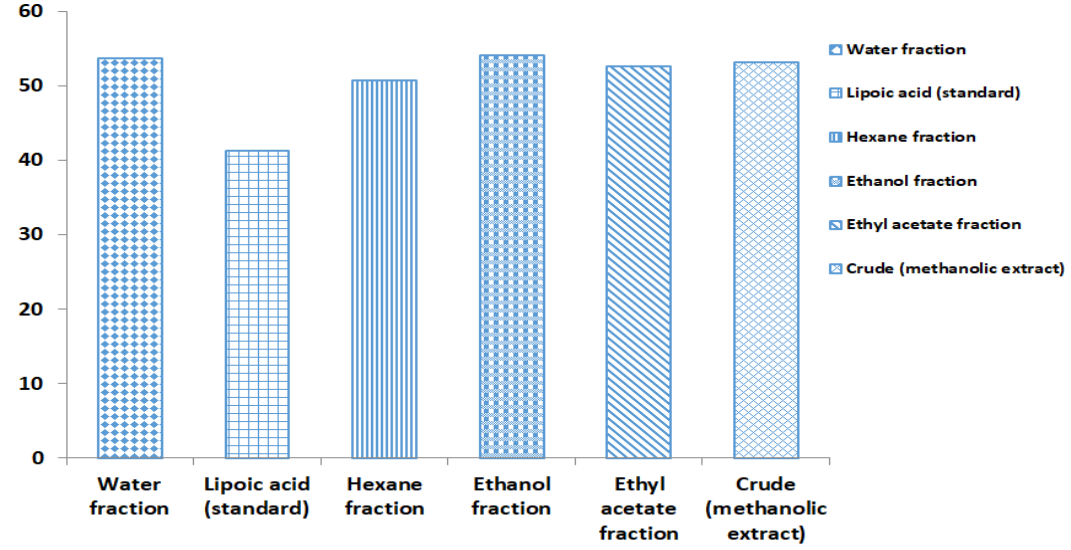

Figure 3. Radical scavenging activities of Singlet oxygen scavenging of *Apium graveolens* crude extract and fractions compared with Lipoic acid (standard)

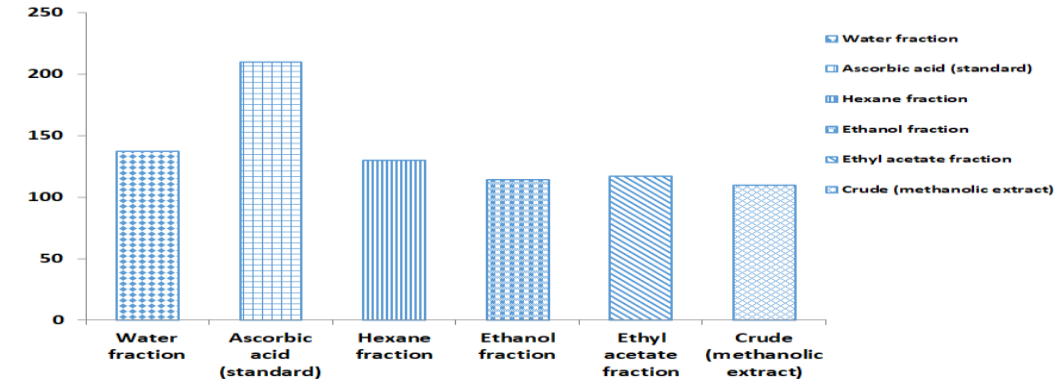

Figure 4. Radical scavenging activities of Hypochlorous acid scavenging of *Apium graveolens* crude extract and fractions compared with Ascorbic acid (standard)

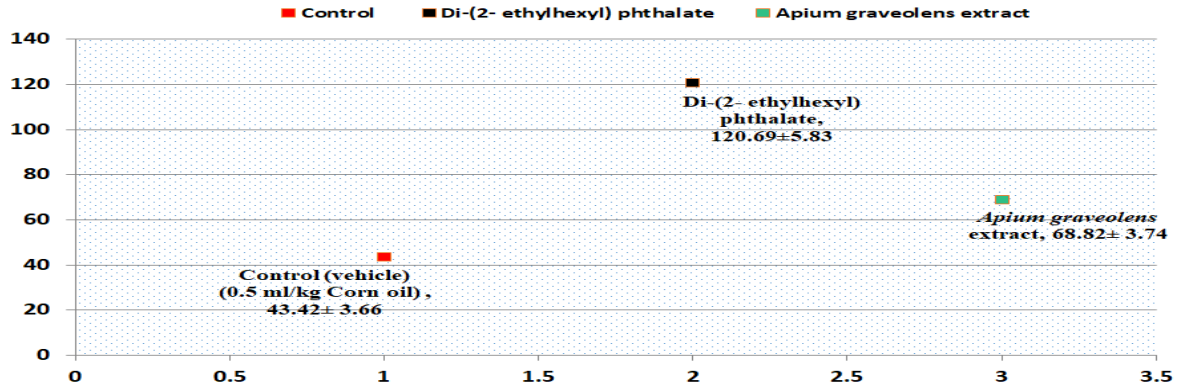

Figure 5. Effect of oral administration of bioactive secondary metabolites of *Apium graveolens* extract and Di-(2- ethylhexyl) phthalate on serum glutamate-pyruvate transaminase enzyme

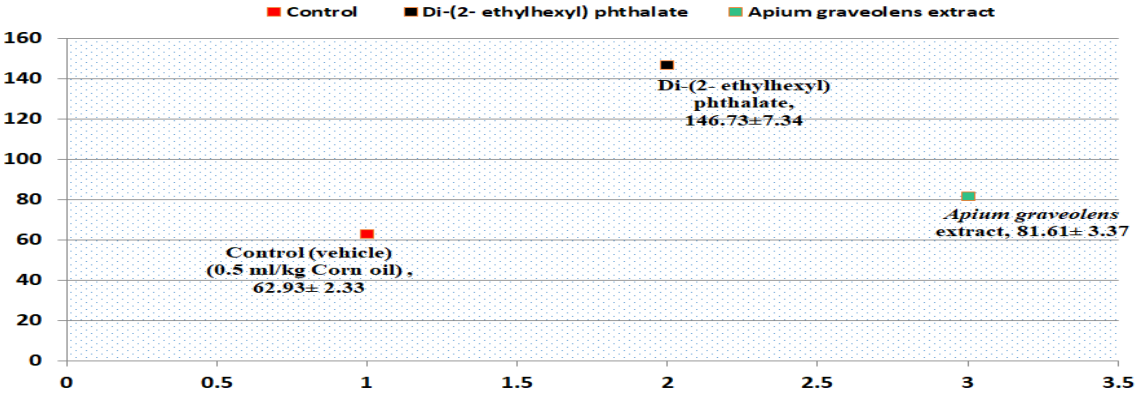

Figure 6. Effect of oral administration of bioactive secondary metabolites of *Apium graveolens* extract and Di-(2- ethylhexyl) phthalate on serum glutamate-oxaloacetate transaminase enzyme

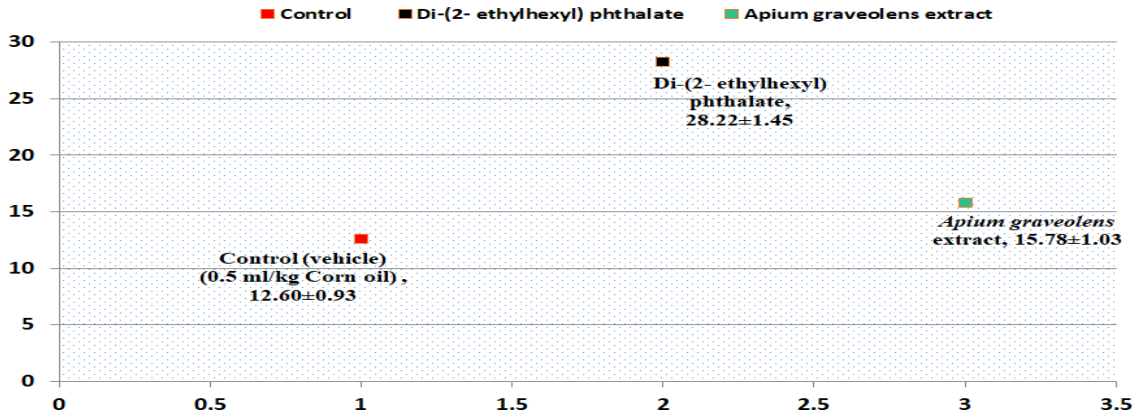

Figure 7. Effect of oral administration of bioactive secondary metabolites of *Apium graveolens* extract and Di-(2- ethylhexyl) phthalate on serum Alkaline phosphatase enzyme

Conclusion

Our results show that various Apium graveolens extracts have strong antioxidant and anti-inflammatory properties. Polyphenolic chemicals, which may have numerous uses in the treatment of disorders associated with oxidative stress, are likely responsible for this antioxidant capacity. In addition, this work provides a rational foundation for understanding the medicinal herb Apium graveolens's usage in treatment by showing, for the first time, that methanol and ethyl acetate extracts of the plant had a dose-dependent effect on decreasing blood pressure in rats. This study lays the framework for future research into the molecular mechanisms that underlie the extracts' biological profile, with the goals of isolating and purifying the extracts' more active principles and understanding how they work.

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