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Original Article

Chemical Composition, Antioxidant, Antimicrobial, and Antidiabetic Activities of Celery (*Apium graveolens* L.)

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Abstract

Background: Secondary metabolites (SM) are not needed for survival, but help the cell (organism) interact with its surroundings. Different kinds of natural active products come from plants because of their special characteristics. Researchers have examined the phenolic and antioxidant compounds in celery (Apium graveolens L.) because it belongs to the apiaceae family.

Objectives: Phytochemical screening and the identification of various natural chemical components in the methanolic leaves extract of Apium graveolens L. by Fourier Transform Infrared Spectroscopy (FTIR) and research on its anti-microbial and anti-diabetic properties were the aims of this study.

Materials and Methods: Cleaned and isolated foreign substances Apium graveolens L. which were bought dried from markets in Babylon Province and then studied at the college science's advanced botanical laboratory in the University of Babylon. An electrical machine crushed the substances and after, the resulting powder was kept in nylon bags in the lab at room temperature until experiments began. About 1 gram of laboratory crushed leaf powder was converted into pellets by mixing with KBr. All samples were checked with three types of tests and pellets made of KBr that were not treated were used as controls.

Results: While getting ready for FTIR analysis, the values measured are Peak Wave number (cm^{-1}), Intensity, Corr. Intensity, Area and Functional group assignment were (667.37, 69.147, 1.522, 2.915, and Alkenes), (894.97, 82.045, 0.457, 1.958, and Alkenes), (1029.99, 61.548, 17.442, 32.156, and alkyl halides), (1238.30, 81.092, 0.518, 2.645, alkyl halides), (1317.38, 81.874, 81.874, 3.182, and alkyl halides), (1373.32, 81. 514, 0.203, 2.255 and alkyl halides), (1519.91, 82.843, 1.227, 3.086, and Aromatic), (1616.35, 77.669, 0.321, 3.636, and Amide), (1743.65, 87.838, 6.121, 2.211, and Ester), (2852.72, 87.591, 2.845, 2.629, and Alkane), (2920.23, 83.176, 5.651, 4.259, and Alkane). The Celery (Apium graveolens) extract and the antibiotics AM-Amikacin, CFO-Cefoxitin and RF-Rifampicin (standards) performed with an antibacterial activity of 26.34±0.23, 23.04± 0.25, 35.13±0.48, 30.19±0.39, and 33.00±0.39 against Enterococcus faecalis, while recorded 20.24±0.16, 19.22±0.20, 30.96±0.41, 27.68±0.36 and 30.83±0.40 for Escherichia coli and recorded 25.47±0.27, 15.58±0.14, 28.94±0.39, 26.07±0.36 and 28.00±0.38 in Enterobacter aerogenes in the same time recorded 17.40±0.11, 23.05±0.26, 32.94±0.43, 34.98±0.46 and 30.71±0.39 respectively for Staphylococcus aureus, and recorded 25.10±0.29, 18.25±0.22, 28.01±0.37, 23.05±0.34 and 27.86±0.36 respectively for Streptococcus pyogenes. Based on the extract used, the activity of Celery proved effective at blocking α-amylase at comparative percentages (79.54±0.52, 48.93±0.26 and 11.13±0.07, respectively). In separate trials, each sample proved to have inhibitory activity against α-glucosidase enzymes (recorded: 54.08±0.26, 22.68±0.12 and 11.70±0.05).

Keywords: Antioxidant, Antimicrobial, Antidiabetic, Celery (*Apium graveolens* L.).

Introduction

At present, lots of diseases infect us each day and to address them, it is important that we discover natural antibiotics that can fight many dangerous bacteria, both gram-negative and gram-positive [1, 2]. Insufficient insulin secretion or insulin resistance leads to high blood sugar levels for a long time which is called diabetes mellitus. Many serious diseases or complications can follow such as arteriosclerosis, high blood pressure, thyroid problems, eye damage, kidney damage and nerve damage. Regular use of antidiabetic drugs can lead to several unwanted effects, so their use is sometimes connected to different health issues. There are many difficulties in managing type 2 diabetes such as less glucose absorption because of lacking insulin, insufficient insulin release and symptoms of high insulin and resistance to insulin. Typical medicines for this problem are glibenclamide, metformin and α-amylase and α-glucosidase inhibitors. Also, because antidiabetic plants have traditionally been used and show generally fewer side effects, discovering their active ingredients may lead to effective new diabetes treatments. Several antidiabetic plants have now been approved and studies have identified how they work to support diabetes management. Many traditional strategies are explored in managing hyperglycemia and medicinal plants are very important in this field. Since olden times, medicinal plants were common for treating public health and different parts were used for this purpose [3]. Practicing natural medicine tends to be reasonably affordable. For a long time, people used plants to manage pain and now the main interest is in their healing effects and their use in disease treatments. According to several studies, using certain herbs and plants may improve conditions linked to infertility, hormone imbalance, disorders of the liver, anemia, renal diseases and disorders of the neurological and mental systems. It is common for plants to have flavonoids and other phenolic compounds and scientists are studying how these compounds affect different diseases such as coronary heart disease, diabetes and cancer. Side effects from medicinal herbs are less than those from chemical drugs and their antioxidant characteristics also help limit the toxicity of drugs. Today many choose herbal drugs instead of chemical drugs mainly because they have fewer side effects. The apiaceae family contains celery (Apium graveolens L), an annual or perennial plant that grows in Europe and in tropical and subtropical Africa and Asia. Even though celery requires lots of moisture, the temperature for it should not be too high [4, 5]. For this reason, the best celery grows best in cold and moderately warm climates. Cardiovascular problems, jaundice, liver and lien diseases, obstruction in the urinary tract, gout and rheumatic diseases may be prevented by celery. Celery extracts made with ethanol have been found to promote both spermatogenesis and better fertility in rats. The properties of celery lower blood glucose, total cholesterol and pressure which aid heart health. Celery has been found in studies to stop the growth of fungi and decrease inflammation. Also, the essential oils contained in cinnamon are antibacterial. The seeds are helpful when treating bronchitis, problems with vision, asthma, common skin disorders, vomiting, fever and tumors. Diuretic celery root is used to help treat colic. Plants provide many natural actives and the type and properties depend on their mechanism. Many of the chemicals in plants, mainly polyphenols, pick up free radicals and support their antioxidant functions. Polyphenols have effects on living organisms. Thanks to their antioxidant effects [6], these foods help prevent free radicals and peroxidation. Since polyphenols have similar actions, at least one of the phenolic groups can pair with hydrogen donors to neutralize free radicals.35 Experts have paid attention to the health benefits of celery antioxidants by studying them through various studies. Celery has been the subject of study by many scientists who have looked at the phenolic and antioxidant compounds it contains. Celery root and its leaves have the function to get rid of OH and DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals, while the plant also lessens the strength of liposomal peroxidation which shows it is protective. Antimicrobials stop the growth of

certain bacteria and kill them. A large amount of antibiotics being used and sometimes used incorrectly has encouraged bacteria to become resistant. There have been continual cases of antibiotic resistance in humans and animals for some time [7]. Therefore, alternative medicines are necessary to treat diseases. Because bacteria are now developing resistance to existing antibiotics, we must look for a different antibacterial agent. Several infections such as those of the respiratory system, on the skin, in the genitourinary tract and in wounds, can be caused by Gram-positive bacteria such as Staphylococcus sp. Staphylococcus aureus is responsible for about half of hospital infections. It can be tough to kill using a variety of antibiotics. Staphylococcus epidermidis which is under the CNS family, is an important resident on human skin and mucous membranes. The bacteria Staphylococcus epidermidis is found most often during clinical cultures and is now recognized as the main cause of nosocomial infections [8, 9]. Many herbal antimicrobials have been created to treat a variety of diseases. Many natural things such as plants and spices are sources of antimicrobials. Many plants used in traditional medicine include celery leaves, betel leaves, papaya leaves, soursop leaves and gambier leaves. Celery (Apium graveolens) is one of the plants that people have used as an antimicrobial agent. Many people turn to a celery seed extract as therapy for rheumatism, rheumatic pain, rheumatic diseases and gout. Our research set out to test antioxidant, antimicrobial, antidiabetic activity and to study Celery (Apium graveolens L.).

Materials and Methods

Samples Preparation

The dried leaves of Apium graveolens were added to a blender and ground into a powder. Every sample powder was stored in polyethylene bags and kept in the deep freezer before testing. Sterile water was used to extract fractions of Apium graveolens by soaking 25 g in 150 ml of sterile water in individual sterile flasks. The flasks stayed in place for two days and they were shaken occasionally. Flask contents were passed through a filter.

Assessment of the antimicrobial activity of compounds derived from secondary metabolites against five harmful microorganisms

At first, put 6 to 7 grams of nutrient broth in a container and mix with 500 ml of water. After that, put it in the autoclave for pouring and just leave it overnight. Apply the bacterial strain the next day, if the test suggests infection, the result is positive and if bacteria is not found, the result is negative. To check how antimicrobial the leaves of Apium graveolens are against microbes, we evaluated the presence of inhibition zones, the areas of these zones and minimum inhibitory concentration (MIC) values. The skills of the candidates were measured in numerical ways as well as in other ways. The plant was found to have strong ability to fight many different microbial strains. Tests of extract from the plant against various microbes found that it had limited antimicrobial capacity.

Fourier transform infrared spectroscopy (FTIR) analysis of *Apium graveolens*

Experimental FTIR spectra were obtained for all GLVs using mainly software run on personal computers from data received from the FTIR instrument. For FTIR experiments, some laboratory crushed leaf samples were mixed with KBr to make pellets and at the same time a thin layer of the mixture was prepared by pressing it firmly. At the same time the information was collected using wave numbers from 4000 cm−1 to 500 cm−1. KBr pellets not treated in any way served as a control as all the experimental samples were analysed using three different tests.

α-amylase inhibitory assay

A few small steps were added to the common procedure to test the α-amylase inhibition of the extract and the fractions. A total of twenty millilitres of α-amylase with two International Units per millilitre was included, next was two hundred millilitres of extract and fractions at a concentration of 0.5 milligramme per millilitre and finally, the solution included five hundred millilitres of 6.8 phosphate buffer with a

phosphate concentration of one hundred millimolar. The mixture was divided into 96 wells in a plate and after 20 minutes at 37 °C, it was processed further. The temperature used during preincubation was 37°C for the incubation. The mixture was then placed back into an incubator at 37 degrees Celsius for 30 minutes After that, 20 litres of 1% soluble starch in 100 mM phosphate buffer pH 6.8 were added as the substrate. Next, the liquid was brought to a boil at a constant pressure for 10 minutes starting from the addition of the DNS colour reagent (100 litres). A Multiplate Reader (Multiska Thermo Scientific, version 1.00.40) followed and absorbance measurements were taken at 540 nanometres. The measurement was done to find out how much the final mixture absorbs. Levels of acarbose from 0.1 to 0.5 mg/ml were used as control concentrations. For comparison, a material that had no experimental processing (no extracts or fractions) was created at the same time. A total of three repetitions were done for each experiment. The applied formula produced results that showed the percentage of inhibition. The IC50 values were obtained by looking at the graphs that showed how much of the fractions inhibited the enzyme activity.

The percentage of inhibition could be determined by applying the following formula:

$$\% \text{ Inhibition} = (Abs_{control} - Abs_{extract}) / Abs_{control} \times 100$$

α-Glucosidase Inhibitory Assay

The inhibitory action of the extract and different fractions on α-glucosidase was examined. Alternatively, minor adaptations were made to the traditional process for doing the analysis. A 96 well plate was chilled and then, for 15 minutes, held at 37 degrees centigrade before adding serum samples to it. Among the mixture were twenty litres total of the nine extracts and fractions, each one at 0.500 mg/mL, ten litres of pure alphabet-glucosidase at one unit per millilitre and fifty litres of the 55 sodium phosphate buffer, at a concentration of 100 mM. Pre incubation steps were performed at 37 degree centigrade. After that, twenty liters of P-NPG containing five millimolar concentration were added and the mix was incubated for twenty more minutes at thirty-seven degrees centigrade. The reaction stopped when 50 litres of a 0.1 M sodium carbonate solution were added in the reactor. The multiplate reader was used to measure the amount of nitrophenol absorbed and the reading was taken at 405 nm. At the same time, the standard substance of acarbose was applied and measured; it was present in the sample under examination in the amount of 0.5 mg/mL. The procedures were performed about three times for comparison of the findings. At the same time, a control experiment was done using no chemical, to see how it affects the process. The studies were repeated three times so that the information is as accurate as possible. Percentage of inhibition was used as a way to measure the activity of α-glucosidase inhibition in the experiment.

$$\textbf{\% Inhibition} = (Abs_{control} - Abs_{extract}) / Abs_{control} \times 100$$

The two symbols, A control and A extract, mean the absorbance of the control and fractions. The IC50 points from the graphs showed the levels of fracture needed to keep enzyme activity down by half.

Antioxidant Activity

Peroxynitrite scavenging

Peroxynitrite (ONOO-) was produced by using the method explained by Beckman et al. [10, 11]. 5 ml of H2O2 (0.7 M) was added to a 5 ml solution of HCl (0.6 M) kept on an ice bath. 5 ml of 0.6 M KNO2 was then added, followed by 5 ml of ice-cold 1.2 M NaOH. H2O2 was allowed to react with granular MnO2 prewashed with NaOH and then both were stored overnight at -20°C. The peroxynitrite solution from the frozen mixture was taken from the top and the level of peroxynitrite was measured using a spectrophotometer at 302 nm (with an extinction value of 1670 M-1 cm-1). The Evans Blue bleaching assay was applied to check how much peroxynitrite the product can scavenge. A standard method for the assay was followed, but with a slight change. The components in the mixture were 50 mM phosphate buffer (pH 7.4), 0.1 mM DTPA, 90

mM NaCl, 5 mM KCl, 12.5 µM Evans Blue, a range of plant extract concentrations (0–200 µg/ml) and 1 mM peroxynitrite in 1 ml of solution. After waiting 30 min at 25°C, the absorbance was taken at a wavelength of 611 nm. The amount of scavenging by ONOO- was worked out by comparing the ONOO- exposure treatment with the blank sample. Tests were completed in pairs six times. Gallic acid was the compound used as the standard.

Singlet oxygen scavenging

A reportable spectrophotometric technique was used to determine the production of singlet oxygen (1O2) by assessing the bleaching of N, N-dimethyl-4-nitrosoaniline (RNO). The generation of singlet oxygen was done by reacting NaOCl with H2O2 and the bleaching of RNO was checked at the wavelength of 440 nm. The mixture for the reaction had 45 mM phosphate buffer (pH 7.1), 50 mM NaOCl, 50 mM H2O2, 50 mM histidine, 10 µM RNO and several doses (0–200 µg/ml) of sample in a 2 ml volume. Reaction mixture was incubated at 30°C for 40 min and changes of RNO absorbance at 440 nm were studied. The activity of scavenging of the sample was measured and then compared with known activity of lipoic acid. The test was carried out 12 times in all [12].

Hypochlorous acid scavenging

Hypochlorous acid (HOCl) was made just before the experiment by adjusting the pH of a 10% (v/v) NaOCl solution to 6.2 with 0.6 M H2SO4 and then the concentration of HOCl was found by measuring its absorbance at 235 nm. The assay was done using the procedure set by Aruoma and Halliwell with a few changes. The role of scavengers was tested by tracking reductions in the absorbance of catalase at 404 nm. In the reaction mixture, 1 ml contained phosphate buffer at 50 mM (pH 6.8), catalase at 7.2 µM, HOCl at 8.4 mM and different concentrations (0–100 µg/ml) of the plant extract. For 20 min at 25°C, the mixture was left undisturbed and then its absorbance was checked using an appropriate blank as reference. Every test was run six times [13].

Statistical Analysis

Data were recorded as the mean value of the Inhibition zone diameter, while the standard error of mean (SEM) was used as a measure of variability. To look at the recorded results, a one-way ANOVA procedure was used. All data was analyzed using SPSS version 20.0.

Results and Discussion

Different organic molecules known as secondary metabolites can be made by many plants. The specific structures of carbon skeletons are an important basic feature of plant secondary metabolites. Although a cell (organism) does not require secondary metabolites to exist, they assist in the cell's (organism's) interaction with its environment and ensure the organism's survival in its place of life. The way SMs are formed is usually specific to cells, tissues and organs and these molecules are low molecular weight. The amount and kinds of these compounds change somewhat among individuals of the same kind of plant. They help plants resist different forms of stress (bacteria, fungi, nematodes, insects, heat, moisture changes, shade, injury or toxic heavy metals) [14, 15]. Many people take advantage of the economic value by using SMs as drugs, flavours, fragrances, insecticides and dyes. SMs in plants can be sorted into three main categories (Terpenoids, Polyketides and Phenypropanoids) based on their methods of biosynthesis. Alkaloids are another kind of SMs that are nitrogenous organic compounds. They are formed mainly from amino acids like tryptophan, tyrosine, phenylalanine, lysine and arginine and different special enzymes are needed for their synthesis. Many important medications in modern medicine are alkaloids. Cellular or sub-cellular places are used to organize biosynthesis. SMs may be transported away from their original sites and can build up there. There are hundreds of thousands of low molecular weight organic compounds made by plants. Peak wave number cm^{-1}, intensity, correlation intensity, area, and functional group

assignment were all included in the preparation for FTIR analysis: (667.37, 69.147, 1.522, 2.915, and Alkenes), (894.97, 82.045, 0.457, 1.958, and Alkenes), (1029.99, 61.548, 17.442, 32.156, and alkyl halides), (1238.30, 81.092, 0.518, 2.645, alkyl halides), (1317.38, 81.874, 81.874, 3.182, and alkyl halides), (1373.32, 81. 514, 0.203, 2.255 and alkyl halides), (1519.91, 82.843, 1.227, 3.086, and Aromatic), (1616.35, 77.669, 0.321, 3.636, and Amide), (1743.65, 87.838, 6.121, 2.211, and Ester), (2852.72, 87.591, 2.845, 2.629, and Alkane), (2920.23, 83.176, 5.651, 4.259, and Alkane). Many useful natural products called secondary metabolites are created through secondary metabolism inside plants. Some metabolites are formed during taxon changes and developmental stages in plants which indicates they are secondary metabolites. Higher production of secondary metabolites during In Vitro growth is usually seen in differentiated tissues than in undifferentiated or only slightly differentiated ones. These metabolites make things convenient for us such as being able to rescue and use them and they are ideal for plants that are difficult or costly to grow [16, 17]. Many other cases could be discussed as the field of plant metabolic engineering is very active right now. Even though metabolic engineering is a major advance, just changing the genes will not eliminate all the obstacles that have stopped plant secondary metabolites from succeeding commercially. Information about how compounds found in plants and cultures are made is not well-developed and that is why techniques are needed to study this at the cell and molecular level. Because plant cells in culture are so complex and misunderstood, each specific case study is needed to explain the issues in the production of secondary metabolites. Experiments have resulted in many useful secondary phytochemicals from both kinds of cultures, though in other instances production comes from more complete micro plant or organ cultures. Every plant has primary metabolites which help with important metabolic jobs like nutrition and reproduction. Sometimes it can be difficult to tell primary metabolites apart from secondary ones. An example is that terpenoids include primary and secondary metabolites and one compound can play both types of roles. There are many secondary metabolites from various metabolite groups that can be very quick to develop under stressful conditions. Another role of carotenoids and flavonoids is to give pigment to flower and seed which helps draw in the insects and animals that pollinate or spread seeds. For this reason, they are involved in the reproduction of plants. The building blocks of nucleic acids, proteins, carbohydrates, fats and lipids in plants are called plant primary products and they are significant for the structure, physiology and genetics [18] of plants. Secondary metabolites tend to be present in less proportion and have lower concentrations than primary metabolites. The carboxylic acids of the Krebs cycle are produced during the actions of primary metabolism. Secondary metabolites are not necessary for life, but they help the species survive better. Also, certain species are identified by their bacteria content which is often used to arrange and classify plants based on how their secondary chemicals change within a given species (chemotaxonomy). Terpenes, Phenolics, N (Nitrogen) and S (sulphur) containing compounds are the main chemical groups in plants' secondary metabolites.

Standard antibiotics AM-Amikacin, CFO-Cefoxitin, and RF-Rifampicin, as well as bioactive natural components of celery (Apium graveolens), were found to exhibit antibacterial activity: 26.34±0.23, 23.04± 0.25, 35.13±0.48, 30.19±0.39, and 33.00±0.39 against Enterococcus faecalis, while recorded 20.24±0.16, 19.22±0.20, 30.96±0.41, 27.68±0.36 and 30.83±0.40 for Escherichia coli and recorded 25.47±0.27, 15.58±0.14, 28.94±0.39, 26.07±0.36 and 28.00±0.38 in Enterobacter aerogenes in the same time recorded 17.40±0.11, 23.05±0.26, 32.94±0.43, 34.98±0.46 and 30.71±0.39 respectively for Staphylococcus aureus, and recorded 25.10±0.29, 18.25±0.22, 28.01±0.37, 23.05±0.34 and 27.86±0.36 respectively for Streptococcus pyogenes (Figure 2-6).

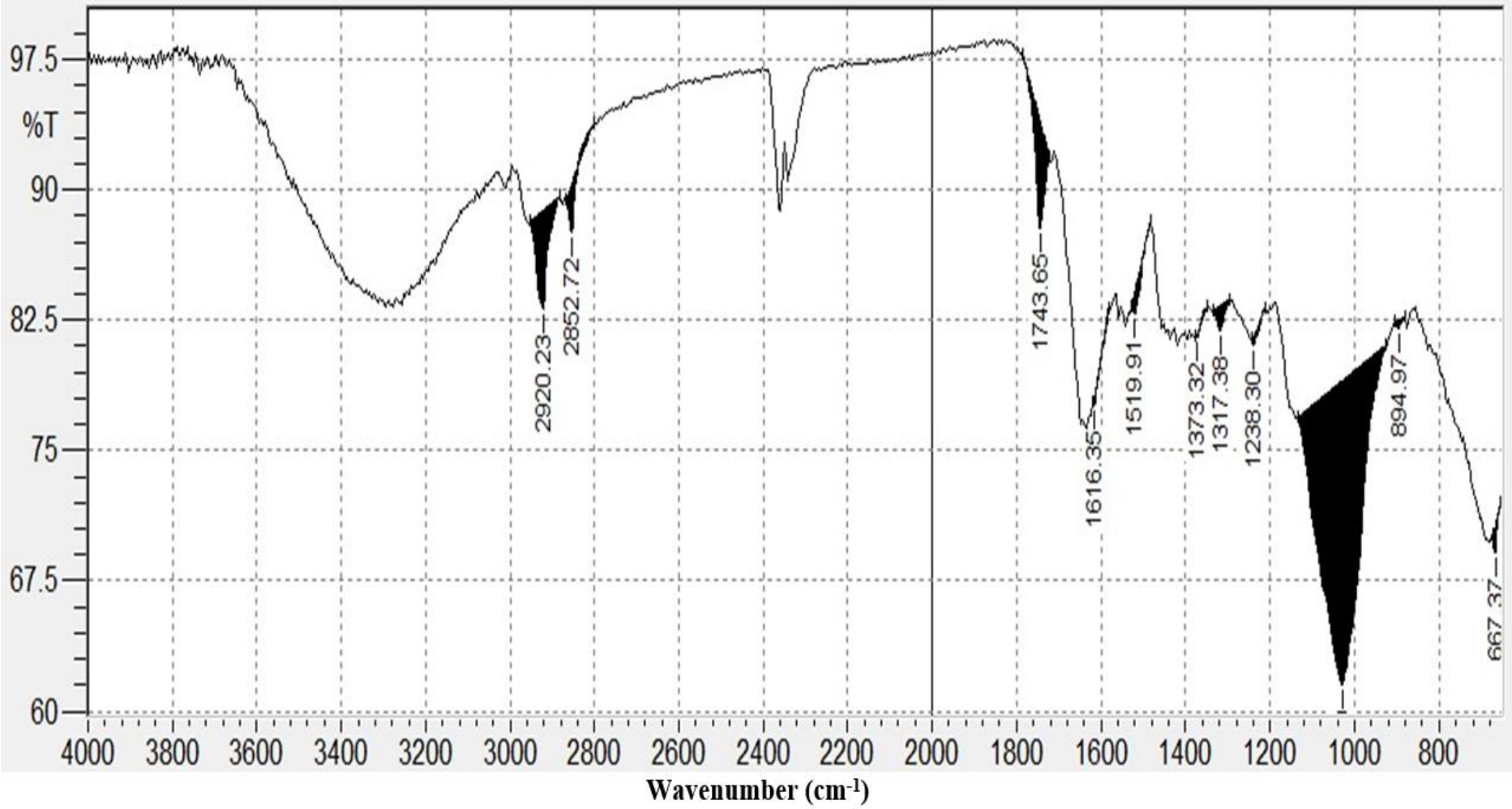

Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of seed extract of Celery (*Apium graveolens* L.).

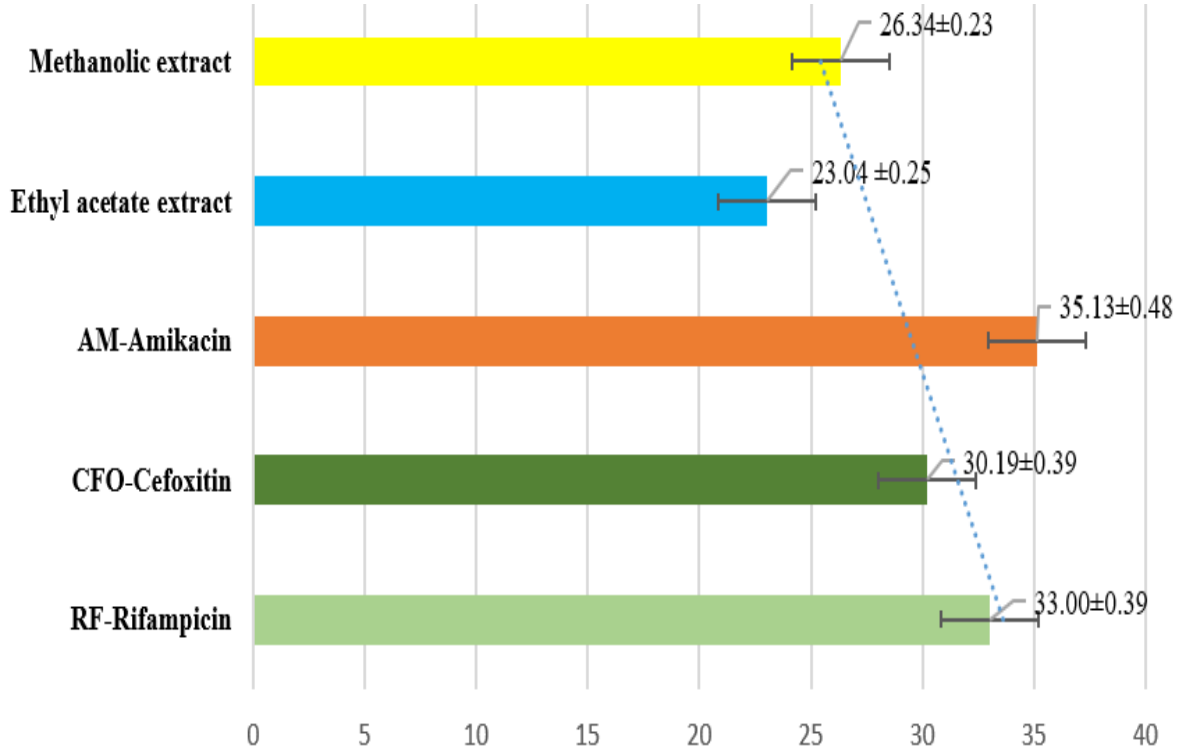

Figure 2. Bioactive natural compounds of Celery (Apium graveolens), antibiotics AM-Amikacin, CFO-Cefoxitin, and RF-Rifampicin (standards) as antibacterial activity against *Enterococcus faecalis*

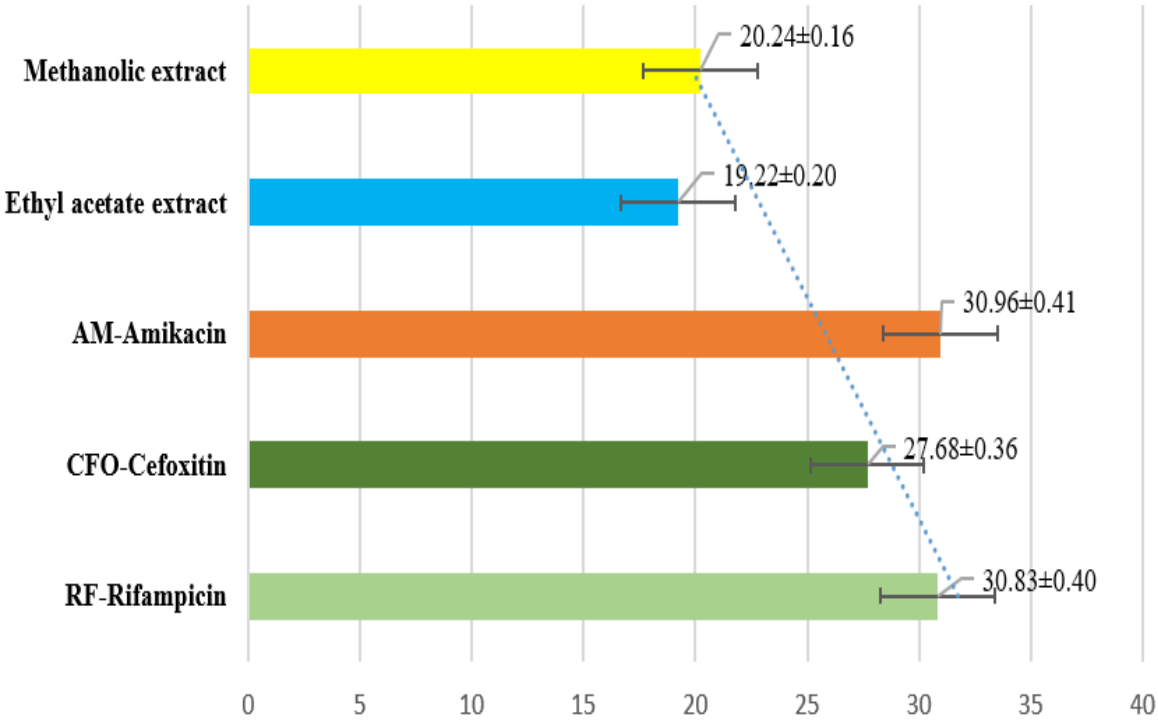

Figure 3. Bioactive natural compounds of Celery (Apium graveolens), antibiotics AM-Amikacin, CFO-Cefoxitin, and RF-Rifampicin (standards) as antibacterial activity against *Escherichia coli*

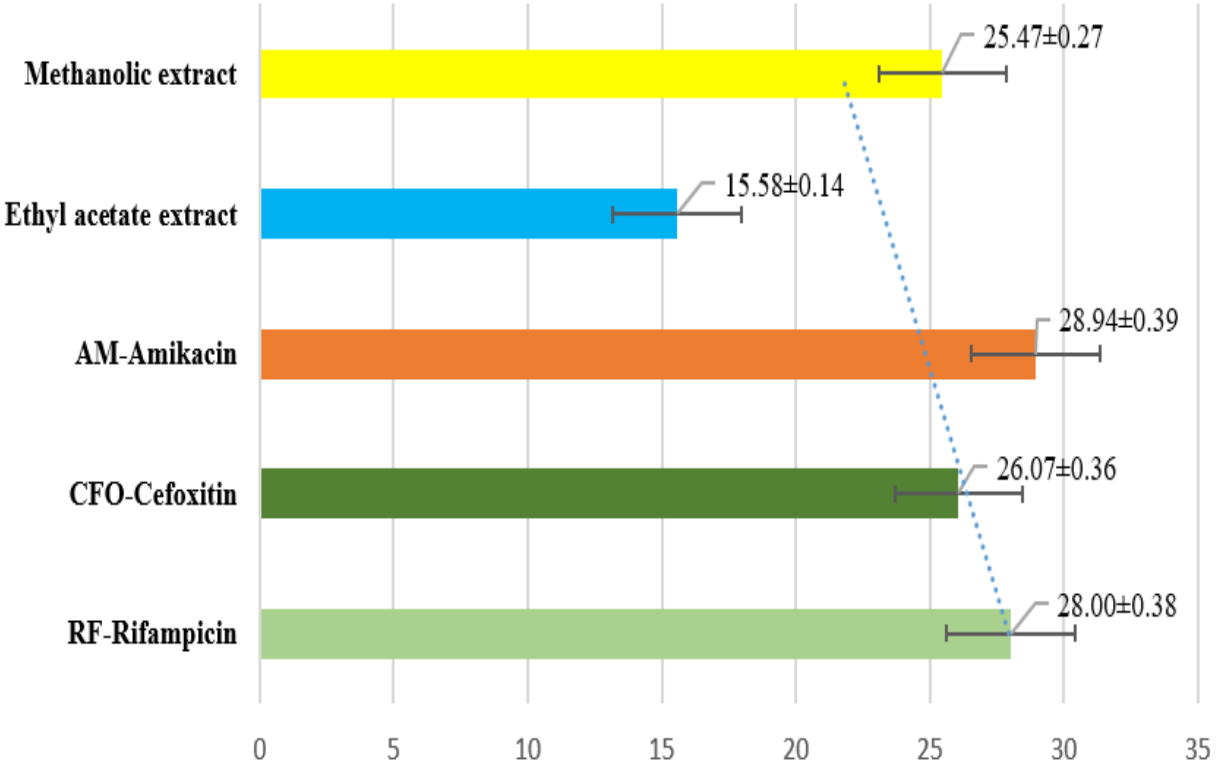

Figure 4. Bioactive natural compounds of Celery (Apium graveolens), antibiotics AM-Amikacin, CFO-Cefoxitin, and RF-Rifampicin (standards) as antibacterial activity against *Enterobacter aerogenes*

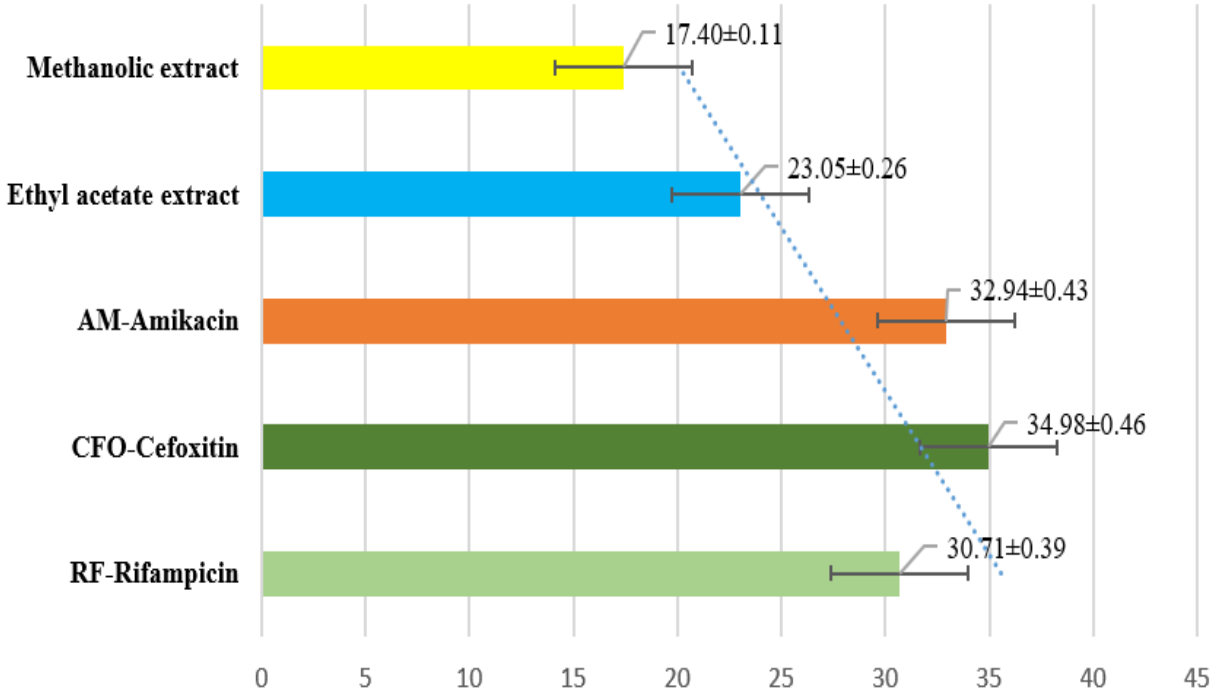

Figure 5. Bioactive natural compounds of Celery (Apium graveolens), antibiotics AM-Amikacin, CFO-Cefoxitin, and RF-Rifampicin (standards) as antibacterial activity against *Staphylococcus aureus*

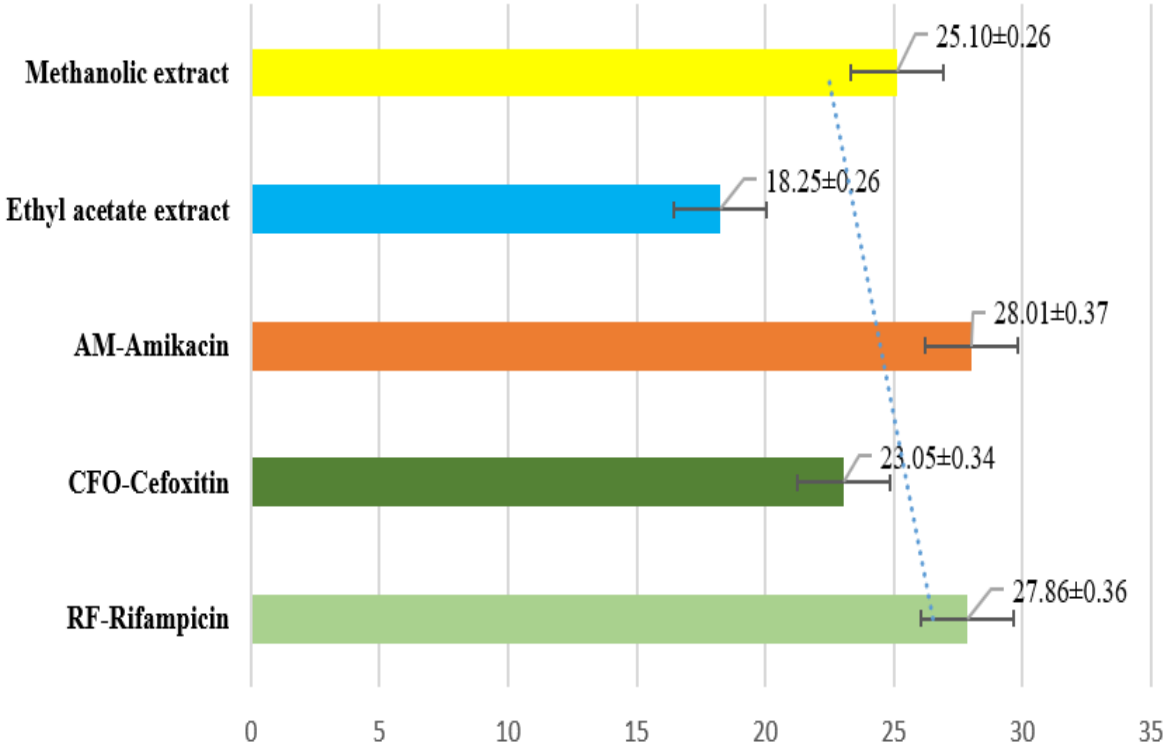

Figure 6. Bioactive natural compounds of Celery (Apium graveolens), antibiotics AM-Amikacin, CFO-Cefoxitin, and RF-Rifampicin (standards) as antibacterial activity against *Streptococcus pyogenes*

These figures indicate that the Celery (Apium graveolens) extracts were able to block the actions of α-amylase and α-glucosidase enzymes, as the extracts seen in Figures 7 and 8 showed. Using the enzymatic inhibitor assay on Celery (Apium graveolens) fractions, it was found that dose and the particular fraction influenced the inhibitory activities. There was the greatest blockage for the dose that was applied in the highest amount, whereas the lowest dose had the most relaxation. Celery (Apium graveolens) extract and its fractions show the ability to reduce the activities of α-amylase and α-glucosidase based on how they were extracted (crude methanol extract, ethyl acetate fraction and Acarbose) recorded (79.54±0.52, 48.93±0.26, and 11.13 ± 0.07) respectively inhibitory potency against α-amylase. While recorded (54.08±0.26, 22.68±0.12, and 11.70±0.05) respectively inhibitory potency against α- glucosidase activity. Apium graveolens contains polyphenols that contribute to lower blood sugar in several ways by regulating homeostasis and enhancing the response to insulin. They have a positive effect on insulin sensitivity by controlling how insulin receptors and IRS-1 work which helps the body react better to insulin. These chemicals prevent some digestive enzymes from acting which reduces the amount of blood sugar that peaks after a meal. They stimulate the work of glucose transporters such as GLUT4 which helps more glucose reach the muscles and fat. Also, celery polyphenols turn on the AMPK pathway which makes the body burn more glucose and fat and lowers the chance of developing insulin resistance. These properties of polyphenols reduce chronic inflammation and the damaging effects of oxidative radicals which help protect insulin signaling and pancreatic β-cell function [19-21]. Celery polyphenols have an influence on the way lipids are processed in the body, shielding it from non-alcoholic fatty liver disease (NAFLD) and supporting better metabolic regulation. They might also preserve how the kidneys work by decreasing oxidative damage and inflammation which could prevent diabetic nephropathy. They join forces to create a thorough way to handle diabetes and its side effects.

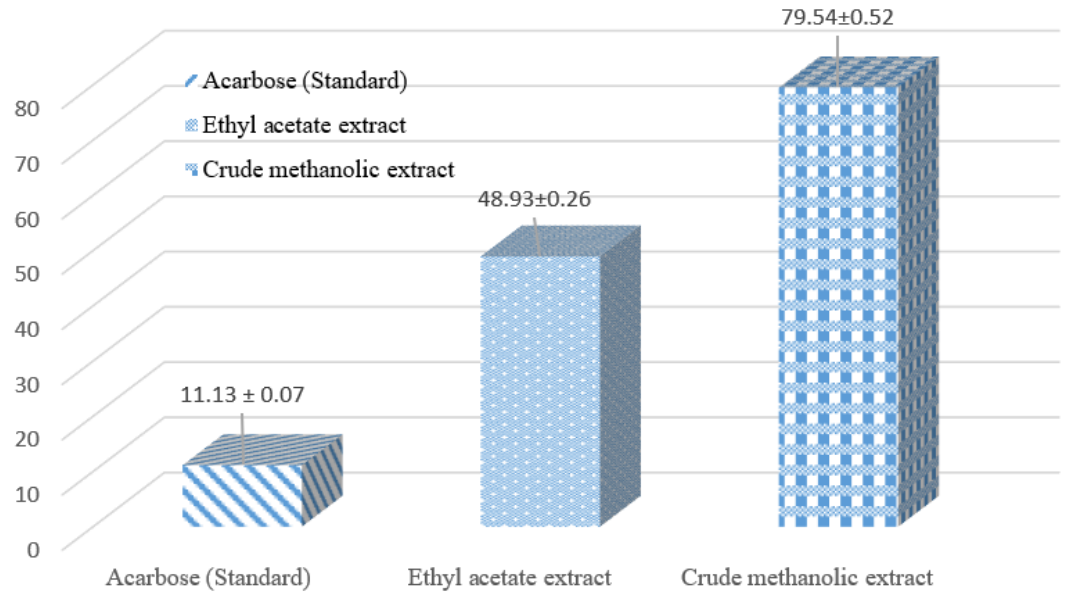

Figure 7. Antidiabetic activities of Celery (Apium graveolens) extract fractions' inhibitory efficacy against α-amylase

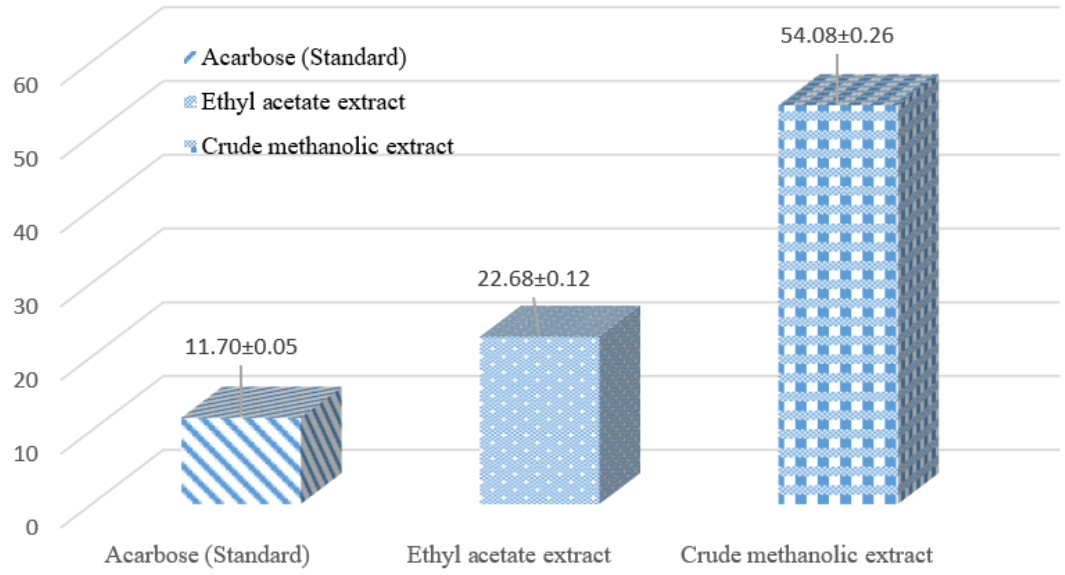

Figure 8. Antidiabetic activities of Celery (Apium graveolens) extract fractions' inhibitory efficacy against α-glucosidase

The capacity of Celery's (Apium graveolens) extracts and Gallic acid to prevent the effects of oxidative stress by scavenging peroxynitrite, singlet oxygen and hypochlorous acid. Data were reported on various extract types: crude, ethyl acetate, ethanol and standard recorded 702.74±18.07, 657.54± 15.94 and Gallic acid (standard) 816.91 ± 21.08 for Peroxynitrite scavenging (Figure 9). Although peroxynitrite (ONOO-) is more stable than most free radicals, once it picks up a proton, it becomes the active form known as peroxynitrous acid (ONOOH). Excess ONOO- creates damage to tissues and other structures. Peroxynitrite makes Evans Blue turn colorless by oxidizing it. Based on the current findings, the plant extract reduces Evans Blue bleaching by scavenging peroxynitrite and it is more potent than gallic acid. While we recorded 45.01 ± 3.70, 44.53 ± 3.68 and Lipoic acid (standard) 33.09 ± 2.71 for the scavenging of

singlet oxygen (Figure 10). At the same time, you should also obtain the following results for hypochlorous acid scavenging potency: 99.24 ± 4.41, 106.75 ± 5.1 and 199.87 ± 7.45 for Ascorbic acid (standard). Figure 11 reveals that the percentages by which crude and fraction extracts stopped Nitric oxide radical activity were greater ($P<0.05$) than what Ascorbic acid stopped on its own.

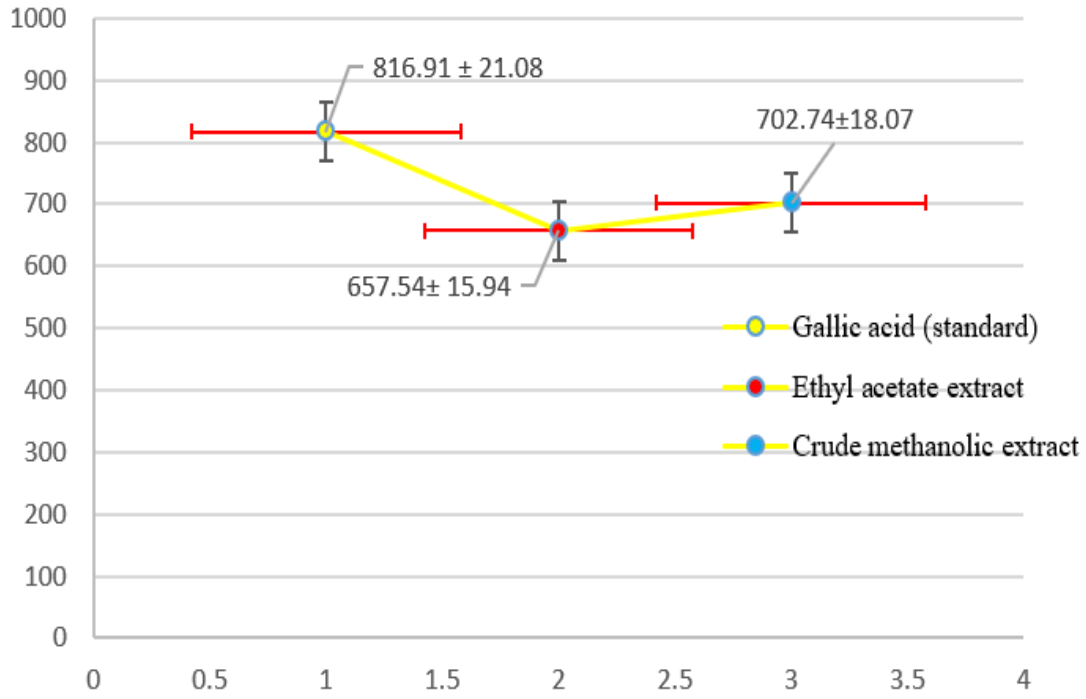

Figure 9. Antioxidant activity [Peroxynitrite scavenging] of Celery (Apium graveolens) extract fractions and Gallic acid (standard)

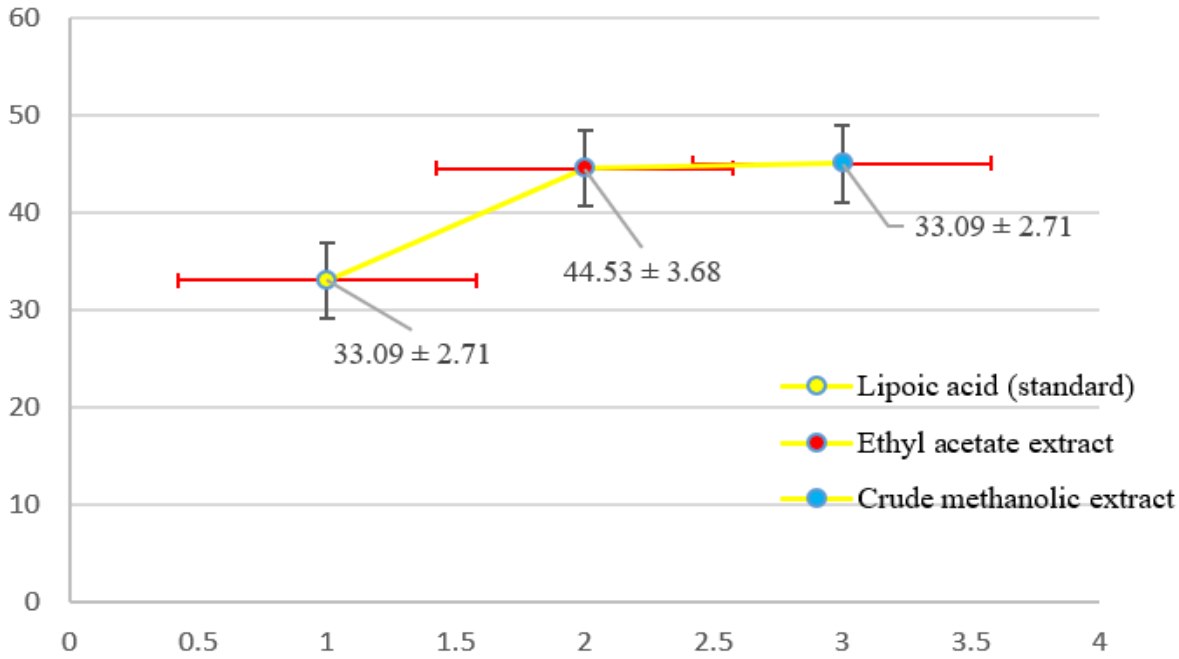

Figure 10. Antioxidant activity [Singlet oxygen scavenging] of Celery (Apium graveolens) extract fractions and Lipoic acid (standard)

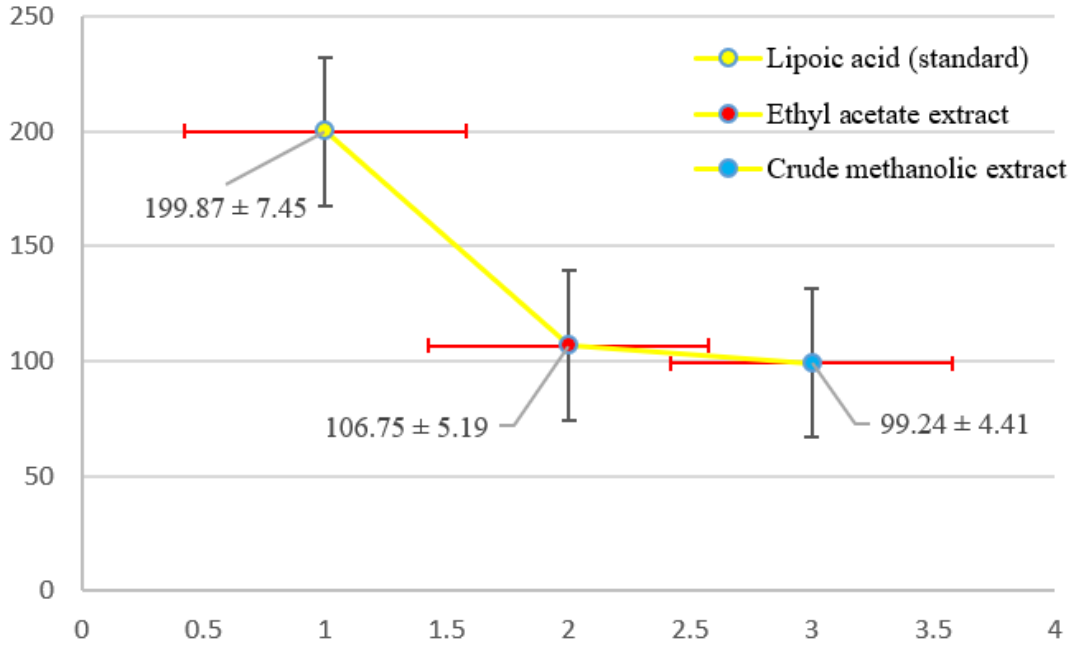

Figure 11. Antioxidant activity [Hypochlorous acid scavenging] of Celery (Apium graveolens) extract fractions and Ascorbic acid (standard)

Many plants make a variety of secondary products with a Phenol group which is a hydroxyl functional group found on smelly compounds in nature. They may act as part of the plant's defence and help prevent damage from pests and disease like root parasitic nematodes. Extra ozone caused the phenolic content of leaves to go up and caused only small changes in the concentrations of each compound. Many different kinds of vascular plants contain coumarin and these phytochemicals seem to work in various ways to protect them against insect pests and fungi. Bacteria, fungi and plants have the shikimic acid pathway, but not animals do. Some coumarin derivatives are more effective against soil borne plant disease fungi and are more stable than the coumarin compounds alone. Furano is another coumarin compound and it has special importance for plant toxicity because it is widespread in umbelliferae family plants such as celery, parsnip and parsley. SO2 treatment is rare in plants due to the use of psoraline, basic linear furacoumarin which helps with fungal protection. By oxidizing three different alcohols called coniferyl, coumaryl and synapyl via an enzyme called peroxidises, ligin is formed from many free radicals (ROS) which randomly connect to each other [22, 23]. This wood resists attempts by herbivores to eat it and also is too tough for insect pests to easily digest. Lignification stops the spread of pathogen and is commonly brought on by infection or injury. Plants use flavonoids for processes such as colour and protection. Besides proanthocyanins, flavones and anthocyanins, flowers have another group of flavanoids called flavanones and flavanols which collect in the epidermis and absorb harmful UV-B rays but still allow PAR light through. Plants grown under increased UV-B light have been shown to increase their production of flavanones and flavanols, suggesting flavonoids might help shield plants against harmful UV-B. Isoflavanoids are formed from naringenin, a common flavanones intermediate in plants and participate in the development and protection of plants. The legumes release these compounds and they help in the growth of nitrogen fixing nodules by teaming up with symbiotic rhizobia. Furthermore, these flavanoids appear to be useful in setting off mechanisms to eliminate reactive oxygen species (ROS). Examining the activity of antioxidant enzymes revealed that SOD, CAT, POX, APX, GPX and GR, having less activity than peroxidases, were the main active enzymes under Cu++ stress. Plants produce tannins which are

found in the second group of plant phenolic polymers that safeguard them [24]. Tannins which are found everywhere, lessen the chances of many herbivores surviving and also discourage many animals from eating the food. Plant samples must be chosen and prepared with care for extraction to work properly. My work during extraction is to decrease interference caused by compounds that may be taken together with the target, prevent any kind of contamination of the extract and make sure that the important metabolites and their structures are kept intact. To understand what makes a plant or its extracts active, researchers from many scientific fields often have to pull out the active compounds from the plant with solvents, as the first step in separating and identifying the responsible chemical. This research was started mostly because traditional pharmacopeias are built around plants and many significant medicine drugs are made from them. The increased notice that secondary products from organisms, in particular plants, are important for the environment, especially as messengers and for defense, leads to greater interest. It is quickly found by investigators that the preparation of plant material for testing and analysis cannot be done simply; experts should have excellent lab skills for this process.

Numerous secondary compounds help the plant by signalling, affecting other cells and moderating their metabolic processes. Certain flower colors allow plants to notify pollinators or to prevent animals from eating the plants which they achieve using special phytoalexines after being attacked by fungi. In some cases, plants use secondary metabolites to attract certain insects that will pollinate them and they use them to attract animals that disperse their seeds. At the moment, terpenoids, alkaloids and flavonoids are being used as medicine or dietary supplements because they seem to be effective in treating or stopping many diseases and some of these may even stop or inhibit cancer. Around 74% of pharmaceutical compounds from plants are thought to come from using knowledge about local remedies and around 14% to 28% of the species studied by herbalists are used in medicine. Secondary metabolites are created as part of cell function, differing between some groups of organisms and not essential for survival and they are formed by a wider variety of pathways than normal metabolites. The presence of monoterpenes or essential oils keeps the plants protected, mainly by guarding against both insect pests and pathogenic fungi. They are important in plant-plant exchanges and pull in pollinators. In addition to signalling, they indicate how related species are by their roles in the body. Some secondary compounds like cyanogenic glycosides, isoflavoids and alkaloids are likewise toxic to animals. Investigations to discover a less harmful compound for the treatment of bacterial infections and cancer keep continuing. Traditional medical treatments using plant-derived drugs are found in many countries for bacterial infections and cancers. Experts at the WHO conclude that plant-derived medicines provide major support for the essential health needs of about 75% of the world's rural population in less advanced countries. Herbal medicine's current growth may depend on the strong activities of active compounds found in plants. Many recent allopathy drugs might not be good for health and this necessitates finding effective alternatives; thankfully, compounds found in plants are a good option and safe, easy to practice and easily available. At this time, several pharma companies and research groups study plant-based substances as they are easy to find and useful for medicine. For incidence, several researches have explored plants and their key ingredients which have largely shaped effective antioxidants, anticancer drugs and antimicrobial agents. Study outcomes indicate that around 60% of the modern anti-cancer and antimicrobial drugs come from plants. Crude extracts from A. graveolens have the ability to kill many Gram-positive and negative bacteria. It is known that certain plant extracts and their bioactive substances have been shown to be antimicrobial. Especially among phytochemicals, flavonoids have been proven to show antibacterial properties. How antibacterial flavonoids work is well-known to include preventing changes in DNA, weakening bacteria's membranes and lowering their energy production. It is well known from studies that high

levels of ROS and RNS in combination with low natural antioxidant enzymes have been found in cancer in humans. A number of tumor cells found in the human body are known to be pro-oxidant, raise inflammation and increase stress related to free radicals. Rising these oxidative stresses gives tumor cells the ability to survive longer, as they increase mutations, redox responses and proinflammatory signals, chemokines, NF-kB and cytokines. Antioxidants alters the balance of oxidation reactions inside cells and makes cytotoxic therapy more effective. Various parts of A. graveolens extract in this study showed good antioxidant effects by scavenging NO, DPPH, ABTS, Lipid peroxidation and hydrogen peroxide [25]. Previous studies showed that extracts from the plant with antioxidant content were able to destroy cancer cells in cell cultures and had a positive effect against tumors in several animal tests. What enables plant-derived compounds to be cytotoxic and anticancer is often seen as activation of apoptosis, a reduction in proinflammatory signals and/or a blockage of angiogenesis.

Conclusion

This research found that Apium graveolens could help manage hyperglycemia. A decline in blood glucose levels during the treatment shows that the extract might be a beneficial option for people with diabetes. The effects of the extract were noticeable in the lowering of lipid measurements which may mean that it works to control diabetes-related lipid issues. Considering the effects of uncontrolled diabetes, Apium graveolens could help people with diabetes avoid one of its frequent complications. Combining these studies, it is possible to suggest that Apium graveolens might help to control high blood sugar and protect from diabetes side effects. Still, despite the positive results, extra studies are required to determine how effective and safe it can be in treating humans. It is also necessary to conduct complete toxicology studies to make sure that Apium graveolens can be safely consumed and used as medicine by people. A methanolic extract of Celery (Apium graveolens L.) was tested and found to have bioactive phytochemical materials. Bioactive chemical compounds are identified by using their peak area, the time they take to separate, their molecular weight and their molecular formula. Getting ready for FTIR analysis, there were thirteen functional groups to be considered: peak (wave number cm-1), (type of intensity) and (bond and group assignment). Bioactive substances in celery (Apium graveolens L.) were discovered to act nicely against Enterococcus faecalis.

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