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Original Research Article

The Study Examines Antimicrobial along with Antioxidant [Hypochlorous acid, Hydroxyl Radical Scavenging] Properties of Celery (*Apium graveolens* L.) and Monitoring Its Essential Chemical Compounds

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Abstract: Natural active products from plants vary depending on the chemical composition each has. Because extracts and chemicals from plants have activity in test tubes and animal models, people now see them as better alternatives for medicine. Due to coumarin, essential oils, and a number of acids along with apigenin, luteolin, and kaempferol, Apium graveolens helps to eliminate free radicals in the body. Celery made up of different chemicals and different concentrations is capable of treating a range of diseases. ***Objective*:** In this study, the antibacterial and antioxidant capabilities of celery (*Apium graveolens* L.) were examined. The antioxidant properties included hypochlorous acid and hydroxyl radical scavenging. ***Methods*:** The sample powder was mixed with 150 ml methanol and allowed to stand in the shaker for 16 hours. Filter paper made by Whatman No.1 was used to separate the extract made from the plant. Phytochemical analysis was done on the filter solution obtained. The mixture was once again treated with sodium sulphate to get rid of any remaining water. The investigators assessed the antifungal actions of Celery extracts and the antibiotics Voriconazole (VCZ), Fluconazole (FCZ) and Amphotericin B (AmB). At this point, the researcher measures the size of the inhibition zone. ***Results*:** Examination of different compounds relies more on chromatography than on other analytical instruments. Many times, it is applied to accurately analyze and measure the sample substance. It mainly helps separate and study various compounds mixed in a mixture. The main components with their likelihood, the molecules involved, and the mass weight found in the mass spectrum. Twelve compounds found to be present in the ethanolic extract were D-limonene, beta-Myrcene, (-)-trans-Caryophyllene, (+-)-Linalool, (-)-alpha-Thujene, 4-Carvomenthenol, P-CYMENE, BETA-PINENE, (E)-3-Isobutyliden-ephthalide, E)-beta-ocimene, ,8-Octanedicarboxylic acid, and (-)-trans-Caryophyllene. Celery (*Apium graveolens* L.) in ethyl acetate and ethanolic extracts as well as certain antibiotics VCZ, FCZ and AmB, all have antifungal properties. The metabolites of Celery (*Apium graveolens* L.) were very active against *Aspergillus flavus* (25.11 ± 0.51). The antioxidant ability [Hypochlorous acid and Hydroxyl Radical Scavenging] of fruit extract (Ethyl acetate, Ethanol and standards) of *Apium graveolens*. Types of extracts such as ethyl acetate fraction, ethanol fraction, and standard were carefully documented: 107.49 ± 5.04, 121.53 ± 6.71 and Ascorbic acid (standard) 201.89 ± 8.06 respectively of Hypochlorous acid radical scavenging. While recorded 307.99±26.00, 238.20± 21.07, and Mannitol (standard) 541.98± 30.37 respectively Hydroxyl radical scavenging potential.

Keywords: Hypochlorous Acid, Hydroxyl Radical Scavenging, Antimicrobial Properties, *Apium Graveolens* L.

INTRODUCTION

Medical use of plants by our ancestors goes back far into history. Many research articles demonstrate that using some herbs and different medicinal plant parts can positively influence cancer, infectious diseases [1-3], diabetes, and atherosclerosis. A lot of studies have examined the antioxidant effects of phenolic and alkaloid compounds found in plants, especially on cancer, diabetes, liver problems, and coronary heart diseases. Many people turn to herbal drugs today since they usually cause fewer side effects than chemical medicines. Celery (*Apium graveolens* L) comes from the apiaceae

group, and it's one of the annual or perennial plants that flourishes across Europe, Africa and Asia. In the making of certain foods with a special fragrance and taste, celery seeds are commonly added as a condiment [4, 5]. A number of phthalide derivatives in the celery essential oil are responsible for its typical scent. Celery (*Apium graveolens*) has been used in traditional medicine because of its many health benefits. Eating celery may help deal with arthritis, rheumatism, gout, and urinary tract inflammation, even in cases of rheumatoid arthritis that often brings mental depression. Antioxidants that can get rid of radicals are believed to help protect the body from free radical damage. Therefore, these molecules reduce the chance of developing coronary, vascular, and tumor diseases since they prevent harmful oxidative reactions. Free radicals trigger oxidation, which causes harm to lipids or DNA among other tissues [6-9]. However, the usual synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) are not allowed to be used much since they can be harmful and cause cancer. Now that synthetic antioxidants are removed from food products, everyone is looking for more natural antioxidants [10, 11]. Even in ancient days, people used parts of medicinal plants to address multiple health conditions. Applying natural treatments does not cost much. A number of investigations found that different herbs and medicinal plants positively affect infertility, hormone disorders, liver malfunctions, anemia, renal diseases, and problems of the brain and mind [12, 13]. It has been shown in many researches that flavonoids and similar compounds found in plants, such as antioxidants, may act in the prevention of coronary heart diseases, diabetes, and cancer [14]. Examples of the phytochemicals in celery are carbohydrates, phenols, apparently flavonoids, alkaloids, and steroids. Because celery contains limonene, selinene, frocoumarin glycosides, flavonoids, vitamin A, and vitamin C, it is the most commonly used plant in traditional medicine. Celery acts as a prevention for diseases of the heart, jaundice, liver and liver stones, problems in the urinary tract, gout, and rheumatic difficulties. Experiments in rats prove that ethanol extracts from celery leaves can boost sperm production and their fertility. Eating celery decreases glucose, lowers blood lipids, and control blood pressure, all of which may help the heart. Experiments prove that celery contains substances with antifungal and anti-inflammatory abilities. Besides, the essential oils from these plants kill bacteria. Elecampane is helpful in treating bronchitis, asthenopia, asthma, chronic skin problems that include psoriasis, vomiting, fever, and tumors. The root of celery is considered diuretic and it is used for dealing with colic. Plants are a main provider of active natural products that differ according to their structure and functions. Many phytochemical compounds and especially polyphenols are responsible for gathering free radicals and antioxidant functions in plants. It has been established that polyphenols bring biological effects [2-20]. Using these two approaches, especially their antioxidants, helps to avert free radicals and prevent the spread of peroxidation. Since polyphenols are similar in chemical structure, some of their groups can interact with hydrogen donors and fight off free radicals. There have been multiple studies about celery's antioxidants. Celery's phenolic and antioxidant substances have caught the attention of several scientists. The presence of celery root and its leaves is able to decrease OH and DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals, and the plant is also able to weaken liposomal peroxidation, which is a sign of its protection. The sample contained plenty of apigenin and the main phenolic acid found was p-coumaric acid. Studied plants contained a lot of phenolic compounds and had high antioxidant activity. Among the extracted compounds, methanol itself had the highest amount of antioxidant activity. There was almost 10% difference in antioxidant activity between the methanol extract (63.28% + 0.86%) and the diethyl ether extract (54.04% + 0.21%). Once again, the antioxidant activity of the plant's seed was the highest in its whole methanol extract. Supplementing with added luteolin and flavonoids cut down the levels of free radicals, but on the other hand raised SOD, an enzyme protecting the body from its negative effects. For this reason, these compounds could be the reason behind celery seeds exhibiting antioxidant properties [21-23]. Tests on the photochemistry of celery phytochemicals in the presence of flavonoid, tannins, saponins, and terpenoids were found to show the absence of terpenoids. Effectiveness of antioxidants present in the celery and seeds powder volatile oils was studied through Rancimat and DPPH. The test outcome found that every added essential oil that had a different concentration possessed antioxidant activity [24, 25] so all the mixtures or singularly added essential oils had an antioxidant effect. The main objective of this work was to test the antimicrobial activity and antioxidant properties (involving hypochlorous acid and hydroxyl radical scavenging) of Celery (*Apium graveolens* L.).

MATERIALS AND METHODS

The leaves were bought at an Hilla city market, located in the middle of Iraq. After all dirty material was taken off and the leaves were clean, they were immediately put into an airtight container to avoid the influences of humid air. About 30 grams of plant sample including its powder were soaked in 150 ml of methanol for a span of 16 hours in a rotatory shaker. Whatman No.1 filter paper was chosen to separate the extract of the plant. The filtrates were analyzed further to find phytochemicals. It was handed over for a second time to sodium sulphate to get rid of leftover moisture.

GC/MS Investigation and Identification of Components:

Study of the active substances in the bioactive samples was carried out through GC/MS (Shimadzu QP-2010). Hydrogen was used as the gas in the equipment. Sample was split less into the GC using 1 mL, Injector and interface heater-temperature was 270°C, the oven-temperature went from 50 to 280°C at a speed of 3°C in a minute, and electron energy for the mass spectrometer was set at 70 eV with the ionizer at 230°C. By looking at a homologous series of n-alkanes (C8-C28), as well as with NIST and WILEY MS library searches, RI is determined. The number of individual organic molecules was found by measuring the peak area with GC [26, 27].

Investigating the Antifungal Effects of a Metabolite-Rich *Apium graveolens* L. Leaf Extract

For this purpose, twenty food samples have been collected from Babylon and Karbala Province to find out the fungi involved in the poison. To check and study the samples, the samples were sent to the specialized mycology lab of Babylon University. *Apium graveolens* L. leaf metabolite extract was studied for antifungal qualities with the help of the mixing technique and SDA. As a result, 1 mL was put into a Petri dish. The dishes were set aside after the SDA medium was added on top. Also, a 5 mL disc was produced from each fungus with a sterile borer and then inoculated on the culture medium [28, 29]. Then, the dishes with the petry are kept at 25°C ± 2 for 7 days. The size of the zone stopped by the inhibiting activity of the extracts, which developed on the agar, was checked and noted in millimeters (mm).

Hydroxyl Radical Scavenging

Assays count on making the product of 2-deoxyribose together with TBA. Chemicals in a sample produced hydroxyl radical by reacting as the Fenton reagent. There was 2.8 mM 2-deoxy-2-ribose, a pH 7.4 buffer solution, ascorbic acid (100 μM) and the reference or test sample present at different concentrations (0–200 μg/ml) in a final volume of 1 ml. The mixture was incubated at 37°C for 1 h after which 0.5 ml of it was added to 1 ml of 1% aqueous TBA and all this was incubated at 90°C for 15 min to develop the color. When the sample reached room temperature, its absorbance was measured at 532 nm with a suitable blank [30, 31]. All the tests were done six times. Mannitol has been regularly used to ensure the effectiveness of OH. scavengers, so we chose it as our positive control. The extent of inhibition was found by comparing the test with the blank solutions.

Hypochlorous acid Scavenging

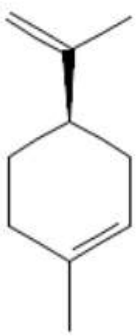
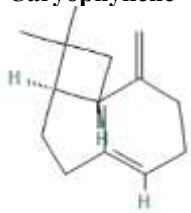
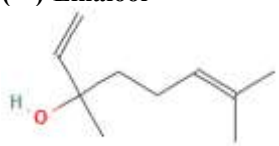
To prepare HOCl, 0.6 M H2SO4 was used to make the NaOCl solution's pH 6.2, and then its concentration was measured at 235 nm according to its molar extinction coefficient of 100 M-1 cm-1. The scavenging of free radicals was measured by checking for a drop in catalase absorbance at 404 nm. In 1 ml total volume, the reaction system had 50 mM phosphate buffer, and varying amounts of plant extract. After 20 min at 25°C, the samples were measured against a plain blank to get the absorbance. The test was performed six times for every experiment. In addition, ascorbic acid was added as a reference in this study [32].

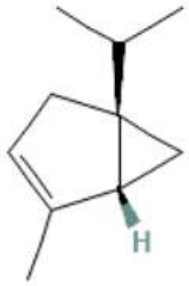
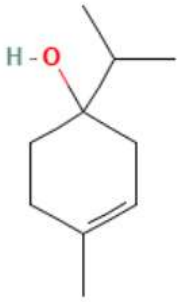
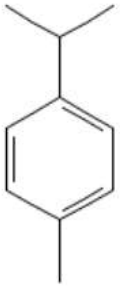
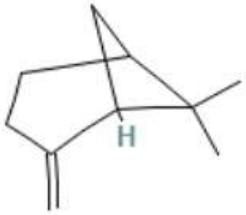
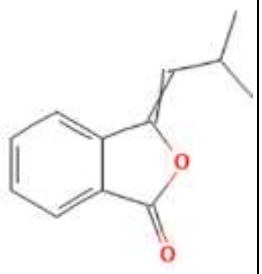
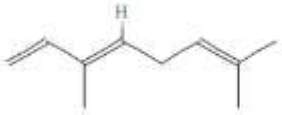
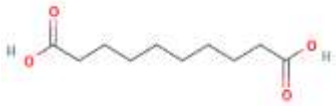
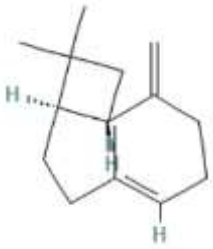
Statistical Analysis

All the tests' mean values and standard deviations were determined by the statistical software SPSS 16.0. The measurements for IC50 were carried out three times, and Microsoft Excel 2008 was used to get IC50 by drawing a graph of inhibition percentage versus oil concentration and then using linear regression.

RESULTS AND DISCUSSION

Chromatography is more important than most other instruments when it comes to examining different compounds. It helps to accurately find and analyze the sample substance in a precise manner. The technique is mainly for separating and studying mixtures made of organic compounds. When chromatography combines with other analysis techniques, it becomes simpler to recognize and identify different compounds in mixtures. A combination of GC and mass spectrometry (GC-MS) ensures an accurate result and the steps used to measure Luteolin are based on those for finding any flavonoid. For the analysis of Luteolin's derivatives, scientists have used Mass Spectrometry. Scientists use the key aspects called active principles along with the possible outcomes, the chemicals' formulas, and their weight in the mass spectrum. Among the twelve substances detected in the ethanolic extract, there were D-limonene, beta-Myrcene, (-)-trans-Caryophyllene, (+-)-Linalool, (-)-alpha-Thujene, 4-Carvomenthenol, P-CYMENE, BETA-PINENE, (E)-3-Isobutyliden-ephthalide, E)-beta-ocimene, ,8-Octanedicarboxylic acid, and (-)-trans-Caryophyllene.

D-limonene	**beta-Myrcene**	**(-)-trans-Caryophyllene**	**(+-)-Linalool**
 **M.W:136.23 g/mol**	 **$C_{10}H_{16}$** **M.W: 136.23 g/mol**	 **$C_{15}H_{24}$** **M.W: 204.35 g/mol**	 **$C_{10}H_{18}O$** **M.W: 154.25 g/mol**

(-)-alpha-Thujene  **$C_{10}H_{16}$** **M.W:136.23 g/mol**	**4-Carvomenthenol**  **$C_{10}H_{18}O$** **M.W: 154.25 g/mol**	**P-CYMENE**  **$C_{10}H_{14}$** **M.W: 134.22 g/mol**	**BETA-PINENE**  **$C_{10}H_{16}$** **M.W: 136.23 g/mol**
(E)-3-Isobutyliden-ephthalide  **$C_{12}H_{12}O_2$** **M.W: 188.22 g/mol**	**(E)-beta-ocimene**  **$C_{10}H_{16}$** **M.W: 136.23 g/mol**	**1,8-Octanedicarboxylic acid**  **$C_{10}H_{18}O_4$** **M.W: 202.25 g/mol**	**(-)-trans-Caryophyllene**  **$C_{15}H_{24}$** **M.W: 204.35 g/mol**

Comparison of the antifungal effects of the common antibiotics Voriconazole (VCZ), Fluconazole (FCZ), and Amphotericin B (AmB) with those of the ethanolic fractions and secondary metabolites of celery (*Apium graveolens* L.) was noted: (15.00 ± 0.29, 18.06 ± 0.31, 22.79 ± 0.45, 30.00 ± 0.54 and 27.18 ± 0.49 respectively. Against *Aspergillus flavus* recorded (23.91 ± 0.48, 25.11 ± 0.51, 29.11 ± 0.45, 25.97 ± 0.41, and 20.00 ± 0.39). Antifungal activity of secondary metabolites against *Trichophyton rubrum* recorded (16.85 ± 0.32, 21.00 ± 0.43, 21.80 ± 0.40, 30.15 ± 0.54 and 24.29 ± 0.46). while recorded (23.12 ± 0.47, 15.09 ± 0.29, 33.00 ± 0.57, 31.14 ± 0.54 and 29.00 ± 0.49) in *Fusarium oxyporum*. In the same time recorded (11.00 ± 0.23, 18.45 ± 0.32, 25.70 ± 0.41, 28.00 ± 0.51 and 32.95 ± 0.55) *Cladosporium herbarum*. The active metabolites of celery (*Apium graveolens* L.) were extremely effective against *Aspergillus flavus* (25.11 ± 0.51). Hydroxyl radical scavenging and hypochlorous acid antioxidant activity of *Apium graveolens* fruit extract (Ethyl acetate, ethanol, and standards). A variety of extract types were documented, including ethyl acetate fraction, ethanol fraction and standard recorded 107.49 ± 5.04, 121.53 ± 6.71 and Ascorbic acid (standard) 201.89 ± 8.06 respectively of Hypochlorous acid radical scavenging. While recorded 307.99±26.00, 238.20± 21.07, and Mannitol (standard) 541.98± 30.37 respectively Hydroxyl radical scavenging potential. The inhibition effect that Crude Oil and others had against Hydroxyl radicals was higher ($P<0.05$) than that of the standard Mannitol. Essential oils are made of volatile substances that mix together, coming from plants after they are distilled. Apiaceae consists of plants that stand out because of their big size, world-wide distribution, and usefulness. Almost all of the plants in this known by their aromatic qualities, especially *Apium graveolens* (A. graveolens). Most people call *A. graveolens* celery, which comes from the family Apiaceae. Celery plants have strong roots that have many succulent branches. It is also common to take the root as a stimulant, and its juice is well known for helping with joints pain [33, 34]. Celery's roots have been used to ease digestion, boost the liver, and to remove urinary stones. You can use celery seeds for pain (analgesic) purposes, but the fruits and aerial parts are useful for treating mild anxiety, tiredness, and a cough. Different reports document the main substances found in EO of A. graveolens and the ones capable of fighting microbes. Using *A. graveolens* oil as therapy is possible for candidoses, dermatophytoses and aspergilloses. With the creation of chemical substances, plants prevent themselves from getting sick by pathogens. Plants release many chemicals that have a wide range of effects against a variety of microorganisms. Also, phthalides are the major active substances in the *A. graveolens* EO. They are helpful against cancer, high levels of blood pressure, and cholesterol. Out of all, sedanolide is the most powerful phthalide and it helps to decrease tumor levels in patients with cancer [5-38]. A. graveolens seed oil has a significant ingredient called 3-n-butyl phthalide (NBP) which causes the tumour's detoxifying enzymes known as glutathione S transferase (GST) to work on the target tissues. For that reason, secondary metabolites/phytochemicals may serve as the basis for developing new pharmaceuticals. Several researches found evidence of the antioxidant effects in *A. graveolens*. Flavonoids, phenolic acids and other compounds called transiopropanoids found in green tea use the process of scavenging free radicals, which results in excellent antioxidant activity. The free-radicals and lipid-peroxidation are under polyphenol-control during antioxidant activity.

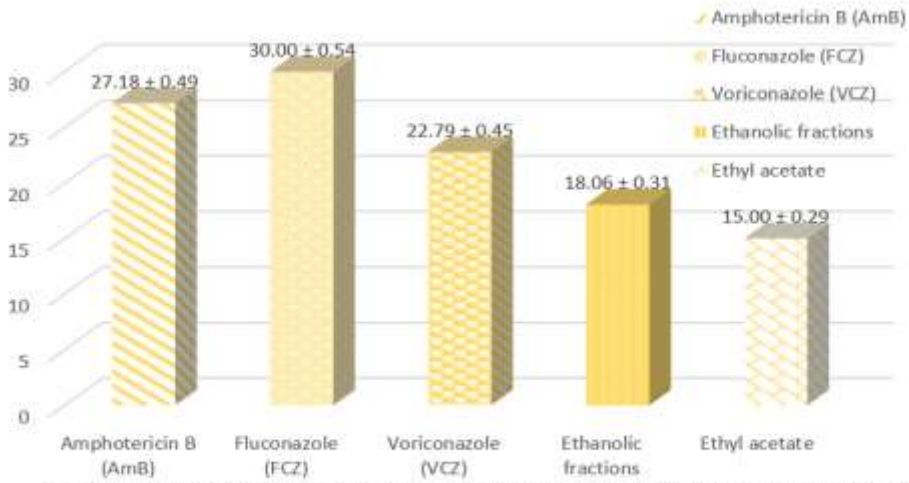


Fig. 1: Bioactivity of the secondary metabolites extract of Celery (Apium graveolens L.) and standard antibiotics Voriconazole (VCZ), Fluconazole (FCZ) and Amphotericin B (AmB) against Alternaria alternaria

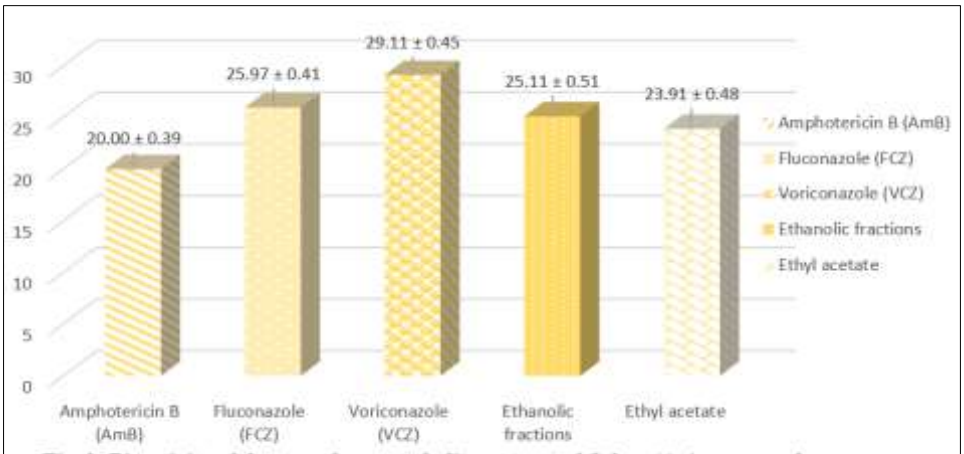


Fig. 2: Bioactivity of the secondary metabolites extract of Celery (Apium graveolens L.) and standard antibiotics Voriconazole (VCZ), Fluconazole (FCZ) and Amphotericin B (AmB) against Aspergillus flavus

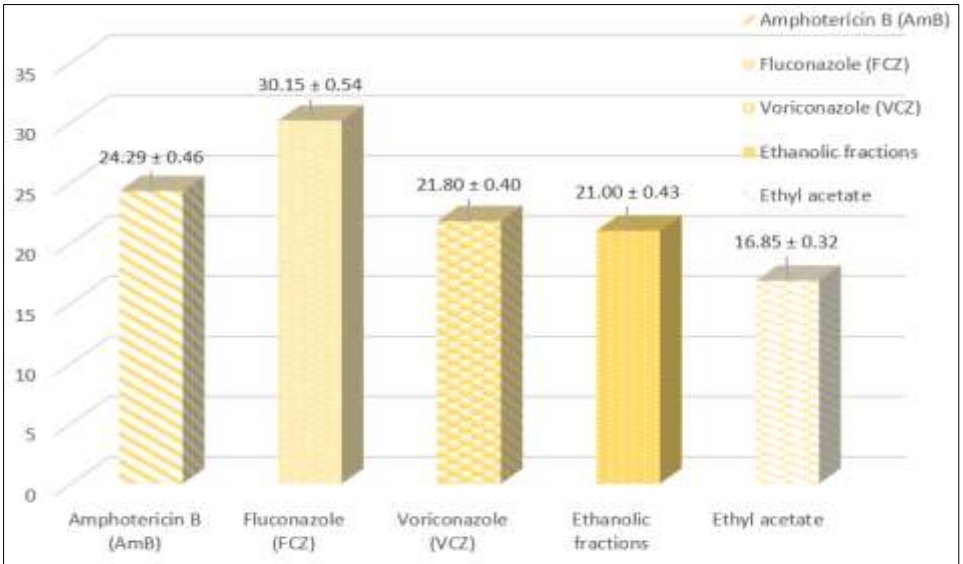


Fig. 3: Bioactivity of the secondary metabolites extract of Celery (Apium graveolens L.) and standard antibiotics Voriconazole (VCZ), Fluconazole (FCZ) and Amphotericin B (AmB) against Trichophyton rubrum

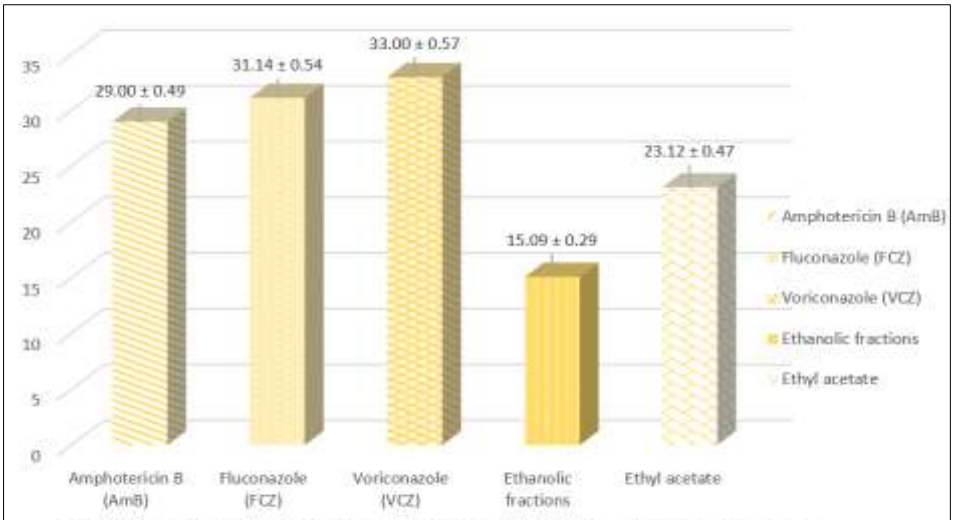


Fig. 4: Bioactivity of the secondary metabolites extract of Celery (Apium graveolens L.) and standard antibiotics Voriconazole (VCZ), Fluconazole (FCZ) and Amphotericin B (AmB) against Fusarium oxyporum

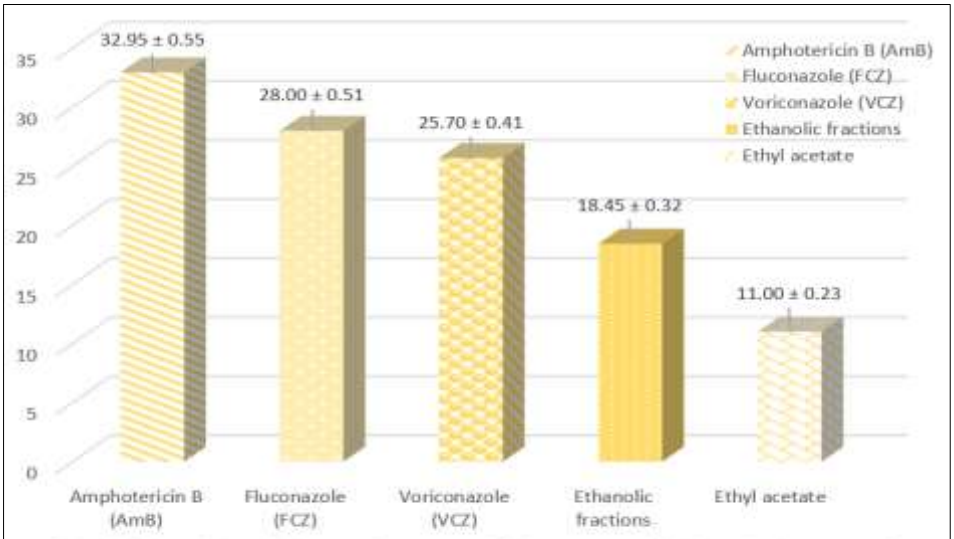


Fig. 5: Bioactivity of the secondary metabolites extract of Celery (Apium graveolens L.) and standard antibiotics Voriconazole (VCZ), Fluconazole (FCZ) and Amphotericin B (AmB) against Cladosporium herbarum

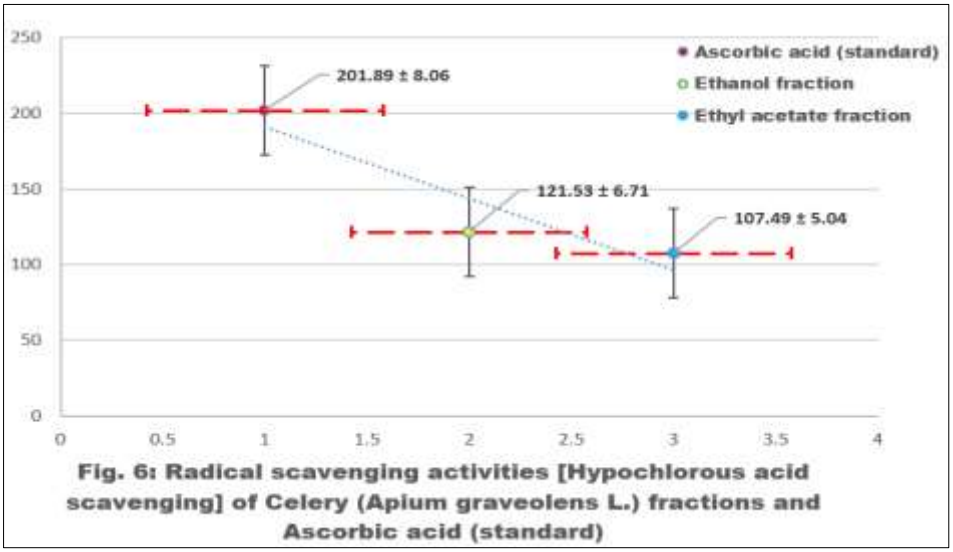


Fig. 6: Radical scavenging activities [Hypochlorous acid scavenging] of Celery (Apium graveolens L.) fractions and Ascorbic acid (standard)

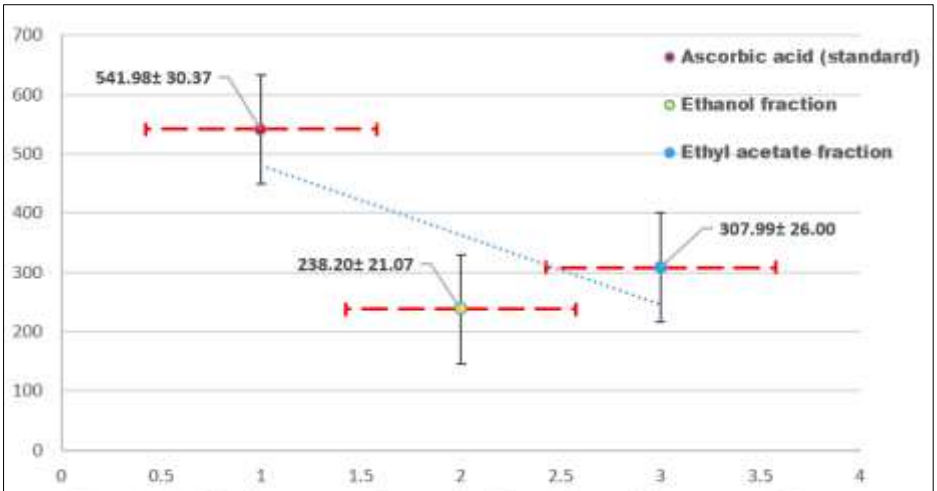


Fig. 7: Radical scavenging activities [Hypochlorous acid scavenging] of Celery (Apium graveolens L.) fractions and Mannitol (standard)

Fungicides and drugs are mainly used to control the disease. Using new strategies is required because fungi are immune to fungicides, environmental problems keep increasing, and people dislike the use of synthetic chemicals. Using natural plant products and secondary metabolites in the place of synthetic drugs and fungicides is better for the environment and is safer [7-42]. In place of using synthetic fungicide/ drugs, essential oils may be effective in handling fungus infection, free radicals, and the formation of cancer cells. EOs taken from plant material have caught researchers' attention due to their incredible abilities. Because of their important constituents, *A. graveolens* essential oil may help explain the various bioactive effects. Minor components in EOs might also play a role in how effective they are. Based on test results, EO can hinder the mycelia growth of three tested fungi only at a certain concentration. This reveals that *A. graveolens* EO is important for fighting against fungal plant diseases and can be used instead of synthetic fungicides for the environment's safety. The means by which EO fights fungi may result from its main phytoconstituents, like p-cymene, limonene, and myrcene, just as previous studies have proven. It has been researched how limonene functions as an anticarcinogenic, an antimicrobial and an antidiabetic agent. According to another experiment, p-limonene helped reduce inflammation by decreasing the expression of IL-6, IL1β, and TNF-α in a dose-dependent way. Additionally, studies prove that increasing the amount of limonene led to a reduced number of LS174T colonic cancer cells [43-46]. Myrcene is an ingredient used for scent and flavor adding in processing food products and beverages. Companies use pheromones in particular for producing scents and flavours, using them as the original starting material. There is evidence that they play roles like protecting cells from damage, fighting bacteria, dealing with different forms of cancer, alleviating pain, inducing calmness, and aiding against diabetes. Possibly, the limonene and myrcene in *A. graveolens* cause a cytotoxic effect due to their active effects against cancer and other actions together with EO compounds [49-51]. Also, it has been noticed that NBP boosts antioxidant capacity by activating scavenger enzymes and lessening lipid peroxidation, blood sugar, water intake and glucose levels, and increases serum insulin in diabetic rats. In addition, the drugs contained in NBP could impede inflammation and oxidative reaction, promote circulation in microvessels, guard against the loss of mitochondrial function, and stop nerve cells from dying [52, 53].

CONCLUSION

The study looked into additional aspects of celery leaves. Celery's antioxidant effectiveness is well known, and various chemicals in the plant can play different roles in treating health problems. The next research should focus on the other medicinal and industrial uses of celery. It is possible to consider using AG-EO as a natural/ herbal fungicide due to its good results in stopping the growth of soil borne fungi. The presence of secondary metabolites in methanolic extract of *Apium graveolens* causes its medicinal property. Twelve active ingredients were found using (GC-MS) analysis. Many antibiotic drugs and medicines use plant derived bioelements that come from Asteraceae.

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