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Review Article

Antibacterial against Four Pathogenic Bacteria of *Apium graveolens* and Screening Its Natural Chemical Constitutes by GC-MS Technique

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Abstract

Many Asian countries use celery, a member of the Apiaceae family (Apium graveolens L.), as a vegetable and seasoning. Leaf, stem, root, and seed are all parts of the plant that are commonly used in cooking, especially for salads and soups. The unusual perfume and essential oil make it very popular. Thanks to its abundance of secondary metabolites including flavonoids, alkaloids, terpenoids, and phenolic acids, as well as its vitamin and carotenoid content, this plant is a wonderful source of nutrition. Asthma, bronchitis, hypertension, diabetes, gastrointestinal issues, urinary calculi, visceral spasm, impotence, and hepatitis are just a few of the many problems that the plants are traditionally used to treat in folk medicine. Aiming to analyze the chemical composition of (Apium graveolens) and its in vitro antibacterial activity was the objective of this investigation. The capacity of specific compounds to either kill or prevent the growth of dangerous bacteria is known as their antibacterial activity. Conventional antibiotics do this by acting on essential bacterial components like cell walls, while natural chemicals derived from plants, such as extracts, can halt the development of biofilms and bacterial cell division. The following compounds were found in the metabolic profile: palmitate, methyl linoleate, oxirane-2-carboxamide, phytol, alpha-Campholenal, trans-9-octadecenoic acid, n-hexadecanoic acid, 2-cyclopropylethanol, 2-(hexadecanoylamino)acetic acid, L-ascorbic acid, dihexadecanoate, (Z)-9-octadecanoic acid, cyclohexanol, 4-methyl, 7,9-octadecadiynoic acid, 6-Heptadecanol, Oxacyclohexadecane-2,13-dione, and 3-Methyl-1-cyclopentadecanone. The results of the Tri-Comparison test against Staphylococcus aureus were 22.08±0.39, 12.00±0.25, 35.71±0.58, and 28.06±0.53 for the crude methanol and ethanol fraction extracts, respectively, compared to the two standard antibiotics RF-Rifampicin and CRO-Ceftraxone. Regardless, it was found to be associated with Escherichia coli (27.00±0.36, 18.07±0.28, 33.19±0.54 and 24.00±0.41). Record Streptococcus pyogenes at the same time as (23.89±0.40, 19.74±0.29, 30.25±0.52, and 26.15±0.44). In contrast, Pseudomonas aeruginosa was found to have concentrations of (17.03±0.25, 21.59±0.29, 25.85±0.47 and 29.00±0.45). The metabolites of Apium graveolens were highly active against Escherichia coli, with an activity level of 27.00±0.36. Finally, the antibacterial activity of Apium graveolens is significant and has great promise for use in agriculture and the food business. The goal of this research is to identify potential chemicals for use in developing novel, naturally occurring antibacterial medicines with increased efficacy.

Keywords: Pathogenic Bacteria, Apium graveolens, Antibacterial activity, Natural Chemical Constitutes.

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Introduction

As the proportion of drug-resistant microbes continues to rise, medicinal plants are gaining popularity for their antioxidant and antibacterial properties, which make them useful all around the globe. A number of disease classes have evolved multidrug-resistant strains, and antibiotic misuse is a major contributor to this problem[1,2,3]. Several clinically significant chemicals have been validated by substantial research into the antibacterial activities of several medicinal plants and their byproducts. Medicinal, culinary, pharmacological, and alternative uses of plant extracts and derivatives date back hundreds of years[4,5]. Some plant extracts, including those from fruits, vegetables, spices, and herbs, have shown promise as antioxidants, anti-inflammatory agents, anti-cancer agents, anti-aging agents, and microbe fighters. Alkaloids, phenolic acids, terpenes, glycosides, and flavonoids are some of the principal bioactive chemicals that give this plant its antibacterial and antioxidant characteristics. There are a variety of vegetables that offer antioxidant, anti-inflammatory, anti-aging, anti-cancer, and antibacterial characteristics[6,7,8,9]. Using plant extracts and derivatives, previous research has shown that numerous plants have antibacterial and antifungal properties against common fungi and a variety of intestinal bacteria. The essential oil and numerous coumarin-rich chemicals found in A. graveolens seeds give the plant its characteristic perfume. Celery possesses a wide range of pharmacological effects, such as protecting the liver, decreasing cholesterol, fighting inflammation, alleviating pain, protecting the heart, preventing infertility, killing larvae, and repelling mosquitoes. Researchers in Iraq have shown that celery has antibacterial and antioxidant properties. Unfortunately, celery has not been studied for its antioxidant, antibacterial, or cytotoxic properties in Saudi Arabia as yet. There is a great deal of variation in the phytochemical composition of plants both within and across species[10,11]. Numerous external elements govern it, such as longitude, latitude, rainfall, ambient temperature, soil moisture, photoperiods, and physicochemical characteristics of water. Plants are able to withstand salt stress and adapt by increasing their osmotic tension and producing a wide range of phytochemicals, such as essential oils, organic acids, alkaloids, and saponins, which serve to both attract insects and ward off predators. Antimicrobials are chemicals that prevent the growth of bacteria, hence killing the microbes. A problem known as bacterial resistance has emerged due to the overuse and overabundance of antibiotics. Animals and humans alike have been dealing with the problem of antibiotic resistance for quite some time now[12,13,14,15]. That is why finding effective alternatives to conventional medicine is a top priority. There has to be a new antibacterial agent discovered because bacteria are becoming increasingly resistant to existing ones. Many infections, including those of the respiratory system, the skin, the genitourinary tract, and wounds, are caused by Gram-positive bacteria such Staphylococcus sp. One of the most common types of nosocomial infections is caused by Staphylococcus aureus. It can withstand several types of antibiotics. Species that do not produce coagulase An essential commensal microbe of human skin and mucous membranes is Staphylococcus epidermidis, a member of the Staphylococci family. The most common nosocomial pathogen, Staphylococcus epidermidis, is currently the most frequently isolated bacterium in clinical culture[16]. The development of numerous herbal antimicrobials has provided an alternative for the treatment of a wide range of ailments. The majority of antimicrobials come from all-natural sources, like spices and plants. In traditional medicine, the leaves of plants such as celery, betel, papaya, soursop, gambier, and others are employed. Celery (Apium graveolens) is one plant that has been employed as an antibacterial agent. Celery seed extract has a long history of usage as a remedy for gout, rheumatism, pain associated with rheumatism, and other rheumatic disorders[17,18]. Our study's objectives were to screen its natural chemical constituents using GC-MS and to evaluate its antibacterial activity against four pathogenic Apium graveolens bacteria.

Material and Methods

Steps for making the plant essences

We got Apium graveolens L. from the grocery store down the street. In order to prepare the A. graveolens L. plant for solvent extraction, it was sprayed with tap water, then air-dried at 37oC, and then used. Cold maceration by ethanol (70% concentration) was applied to several plant sections. We let the mixture evaporate naturally and stored the leftover crude extract in the freezer for when we need it for further research. Following the previously mentioned standard procedures, the entire plant was hydroethanolically suspended and tested for phytochemicals.

Analyzing GC-MS Components

An approach that utilizes volatile bioactive chemicals is the gas chromatography–mass spectrometry (GC–MS) technique, which involves separating the essential oil components by gas chromatography and then determining their molecular weights by mass fragmentation in MS. When an organic vapor is passed through the mass spectrometer's ionization chamber, the material will be bombarded with high-energy electrons, which will then scatter both the substance's own electrons and the high-energy electrons.

Effectiveness Against Microbes

When it comes to antimicrobial action, the cup-plate approach was used to test for both bacterial and fungal effects. The specified sterile plates were then dispersed with Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, and Pseudomonas aeruginosa. The plates were prepared by using a sterile bore to create holes with a diameter of 5 mm. Following a one-hour incubation period at 4oC to allow the usual antibiotics and plant extracts to diffuse into the agar medium, the plates were incubated at 37ºC for the next twenty-four to forty-eight hours. Determination of the Lowest Effective Concentration: MIC was conducted using two-fold dilution using various plant parts. An equal volume of media was distributed out among the organisms being tested, and test tubes were used in sequence for this purpose. Typically, a two-fold dilution was used after a smaller volume of test medications was added to the tubes. Here, a positive control was maintained by keeping one tube empty of the test medication. For each strain, the cultures were kept in the incubator at the appropriate temperature (for bacteria, this is 37 ℃ for 24 hours; this is required for up to fifteen generations of strain multiplication). The organisms' growth was then observed by turbidity measurements; however, the presence of antimicrobial agents in the tube rendered the tube transparent, preventing the growth of the organisms. Therefore, MIC is the drug concentration in the transparent tube at which no growth was seen at the lowest possible drug concentration.

Microorganisms and Well Diffusion Agar Method.

The present investigation utilized lyophilized and sub-grown bacterial strains S. aureus, E. coli, S. pyogenes, and Pseudomonas aeruginosa, all of which were cultured under sterile conditions at 37 C. After preparing the stock culture for subcultivation on slant nutrient agar, it was washed multiple times in sterile ringer solution. A microbial solution with a concentration of 1.5108 CFU/mL was achieved by measuring changes in the optical density of the suspension at 630 nm, which is half of the McFarland standard. The 6 mm filter paper discs coated with extract were placed on the infected plates after being streaked throughout the Petri dishes containing the microbial suspension. The next step was to incubate them at 37 oC for 24 hours. The radius of the filter paper rounds was measured in centimeters. The next step was to prepare the surface by drilling 6 mm diameter wells in the medium and then adding 20 uL of the extract to each. Millimeters were used to measure the well diameters and the surrounding inhibition zones. The petri dishes were maintained at 37 oC for 24 hours.

Data Analysis by Statistic

The average inhibition zone diameter plus or minus the standard error of the mean was used to

describe the facts that were gathered. The data that were documented were analyzed using one-way ANOVA. The data analysis was conducted using SPSS version 20.0.

Results and Discussion

The chemical components of Apium graveolens (celery) can be identified by GC-MS analysis, which reveals a number of substances in the plant's seeds, leaves, and roots, among other sections. Celery has a long history of usage in traditional medicine for a variety of purposes, including as an antibiotic, antioxidant, and cholesterol-lowering agent. Modern research has shown that these effects are due to the presence of phenolic chemicals, flavonoids, alkaloids, and terpenoids in celery extracts. Quality control, pharmaceutical research, food and fragrance analysis, and the identification of bioactive chemicals are just a few of the many fields that benefit greatly from GC-MS applications in plants, which are essential for assessing volatile and non-volatile organic compounds[19,20]. The chemical makeup of plant extracts, active principles, fragrance profiles, and authenticity and purity of herbal items can be examined using this method. The following compounds were found in the metabolic profile: palmitate, methyl linoleate, oxirane-2-carboxamide, phytol, alpha-Campholenal, trans-9-octadecenoic acid, n-hexadecanoic acid, 2-cyclopropylethanol, 2-(hexadecanoylamino)acetic acid, L-ascorbic acid, dihexadecanoate, (Z)-9-octadecanoic acid, cyclohexanol, 4-methyl, 7,9-octadecadiynoic acid, 6-Heptadecanol, Oxacyclohexadecane-2,13-dione, and 3-Methyl-1-cyclopentadecanone.

Table 1. Apium graveolens bioactive secondary metabolites by using GC-MS technique.

No.	Compound	Molecular Formula and Molecular weight	No.	Compound	Molecular Formula and Molecular weight
1.	Palmitate	MF: $C_{16}H_{32}O_2$ MW: 256.42 g/mol	9.	2-(hexadecanoylamino)acetic acid	MF: $C_{23}H_{45}N_3O_6$ MW: 459 g/mol
2.	Methyl linoleate	MF: $C_{19}H_{34}O_2$ MW: 294.5 g/mol	10.	L-Ascorbic acid, dihexadecanoate	MF: $C_{38}H_{68}O_8$ MW: 652 g/mol
3.	Oxirane-2-carboxamide	MF: $C_3H_5NO_2$ MW: 87 g/mol	11.	(Z)-9-Octadecanoic acid	MF: $C_{18}H_{34}O_2$ MW: 282 g/mol
4.	Phytol	MF: $C_{20}H_{40}O$ MW: 296 g/mol	12.	Cyclohexanol, 4-methyl	MF: $C_{26}H_{33}N_3O_4$ MW: 451 g/mol
5.	alpha-Campholenal	MF: $C_{10}H_{16}O$ MW: 152 g/mol	13.	7,9-Octadecadiynoic acid	MF: $C_{18}H_{28}O_3$ MW: 292 g/mol
6.	trans-9-Octadecenoic acid	MF: $C_{18}H_{30}O_4$ MW: 310 g/mol	14.	6-Heptadecanol	MF: $C_{17}H_{36}O$ MW: 256 g/mol
7.	n-Hexadecanoic acid	MF: $C_{21}H_{42}O_2Si$ MW: 354 g/mol	15.	Oxacyclohexadecane-2,13-dione	MF: $C_{15}H_{26}O_3$ MW: 254 g/mol
8.	2-cyclopropylethanol	MF: $C_5H_{10}O$ MW: 86 g/mol	16.	3-Methyl-1-cyclopentadecanone	MF: $C_{16}H_{30}O$ MW: 238 g/mol

Bioactive chemicals, such as flavonoids and phenolic substances, provide Apium graveolens (celery) its antibacterial characteristics. Research has shown that it can effectively combat a range of bacteria, including common infections such as Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. In addition to its use in antibacterial hand sanitizers and as a topical treatment for skin infections, research indicates that it may have other potential uses[21,22,23]. Traditional medicinal plant knowledge applied to modern research yields a fresh approach that generates medicine encounter rates significantly higher than in random meetings. Glycosides, tannins, saponins, flavonoids, steroids, terpenoids, alkaloids, carbohydrates, proteins, and anthraquinones were identified in the A. graveolens during preliminary phytochemical screening in this study. In the past, it has been stated that A. graveolens extract contains furocoumarins, luteolin-glycosides, β-pinene, β-phellendrene, terpinolene, camphene, γ-terpinene, cumene, limonene, α-pinene, p-cymene, sabinene, and α-thuyene, in addition to related furocoumarins, which possess anticancer, antibacterial, and antifungal action[24]. Crude A. graveolens extracts have potent antimicrobial action, killing a wide variety of bacteria, viruses, and fungi. Evidence suggests that plant extracts and the bioactive components within them possess antibacterial properties. There is evidence that the most potent phytochemicals, particularly flavonoids, have antimicrobial properties. Inhibition of DNA topoisomerase, breakdown of plasma membranes, and impairment of microbial energy metabolism are the established mechanisms by which flavonoids exert their antibacterial activity. It has been proposed that these phytochemical components, which have antibacterial and antifungal properties, can either work alone or in combination with other metabolites to give the plant more capabilities. Both reactive oxygen species (ROS) and reactive nitrogen species (RNS) play important roles in the development and progression of tumors in humans. It is well-documented in carcinogenesis that increased levels of ROS and RNS, as well as decreased levels of endogenous antioxidant enzymes in human tissues, are present. The fact that many human tumor cells are pro-oxidants that increase inflammatory responses and oxidative stress has long been known[25,26,27]. By increasing and activating mutations, redox reaction signaling, proinflammatory factors, chemokines, NF-kB, and cytokines, and oxidative stress, increased oxidative stress enhances the tumor cells' enduring potential. Several bioactive components found in CE may have antioxidant and antibacterial effects. This study shows that at a dosage of 0.1%, CE suppresses the growth of MRSA in vitro. One of the most noticeable components of herbal extracts, including CE, is phenolic compounds. There is promising research on phenolic compounds as antimicrobial agents. They do their work by interacting with peptidoglycans found on the bacterial cell membrane. The binding of phenolic-peptidoglycans alters the permeability and stiffness of the membrane. Additionally, research has shown that inhibiting peptidoglycan formation and β-lactamase, leading to impaired membrane permeability, can be achieved by using a herbal antioxidant derived from Stephania suberosa, which includes alkaloids. Because of these processes, MRSA is unable to colonize test tubes when exposed to low osmotic and high ionic pressure. Reducing bacterial colonization within the wound matrix was one of the potential effects of CE cream shown in the in vivo investigation[28]. Fewer bacteria colonizing the tissue means fewer leukocytes infiltrating and less oxidative stress. In addition, the exogenous antioxidants in the CE cream boost the immune system's and cells' ability to respond to wounds, which speeds up the healing process. The endogenous antioxidant levels in injured tissue cells can be affected by antioxidants like tannins, flavonoids, phenolics, alkaloids, and sterols. During wound healing, cells with higher amounts of endogenous antioxidants are better able to repair, invade, and multiply[29,30]. Furthermore, ROS levels in injured tissue are decreased by the combined actions of radical scavenging activity and elevated endogenous antioxidant levels. This study demonstrated these pathways and found that inflammatory cell infiltration was minimal as compared to the control group.

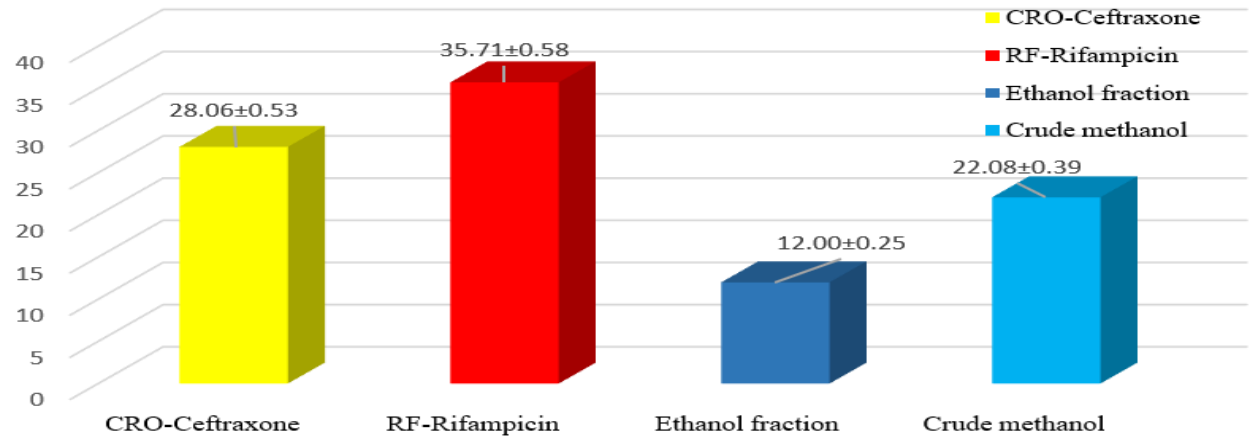

Figure 1. Antimicrobial activity of Crude methanol and Ethanol fraction extract *Apium graveolens* against pathogenic bacteria *Staphylococcus aureus*

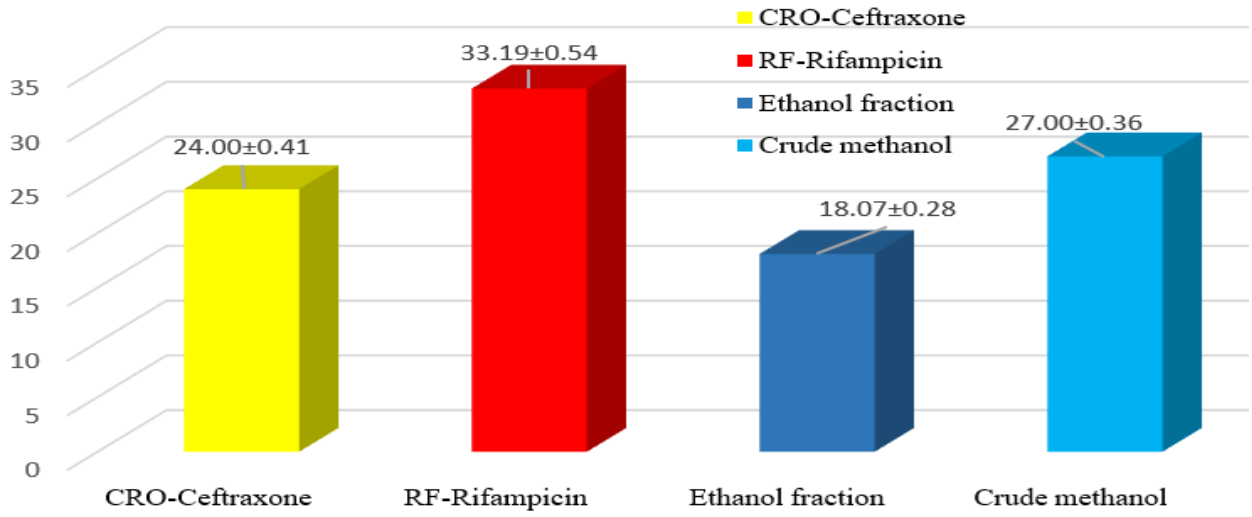

Figure 2. Antimicrobial activity of Crude methanol and Ethanol fraction extract *Apium graveolens* against pathogenic bacteria *Escherichia coli*

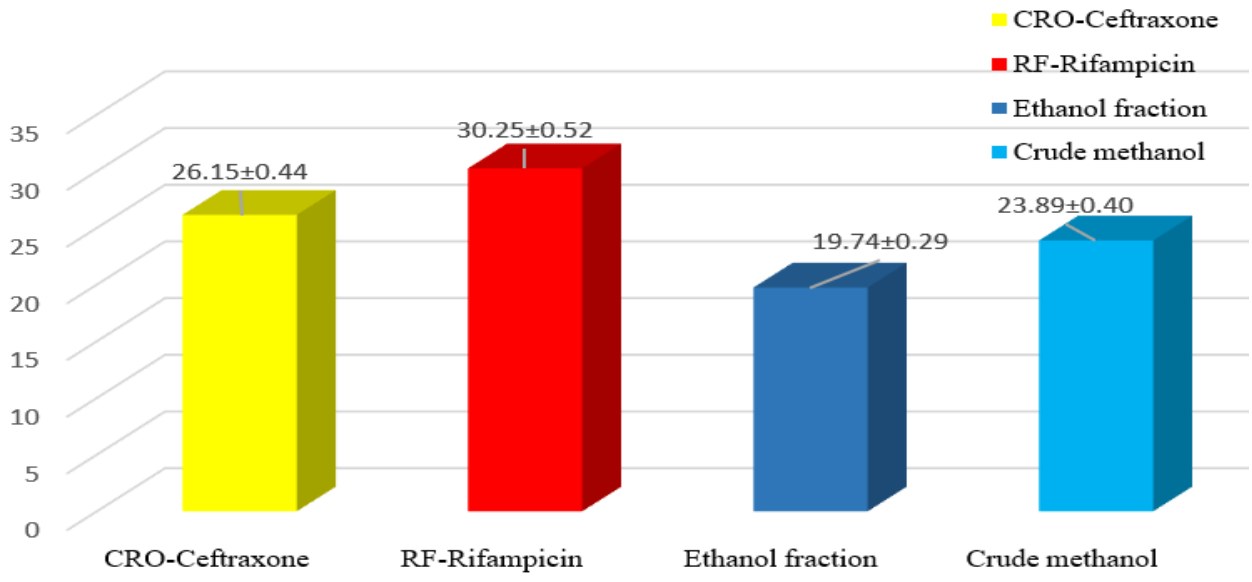

Figure 3. Antimicrobial activity of Crude methanol and Ethanol fraction extract *Apium graveolens* against pathogenic bacteria *Streptococcus pyogenes*

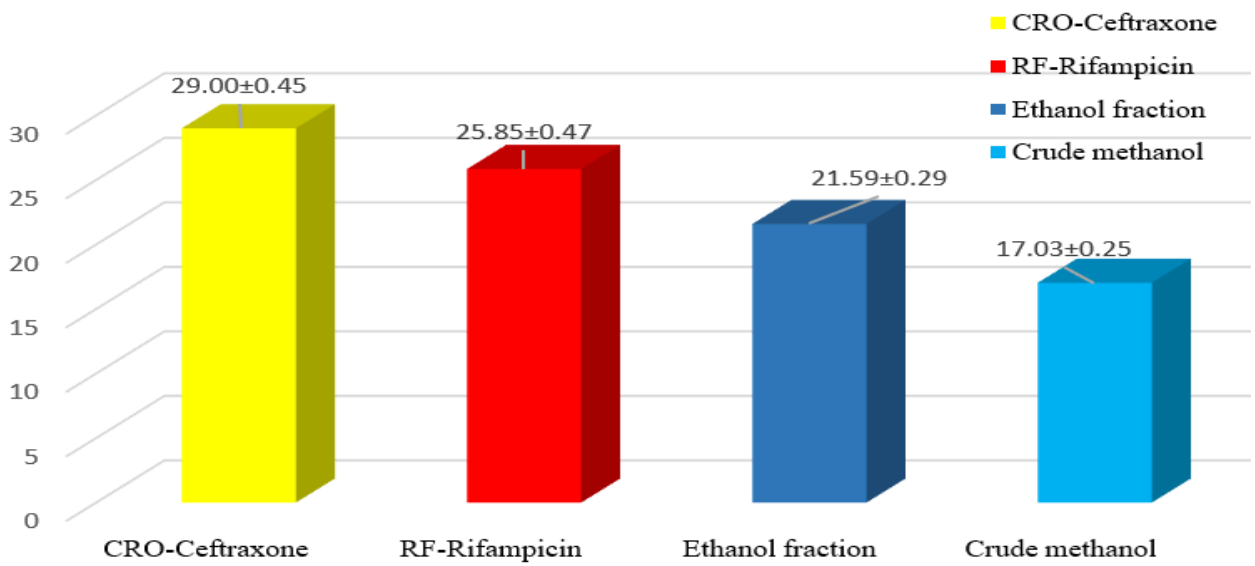

Figure 4. Antimicrobial activity of Crude methanol and Ethanol fraction extract *Apium graveolens* against pathogenic bacteria *Pseudomonas aeruginosa*

The results of the Tri-Comparison test against Staphylococcus aureus were 22.08±0.39, 12.00±0.25, 35.71±0.58, and 28.06±0.53 for the crude methanol and ethanol fraction extracts, respectively, compared to the two standard antibiotics RF-Rifampicin and CRO-Ceftraxone. Regardless, it was found to be associated with Escherichia coli (27.00±0.36, 18.07±0.28, 33.19±0.54 and 24.00±0.41). Record Streptococcus pyogenes at the same time as (23.89±0.40, 19.74±0.29, 30.25±0.52, and 26.15±0.44). In contrast, Pseudomonas aeruginosa was found to have concentrations of (17.03±0.25, 21.59±0.29, 25.85±0.47 and 29.00±0.45). The metabolites of Apium graveolens were highly active against Escherichia coli, with an activity level of 27.00±0.36. We used the paper-disk plate method to measure the inhibition zone and see how the essential oils of Apium graveolens affected the development of bacteria and yeast. The results of this study show that Apium graveolens, when consumed whole, has a wide range of bioactive components that are highly effective against bacteria and other microbes. The identification and description of the active components accountable for bio-efficacy and bioactivity will be the focus of future research. Many research have examined the antioxidant effects of flavonoids and other phenolic compounds, which are abundant in plants. These chemicals have a variety of biological activities and have been studied in relation to conditions like cancer, diabetes, and coronary heart disease. Because of their antioxidant properties, medicinal herbs reduce the toxicity of chemical medications and have fewer adverse effects overall. The minimal amount of negative effects associated with herbal pharmaceuticals is the primary reason why they are being used as an alternative to chemical drugs nowadays. In addition to its two monographs on medicinal plants that include information on pharmacopoeial summaries for quality assurance, the World Health Organization (WHO) promotes the use of safe and effective traditional remedies and practices in both public and private health services. These monographs cover topics such as distribution, identification tests, purity requirements, chemical assays, clinical applications, pharmacology, potential adverse reactions, warnings, and posology. Solely the extract was responsible for the reported antibacterial activity, as the ethanol-free test verified that ethanol was not present. The results of the phytochemical screening confirmed the presence of tannins, alkaloids, flavonoids, and saponins, which align with previous research on the bioactive characteristics of celery. In terms of smell, color, and consistency, the physical examination of the gel formulations showed that the concentration of celery extract affected the

results. A formulation with 100% extract failed the spreadability test due to its liquid consistency, in contrast to formulations with 25%, 50%, and 75% extract, which had semisolid consistency and satisfied ideal spreadability norms. After passing the homogeneity test, all of the formulations were found to be devoid of lumps and coarse grains, and their pH levels were within the safe range for skin application. Previous study has shown that there is a high association between the concentration of the extract and the size of the inhibition zone in the antibacterial test. The classification by Davis and Stout showed that formulations containing 25%, 50%, and 75% celery extract had strong antibacterial activity (<20 mm inhibition zone), whereas the 100% extract formulation and positive control both had very strong antibacterial activity (21.16 mm inhibition zone and 51.45 mm inhibition zone, respectively). Celery extract's antimicrobial properties are due to the secondary metabolites it contains. Bacterial cell walls are disrupted and eventually killed by alkaloids because they interfere with peptidoglycan production. Flavonoids are lipophilic, which means they can harm cell membranes. They also hinder the formation of nucleic acids and disrupt the respiratory chains of bacteria. Biofilm breakdown and cell membrane rupture are caused by saponins penetrating cell membranes through interactions with sterols. Tannins kill bacteria by preventing them from adhering to surfaces, absorbing sugar and amino acids, and growing.

Conclusion

Evidence suggests that an A. graveolens crude extract has antimicrobial properties. For your consideration, the plant extract has bioactive components that are known to prevent the growth of several types of bacteria, including Gram-positive, Gram-negative, and fungal strains. Given its high concentration of antibacterial activity, the facts shown above support the conclusion that A. graveolens is a useful plant for disease prevention. Despite the isolation and identification of most of the bioactive substances enabling such functional trait, it is clear that the molecules vary depending on the species of plant, conditions of collection, and method of analysis used to characterize those features. It is inevitable that A. graveolens will include more strong antibacterial compounds. An excellent source of antioxidants and an effective antimicrobial, celery (A. graveolens) extract is a win-win. Additionally, it improves wound healing process.

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