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RESEARCH ARTICLE

Investigation and Characterization of Alkaloidic Compounds from *Apium graveolens* and Estimation of their Antibacterial Activity

Jumana Najem Ahmed[1], Tahreer Fadil Abbas[2,*], and Abbas Dawwas Matter Al-Maliki[3]

ABSTRACT

***Apium graveolens* is regarded as a traditional food plant that is found around the world and has good therapeutic efficacy against a variety of ailments. Three alkaloidic compounds were isolated and separated. And characterized by using gas chromatography-mass spectroscopy analysis. Also, the mixture of their active chemical compounds was tested qualitatively using the Dragendroff reagent. Various concentrations represented by 0, 25, 35, 50, and 65 mg/ml were prepared from the alkaloidic mixture. They recorded different inhibition diameter values equal to 3, 13, 21, 23, and 25 mm against the growth of *Staphylococcus* bacteria. At the same concentrations, however, the inhibition values against *Bacillus subtillus* bacteria were 11, 21, 23, 25, and 27 mm. It was noticed that the five alkaloidic compounds mixture was more effective against *Bacillus subtillus* than *Staphylococcus aureus* bacteria. The active alkaloidic can, therefore, be applied as a powerful natural treatment for a variety of illnesses brought on by these harmful bacteria.**

Keywords: Active alkaloids, *Apium graveolens*, *Bacillus subtillus*, Synergistic interaction.

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[1] *Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Iraq.*
[2] *Basrah Education Directorate, Iraq.*
[3] *Department of Chemistry, College of Education for Pure Sciences, University of Basrah, Iraq.*

* ***Corresponding Author:***
e-mail: noor.a.ali.748209@gmail.com

1. Introduction

Their clinical and biochemical capacity to treat a wide range of infections and inflammations makes the health and biochemical significance of many traditional and medicinal plants evident. *Apium graveolens* is a traditional, healthy, and medicinal plant spreading in different areas in the world, and it belongs to Apiaceae family. It was carried out in ancient times as a natural therapy for diverse diseases infecting the various organs in the human being body [1], [2]. Chemically, pre-studies proved the presence of multi-active chemical compounds such as phenolics, tannins, alkaloids, terpenes, flavonoids, glycosides, essential oils, xanthenes, and coumarins in the different parts of the celery plant, especially seeds, leaves, and flowers. Additionally, these potent chemical metabolites were successfully used to counteract the biological activity of several harmful microorganisms, including fungus, bacteria, and parasites. The active chemical compounds isolated from the stem of celery were used for treatment of rheumatism, urinary treats inflammation, gout, and arthritis [3], [4]. Metabolic extract to *Apium graveolens* seeds has diverse, effective chemicals represented by steroids, alkaloids, glycosides, and flavonoids [5]. Other studies ensured the existence of phenolics, sesquiterpenes, furocoumarins, and essential oils, and these chemical metabolites were carried out against various diseases and infections [6]. Previous research has indicated the presence of antimicrobial efficacy, ant-parasitic, gastroprotective, neuroprotective, cardioprotective, hypolipidemic, antioxidant, anti-inflammatory, and anti-infertility [7], [8]. Plant alkaloids are active chemical metabolites that are anabolized biochemically via two various pathways represented by amino acids and amines in the presence of required enzymes in all steps of biosynthesis of these chemical compounds [9], [10]. The current investigation aimed on the identification, characterization, and evaluation of the celery plant's alkaloids' antibacterial activity.

2. Materials and Methods

2.1. Preparation of Cold Aqueous Extract

The seeds of *Apium gravolens*, a plant that grows celery, were purchased from the Abu Al-khaseeb market in Basrah, Iraq. Following a thorough cleaning, the seeds were ground into a powder and stored in dark containers until the day of the experiment. A botanical scientist from the biology department of the University of Basrah's College of Education for Pure Sciences in Iraq taxonomized celery. A conical flask containing 25 g of *Apium gravolens* seeds was well blended after being treated with 500 ml of distilled water. After eight hours of stirring at room temperature with a magnetic stirrer and a magnetic bar, the precipitate was extracted by filtering the mixture's contents through a Buchner funnel. After being collected and dried, the filtrate was used to make solid crude [11] with a weight equal to 3.75 g.

2.2. Qualitative Detection of Alkaloids

A solution containing 50 mg of cold aqueous extract of *Apium gravolens* and 1 ml of distilled water was treated with 2 ml of Dragendroff reagent. After giving the mixture a good shake and allowing it to settle, the orange precipitate formed [12].

2.3. Isolation of Alkaloidic Compounds from Celery

Apuim graveolens seed powder weighing 50 g was treated with 500 ml of ethanolic acetic (10% v/v) and thoroughly mixed in a conical flask. Using a magnetic bar, the mixture was agitated for ten hours on a magnetic stirrer. The precipitate was removed after the contents were filtered through a Buchner funnel. After obtaining the filtrate and concentrating it to 25% of its original volume, 5 ml of concentrated sulfuric acid was added to the filter solution and thoroughly shaken. The pH was then brought down to 9 by adding 12 drops of ammonium hydroxide. After thoroughly shaking the contents of the separating funnel and adding 20 ml of chloroform, two layers were generated as part of the extraction process. The organic layer was collected after they were separated from the aqueous layer. The organic layer was then gathered, dried, and formed into a solid crude oil after the aqueous layer was treated twice with 20 ml of chloroform [13], with a production of 2.78 g.

2.4. Separation and Characterization of Celery Alkaloids

Alkaloidic chemicals extracted from *Apium graveolens* seeds were separated and spectrophotometrically analyzed using the Agilent Technologies, Inc., 7890 A-GS system-US gas chromatography mass spectroscopy technique.

2.5. Isolates of Pathogenic Bacteria

Two pathogenic bacteria stains were used in this research, which were represented by *Staphylococcus aureus* and *Bacillus subtillus*. These pathogens were identified by a special microbiologist in a polymer research center at the University of Basrah-Iraq.

2.6. Assessment of Antibacterial Activity of Celery Alkaloids

The antibacterial efficiency of celery alkaloids was investigated in order to assess their biochemical effect and investigate their potential therapeutic properties. In several petridishes, 2 ml of culture medium (Muller Hinton Agar) was added. The media was then combined with 0.1 ml of pathogenic stains. There were bacterial wells in the dishes, and different concentrations (10, 25, 35, 50, and 65 mg/ml) were mixed and put within. These dishes were then placed in an incubator and left for 24 hours at 37 °C. Following that, values for the diameter inhibitory zone were noted [14].

2.7. Determination of Cytoxicity of Alkaloids

The cytoxicity of alkaloidic compounds of *Apium graveolens* was investigated depending on hemolysis of red blood cells method. Stock solution with concentration (200 mg/ml) was prepared, then it was diluted into three concentrations: 0.2, 0.3, and 0.4 mg/ml, where 0.8 ml of each concentration was put in Ependorft tube. Two ml of red blood cells were added into each tube, and at the same time, two Ependorft tubes were prepared 0.8 ml of Ringer physiological solution (negative control) was added into the first tube, whereas tap water (positive control) was added into the second Ependorft tube. Then, 0.2 ml of red blood cells were put in each tube. Finally, all results were recorded after the incubation process for these tubes in the incubator for half an hour at 37 °C [15].

3. Results and Discussion

The importance of different medicinal plants for health and medicine stems from the presence of strong chemical compounds that can treat a wide range of illnesses and infections [16]. So *Apium*

graveolens was chosen in the current research because of it contains diverse active chemical compounds. Cold aqueous and alkaloid extracts were prepared, and their crude weights were 3.75 g and 2.78 g; therefore, the percentages of extraction were equal to 15% and 5.56%, respectively, as shown in Table I.

Because the aqueous extract contains a variety of active chemical components, including alkaloids, the extraction percentage of cold aqueous extract was higher than that of alkaloidic extract. Nonetheless, the high percentage of alkaloids extracted suggests the presence of numerous active alkaloidic substances. Preliminary qualitative detection was established, as shown in Table II, to ensure the purity of alkaloids.

Table II refers clearly to the existence of alkaloids alone in the alkaloidic extract, whereas the other active metabolic compounds were not found. Diverse phytochemical studies belonging to *Apium graveolens* proved the presence of alkaloids as important medicinal materials, and they were used as excellent therapies against different diseases [17], [18]. Besides Dragendroff, there are various reagents to detect alkaloids, such as Mayer, Marquis, and Wagner [19].

To separate and identify the chemical structures of alkaloidic compounds, GC-mass analysis was carried out successfully, where these alkaloids were separated at the beginning by GC technique, and many peaks appeared having various retention times. Then, these separated alkaloids were characterized by using mass spectroscopy, and the chemical structures of all alkaloidic compounds were obtained with their mass spectra. Therefore, these alkaloids were 1-(lmidzol [3], [4] pyridine-6-(3H))-on 1-propanoic and, 4,5,6.7-tetrahydro-5-benzyl, ethyl ester,) 2-(pyrrolizin-1,7–dione-6-carboxylic acid methyl ester) and 3-(a sipidosperidine-3-carboxylic and, 6,7-dihydroxy-16-methoxy, methyl ester, (2β,3β,4β,5a,12β,19a)) as shown in Figs. 1–3.

Because of the medicinal significance of synergistic interaction of active chemical compounds, therefore the alkaloidic compounds were carried out as mixture with diverse concentrations against growth of pathogenic bacterias shown in Table III which indicates to medical activity of the alkaloidic mixture containing three compounds isolated and identified from *Apium graveolens* seeds against two bacterial strains.

TABLE I: Percentages of Extraction of Aqueous and Alkaloids Extracts from *Apium graveolens* Seeds

No.	Extract type	Plant weight (g)	Extract weight (g)	Percentages extraction (%)
1	Aqueous	25	3.75	15
2	Alkaloids	50	2.78	5.56

TABLE II: Preliminary Qualitative Detections of Celery Alkaloids

Reagent kind	Test result	Test indication	Conclusion
Dragendroff	+	Orange p.p.t	Alkaloids are existing
Molisch	–	No vioet ring	Carbohydrates are absent
Benedict	–	No red p.p.t	Reducing sugars are absent
Ferri chloride (1%)	–	No bluish-green color	Phenols are absent
Ethanolic potassium hydroxide (5N)	–	No yellow p.p.t	Flavonoids are absent
Ninhydrin (1%)	–	No violet colur	Amino acids are absent
Lead acetate (1%)	–	No white-light brown p.p.t	Tannins are absent

Note: p.p.t—precipitate.

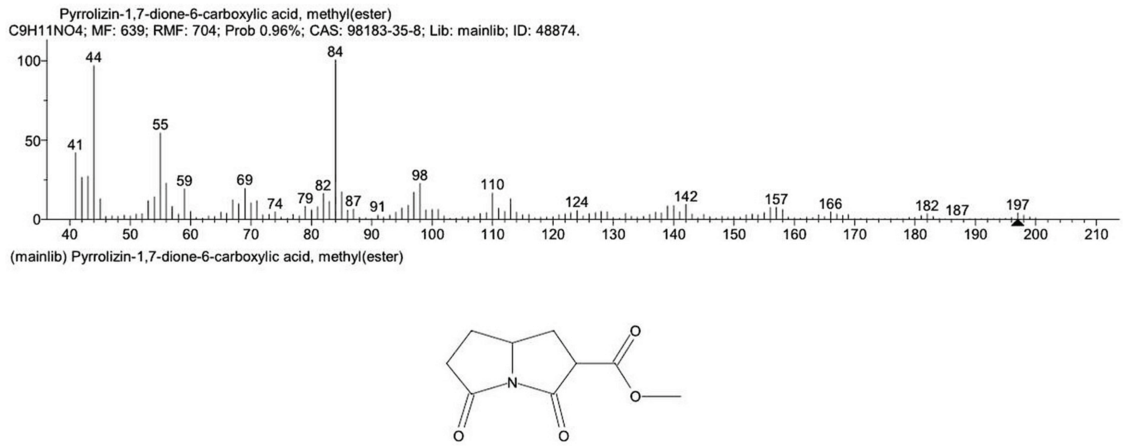

Fig. 1. Mass spectrum of Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester).

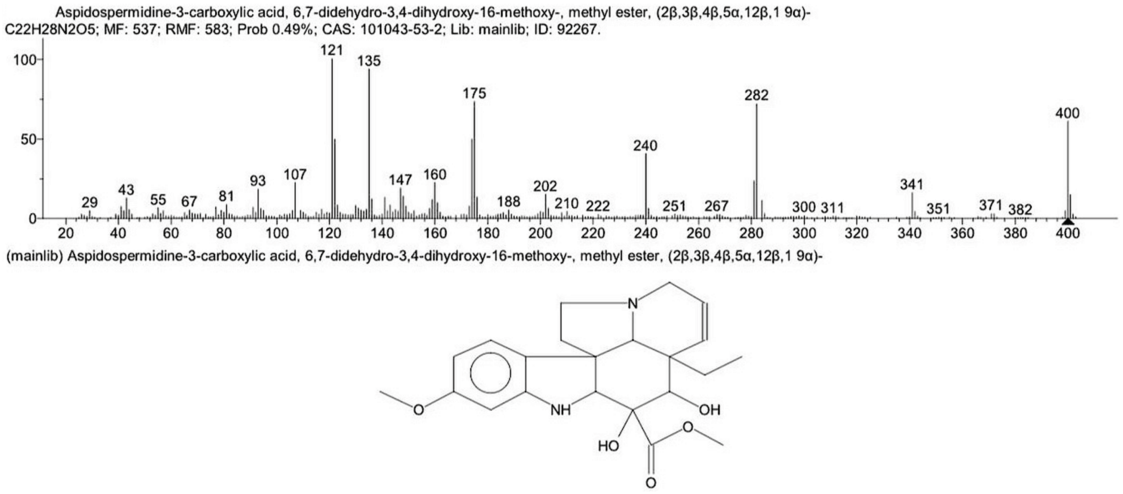

Fig. 2. Mass spectrum of carboxylic acid, 6,7-didehdro-3,4-dihydroxy-16-methoxy-, methyl ester, (2β,3β,4β,5α,12β,19α)-.

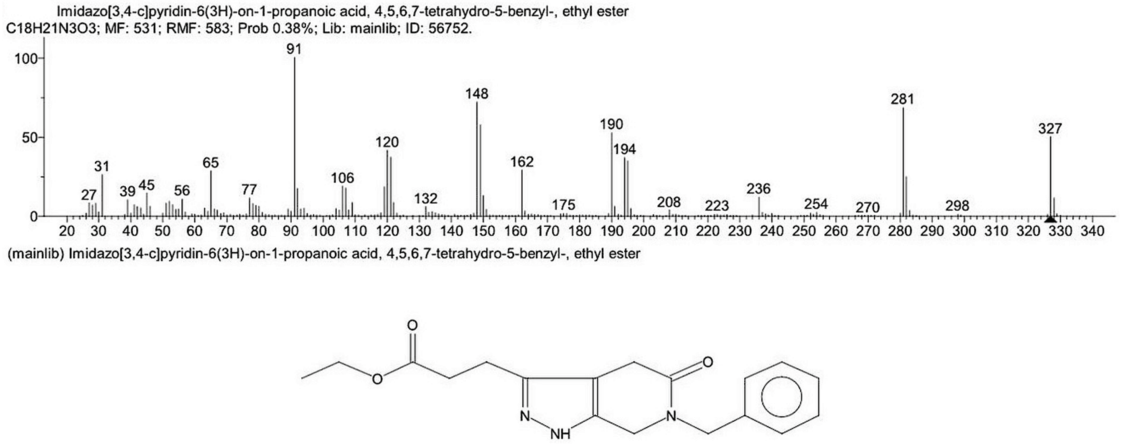

Fig. 3. Mass spectrum of Imidazo(3,4-c)pyridine-6(3H)-on-1-propanoic acid,4,5,6,7-tetrahydro-5 benzyl-,ethyl ester.

TABLE III: Medical Activity of Synergistic Alkaloidic Mixture of Celery Seeds Against Pathogens

Mixture alkaloidic conce (mg\ml)	Inhibition diameters values (mm)	
	Staphylococcus aureus	*Bacillus subtillus*
10	3	11
25	13	21
35	21	23
50	23	25
65	25	27

It was found that the alkaloidic mixture concentrations represented by 10, 25, 35, 50, and 65 mg/ml recorded various values of inhibition diameters equal to 3, 13, 21, 23 and 25 mm, respectively. Staphylococcus aureus bacteria and 11, 21, 23, 25 and 27 mm towards Bacillus subtitles bacteria against. This statement ensures that the increase of concentration led to an increase in the inhibition zone value. The alkaloids as active chemical metabolites act in high activity when they are found as synergistic chemical compounds, leading to an increase in their medicinal efficacy against diverse pathogens [20]. The biological function of alkaloids can be elucidated through hydrogen bonding between the nitrogen atoms found in these active compounds' chemical structures and the hydrogen atoms found in the nucleic acid (DNA and RNA) chemical structures of the living cells of both pathogenic bacteria [21], [22]. Additionally, a variety of studies have shown the chemical capacity of alkaloidic chemicals to impede the metabolism of carbohydrates, proteins, lipids, and amino acids, which destroys the chemical system of pathogenic bacteria cells [23]–[25]. Alkaloids can also destroy the biochemical action of enzymes of pathogenic bacteria by hydrogen bonding with thiol (-SH) group existing in the chemical structure of these enzymes [26], [27].

Table IV proves that the alkaloid extract, which comprises three chemical components, is not harmful to human red blood cells.

TABLE IV: Cytotoxicity of Three Alkaloidic Compounds Mixture Mixture Belonging to Celery Seed

Alkaloidic mixture concentration (mg/ml)	Statues toxic
1:1	T+
1:10	N T−
1:100	N T−
1:1000	N T−
Control negative (blood + Ringer solution)	N T−
Control positive (tap water +blood)	T+++++

Note: T—Toxic and N.T.—Nontoxic.

4. Conclusions

The current research ensured the biochemical importance of the synergistic interaction principle for an alkaloidic mixture containing three chemically active compounds isolated and characterized from *Apium graveolens* seed. The high values of inhibition diameters showed clearly a chemical effect resulting from the medicinal efficacy of these alkaloids, which inhibit the biological growth of both bacteria represented by *Staphylococcus aureus* and *Bacillus subtillus*. Therefore, these microorganisms can cause a variety of ailments that can be treated chemically with celery natural alkaloids.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

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