Journal of Current Medical Research and Opinion

CMRO 09 (05) 4789-4797 (2026)
DOI: https://doi.org/10.52845/CMRO/2026/9-5-1
www.cmro.in

CMRO
ISSN (O) 2589-8779
(P) 2589-8760

Original Research

Antioxidant (Superoxide and Hydroxyl Radical Scavenging), Antidiabetic Potential and Bioactive Chemical Properties of Celery (*Apium graveolens*)

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Abstract

Celery plant, which is also called Apium graveolens Linn, is an Asian plant that belongs to the family of Apiaceae. It can also be used to make many other things that include oil, fresh herbs, seeds and stalks, used in cooking and medicine. Phytochemical studies revealed the presence of numerous phytochemicals including, steroids, flavonoids in addition to vitamins A and C. The essential oils, leaves and seeds of the plant are used in traditional medicine. The paper aimed to discuss bioactive chemical properties and antioxidant activity of celery (Apium graveolens) and its use as an antidiabetic. The compounds present in the methanol extract identified by GC-mass analysis were 1-Pyrenehexadecanoicacid, Cyclohexanol, t-Butyltrifluoroacetate D-Ascorbic acid, 7Z,10Z-hexadecadienoic acid, N-Methyl-N-benzyltetradecanamine, oleic acid, 1-(trifluoromethyl) Cyclopropanemethanol, n-Hexadecanoic acid methyl ester, Butyric acid, 2-tridecyl ester, Butyric acid, 2-tridecyl ester, Phytol oleate, 9,12-Octadecadienoic acid, and cinnamaldehyde. The crude methanolic extract, ethyl acetate fraction, ethanol fraction, and celery (Apium graveolens) standards all demonstrated antioxidant potential, specifically in terms of superoxide radical scavenging, with values of (153.00±4.71, 109.45±5.53, 181.00±6.80, and 572.19±19.00, respectively). The results of hydroxyl radical scavenging were obtained as follows: (21.08±1.04, 27.00±1.46, 25.65±1.40 Quercetin (standard), and 572.19±19.00 respectively). The greatest dose that was studied produced the strongest inhibition, whereas the lowest amount produced the weakest. The inhibitory potency against α-amylase was measured to be (87.01±4.06, 31.00±2.05, 49.68±2.75, and 16.00±1.03) according to the kind of celery extract (Crude methanolic extract), ethyl acetate fraction, ethanol fraction, and acarbose (Standard), respectively. α-Glucosidase activity was measured as follows: (61.94±3.07, 42.81±2.15, 30.45±2.03, and 07.96±0.65, respectively).

Keywords: Antioxidant Antidiabetic Potential, Chemical Properties, Apium graveolens.

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Received: 23.02.2026 | **Revised:** 04.05.2026 | **Accepted:** 13.05.2026 | **Published:** 18-05.2026

Introduction

The prehistoric uses of herbs date back to the time when they were used in the flavouring of food, as a medicine and also as a food preservation method. One of the best preventative measures against sickness is a healthy diet. Celery (apiumgraveolens) contained a wide range of trace elements, volatile oils, glycosides, carbohydrates and alkaloids. Previously conducted pharmacologic studies showed that Apiumgraveolens exhibited a broad range of pharmacologic effects, such as gastrointestinal tract, microbes, hypolipidemia, inflammation and central nervous system effects [1-3]. When you have jaundice, liver disease, lien disease or rheumatic diseases, consuming celery will help. The research indicates that Celery leaf ethanol extracts improve fertility and spermatogenesis in rats. Research has indicated that celery is able to decrease inflammation and combat any fungus [4, 5]. Plant extracts contain phenolic chemicals such as thymol and other components such as linalool and menthol at elevated levels and are linked to antimicrobial effect; this is the reason why plant extracts have diverse antibacterial and antifungal activities. Since they prevent the oxidative degradation of lipids, enhance health maintenance, and protect against cancer and coronary heart disease, the phenolic compounds enhance the nutritional value and quality of the food [6, 7]. The importance of antioxidant chemicals is that food producers are now interested in switching to functional foods with specific health effects. Some of the severe complications of diabetes mellitus include arteriosclerosis, high blood pressure, retinopathy and neuropathy which are recurrent hyperglycemia due to either insufficient production of insulin, resistance to insulin or both. In fact, the use of antidiabetic drugs is fraught with several problems since such medications when used on a regular basis may lead to several adverse side effects [8]. Some of the complications of the disease that complicate the management of type 2 diabetes include reduced insulin-mediated glucose uptake and utilization [9, 10]. Due to their extensive history of application and comparative absence of side effects, antidiabetic plants and the active compounds incorporated in them have immense potential as a source of new drugs to manage diabetes. A number of antidiabetic plants, having clear mechanisms of action, have been approved recently following pharmacological and clinical studies. Many efforts have been made to treat hyperglycemia through the application of conventional approaches, and medicinal plants are not the least. The major aim of the study was to find out the antioxidant (superoxide and hydroxyl radical scavenging) and antidiabetic activity of celery (Apium graveolens) bioactive constituents by using GC-mass analysis.

Materials and Methods

Preparation of Compounds Extracts and Sampling

The Apium graveolens plant obtained its leaves in a Rhyadh market in Saudi Arabia. To ascertain the authenticity of a plant, a herbarium specimen was placed beside the plant. The seeds were ground to a fine powder after being shade-dried at room temperature. The leaves were dried and then milled in the shade and stored in a refrigerator ($4C^{o}$) until it was time to extract the powders. The 60 grams powdered celery leaves were added after 24 hours in a sohxlet apparatus to 200 milliliters of distilled water. It was followed by straining the mixture with Whatman No. 1 filter paper to concentrate the filtrate. Lastly, the concentrated extract was maintained at 4C o until required. The residue was repeated three times with methanol extraction until it became dry. The methanol was dried using a vacuum rotary evaporator to dry it. The extract that was not mixed with methanol was frozen and stored in a dark place until required.

Scavenging Hydroxyl Radicals

The test is based on quantification of the breakdown product of deoxyribose by condensing it with TBA. Radicals were generated in a system of Fe3+, ascorbate, H2O2 and EDTA in what is called Fenton reaction. The reaction mixture included the following concentrations of the deoxy-2-ribose, H_2O_2 (1.0 mM), ascorbic acid (100 μM) and the test sample or reference

chemical (0-200 µg/ml). One hour later, the reaction mixture was incubated at 37C and 1 milliliter of 2.8% TCA, then 1 milliliter of 1% aqueous TBA was added. This was then incubated at 90 o C for fifteen minutes to form the required color [12, 13]. The absorbance at cooled 532 nm using a suitable blank solution. Each test was repeated 6 times. We used mannitol as control since it is a classical OH scavenger. We compared the test and blank solutions to determine the percentage inhibition.

Scavenging Superoxide Radicals

The activity was evaluated against a previously published method that entailed the reduction of NBT. The 1 ml reaction mixture contained various sample solution amounts (0-20 µg/ml) with phosphate buffer (20 mM, pH 7.4). The mixture was incubated at room temperature, 5 minutes later the absorbance at 562 nm was measured against an appropriate blank to determine the extent of formazan that was formed [14, 15]. Every test was run six times. Quercetin was used as a positive control.

α-Amylase Inhibition

The traditional method of the determination of α-amylase inhibitory activity of the extract and fractions was slightly changed. The ingredients were the following: twenty milliliters of 3-alpha-amylase containing two international units per milliliters, two hundred milliliters of extract and fractions with different concentrations of half a milligram per milliliters and finally, half a milligram phosphate concentration was one hundred millimolar, and five hundred milligrams of 6.8 phosphate buffer. The mixture was then poured into 96 wells and left to incubate at 37 ^{o}C over 20 minutes. In the process of preincubation, the incubation temperature was adjusted to 37 ^{o}C. After 30 minutes, the above mixture was put back into an incubator at 37 degrees Celsius. Besides this, 20 liters of a substrate containing 1 percent soluble starch in 100 mM phosphate buffer with pH of 6.8 was added. A constant pressure boil of 10 minutes was made on the liquid after adding 100 liters of DNS color reagent to the liquid. Absorbance of the resulting mixture was to be determined; hence the measurement was done. The control levels were used with acarbose of different standard concentrations of 0.1-0.5 mg/ml. As a point of reference, we synthesised a material that had grown without any experimental procedures (extracts and fractions) all at once. In particular, each of the experiments was repeated three times. The findings have been expressed as a percentage of the inhibition which is the result of using the formula. Graphical examination of the enzyme activity inhibition by the different quantities of the fractions was used to determine the IC50 values.

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

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

Inhibition of alpha-Glucosidase Assay

According to the analysis results, we have known that the extract and fractions inhibit alpha-glucosidase. The analysis was generally done as per the traditional method but with here and there modifications. The temperature that was used for pre-incubation was 37 degrees Celsius. After that, the combination was kept in an incubator set at 37 degrees Celsius for another 20 minutes. Afterwards, 20 liters of P-NPG of five millimolar concentration were added. To stop this process, 50-liter solution of sodium carbonate of concentration 0.1 M was added. The test was used to determine the absorption of the newly released nitrophenol by measuring it at 405 nm using a multiplate reader. Simultaneously, the amount of acarbose in the sample was 0.5 mg/mL and was used as a standard measure. These three tests were repeated approximately three times in order to compare the results of the read. Another experiment was also conducted simultaneously but in the absence of the chemical under study. The studies were repeated thrice in order to ensure that the results are as accurate as possible. The following expression was used to measure the percentage of inhibition of the α-glucosidase inhibitory activity:

% Inhibition = ($Abs_{control}$ − $Abs_{extract}$) /$Abs_{control}$ × 100

Where A extract and A control represent the absorbance and fractionation of the control, respectively. The visual representations were computed to give the IC50 values that were used to represent the amounts of fractions required to inhibit the enzyme activity to 50 percent.

Statistical analysis

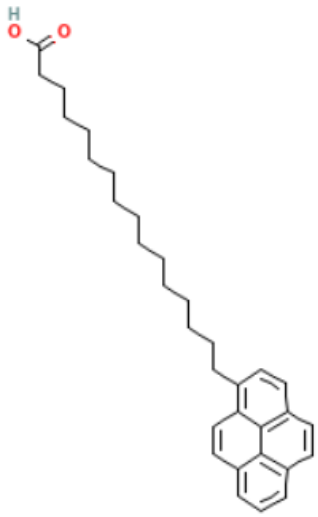
The statistical analysis was done with the help of SPSS Statistics v20.0 program. Standard deviations (SD) and average values (AV) were used to display the data. ANOVA was conducted and the differences between the groups were analyzed by Duncan test at 0.05 level of significance.

Results and Discussion

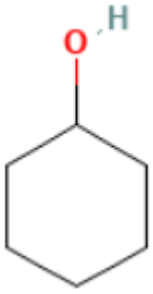
The compounds present in the methanol extract identified by GC-mass analysis as shown in Table 1, were 1-Pyrenehexadecanoicacid, CYCLOHEXANOL, t-Butyltrifluoroacetate D-Ascorbic acid, 7Z,10Z-hexadecadienoic acid, N-Methyl-N-benzyltetradecanamine, 9,12-Octadecadienoic acid ethyl ester, oleic acid, 1-(trifluoromethyl)Cyclopropanemethanol, n-Hexadecanoic acid methyl ester, Butyric acid, 2-tridecyl ester, Butyric acid, 2-tridecyl ester, Phytol oleate, 9,12-Octadecadienoic acid, cinnamaldehyde. Extensive derivatization of polar functional groups effectively converts the metabolomics of non-volatiles polar low-molecular-weight metabolites to volatile form. GC-MS is one of the classic and well-established methods of doing so nowadays. Although the most recent characterization of main plant metabolism is done using GC-MS, other methods contribute significantly to the creation of the complete metabolite picture. Since GC-MS has the ability to profile metabolites with low molecular weights, we discuss its use in plant metabolomics here. Secondary metabolites are produced by plants and these assist them to survive in their natural environment. These microscopic substances have extensive effects to the plant and other organisms. Either, they indicate the behavior of the deciduous or retain perennial growth, initiate the formation of fruits, and abscission. They are also antibacterial, besides their dual ability as attractants and repellents.

Table 1. Celery methanolic crude extract analysed using GC-MS.

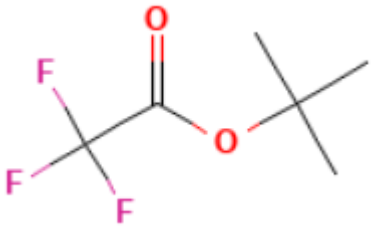
1-Pyrenehexadecanoicacid
MF: $C_{32}H_{40}O_2$
MW: 456.7 g/mol



Cyclohexanol
MF: $C_6H_{12}O$
MW: 100.16 g/mol



t-Butyltrifluoroacetate
MF: $C_6H_9F_3O_2$
MW: 170.13 g/mol

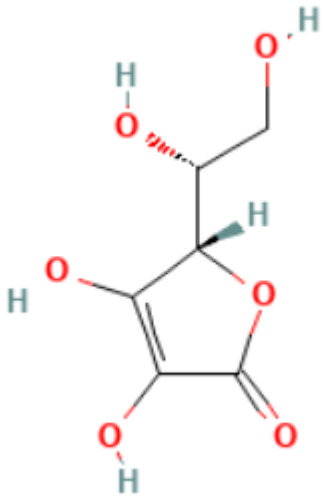


D-Ascorbic acid
MF: $C_6H_8O_6$

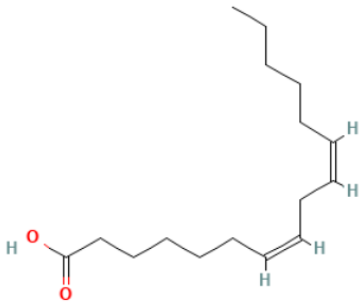
7Z,10Z-hexadecadienoic acid

N-Methyl-N-benzyltetradecanamine
MF: $C_{22}H_{39}N$

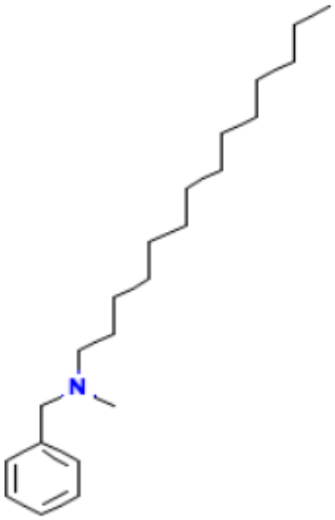
MW: 176.12 g/mol



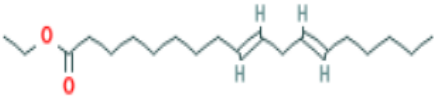
MF: $C_{16}H_{28}O_2$
MW: 252.39 g/mol



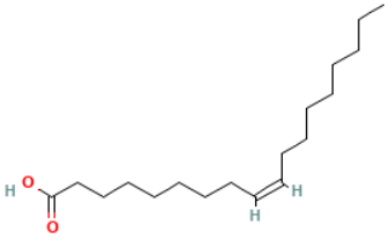
MW: 317.6 g/mol



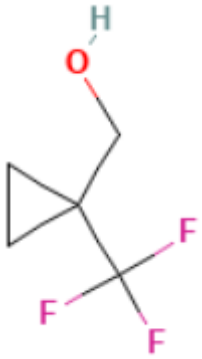
9,12-Octadecadienoic acid ethyl ester
MF: $C_{20}H_{36}O_2$
MW: 308.5 g/mol



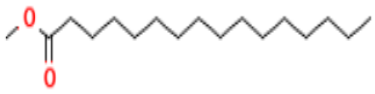
oleic acid
MF: $C_{18}H_{34}O_2$
MW: 282.5 g/mol



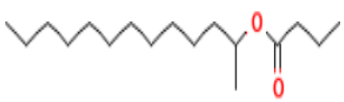
1-(trifluoromethyl)Cyclopropanemethanol
MF: $C_5H_7F_3O$
MW: 140.1 g/mol



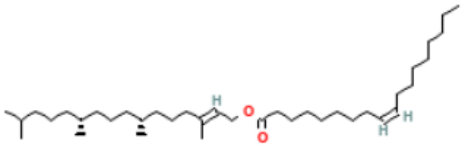
n-Hexadecanoic acid methyl ester
MF: $C_{17}H_{34}O_2$
MW: 270.5 g/mol



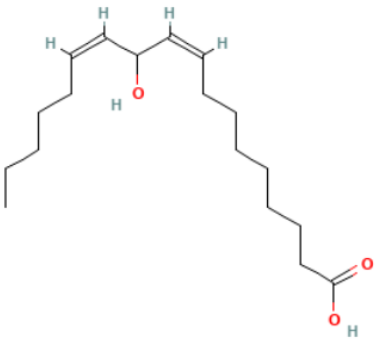
Butyric acid, 2-tridecyl ester
MF: $C_{17}H_{34}O_2$
MW: 270.5 g/mol



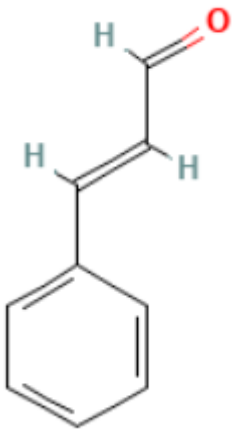
Phytol oleate
MF: $C_{38}H_{72}O_2$
MW: 561.0 g/mol



9,12-Octadecadienoic acid
MF: $C_{18}H_{32}O_3$
MW: 296.4 g/mol



Cinnamaldehyde
MF: C_9H_8O
MW: 132.16 g/mol



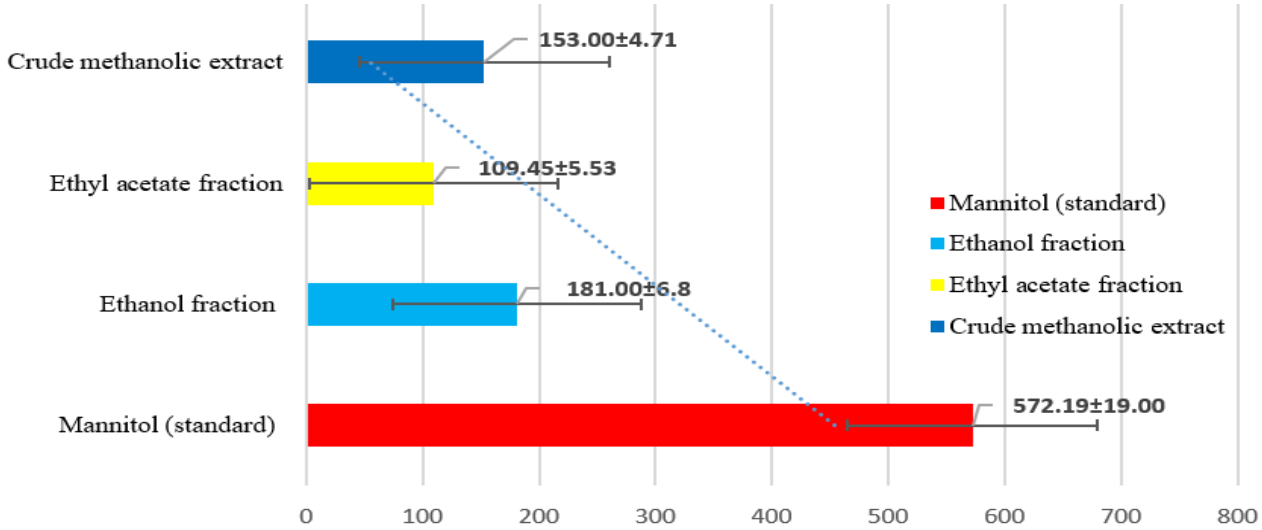


Figure 1. Antioxidant Potential (Superoxide Radical Scavenging) of (Crude methanolic extract, Ethyl acetate fraction, Ethanol fraction and Mannitol (standard) of Celery (*Apium graveolens*)

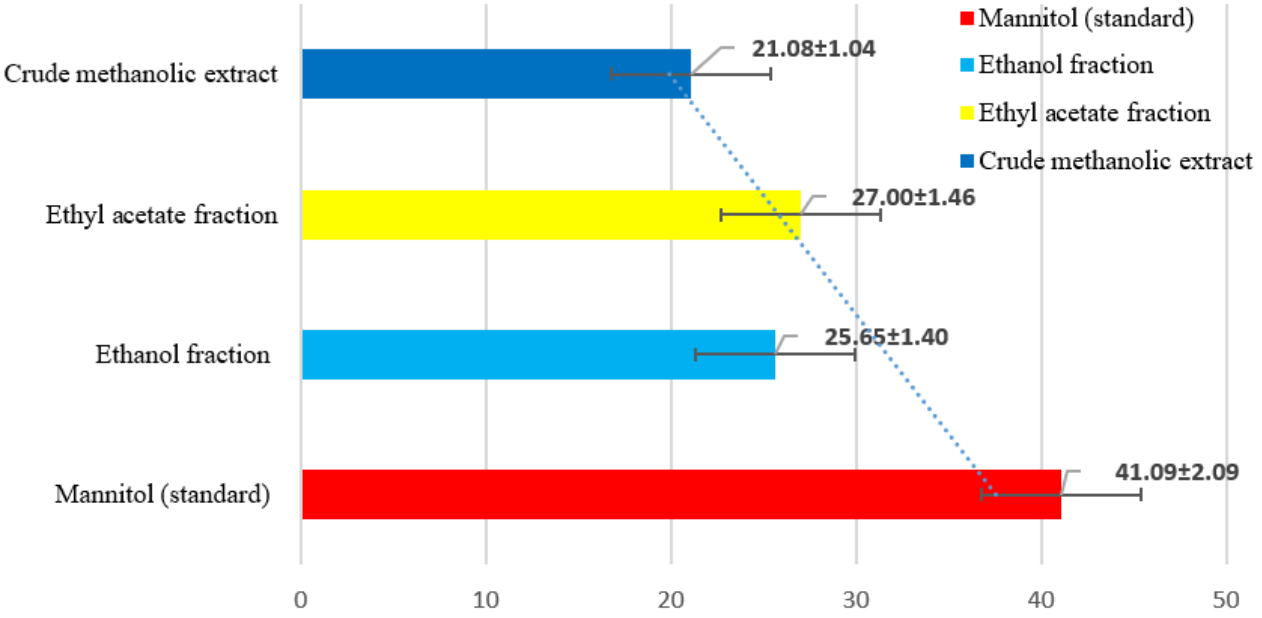


Figure 2. Antioxidant Potential (Hydroxyl Radical Scavenging) of (Crude methanolic extract, Ethyl acetate fraction, Ethanol fraction and Quercetin (standard) of Celery (*Apium graveolens*)

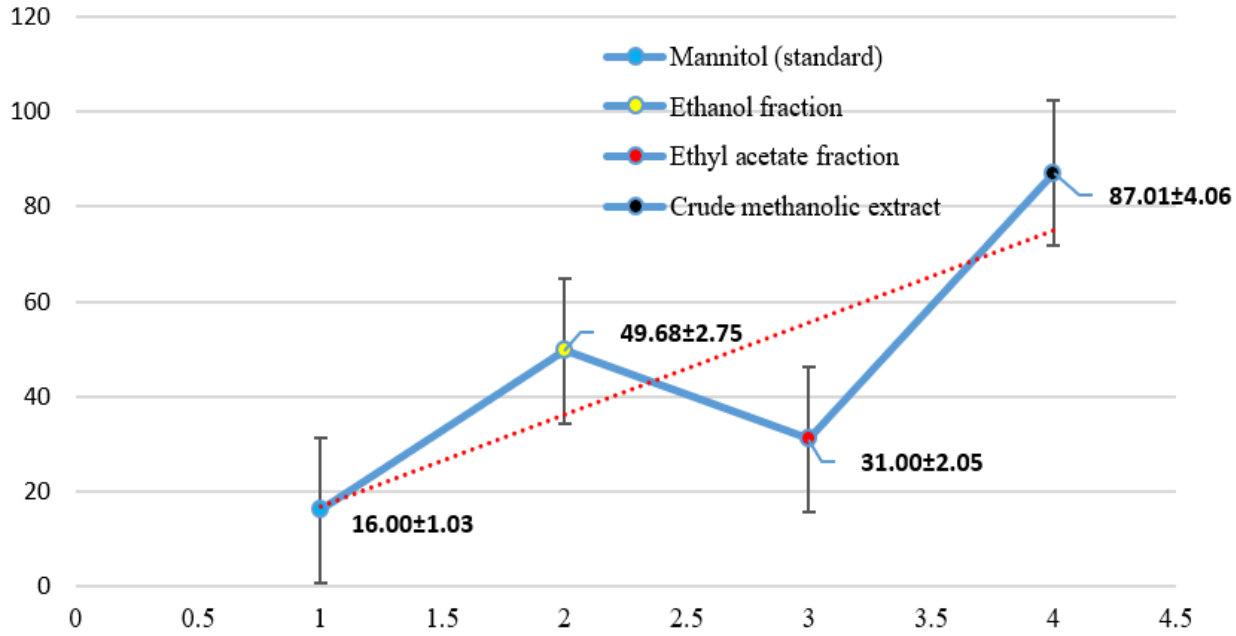


Figure 3. Antidiabetic potential (α-Amylase) of (Crude methanolic extract, ethyl acetate, ethanol fractions and acarbose (standard) of Celery (*Apium graveolens*)

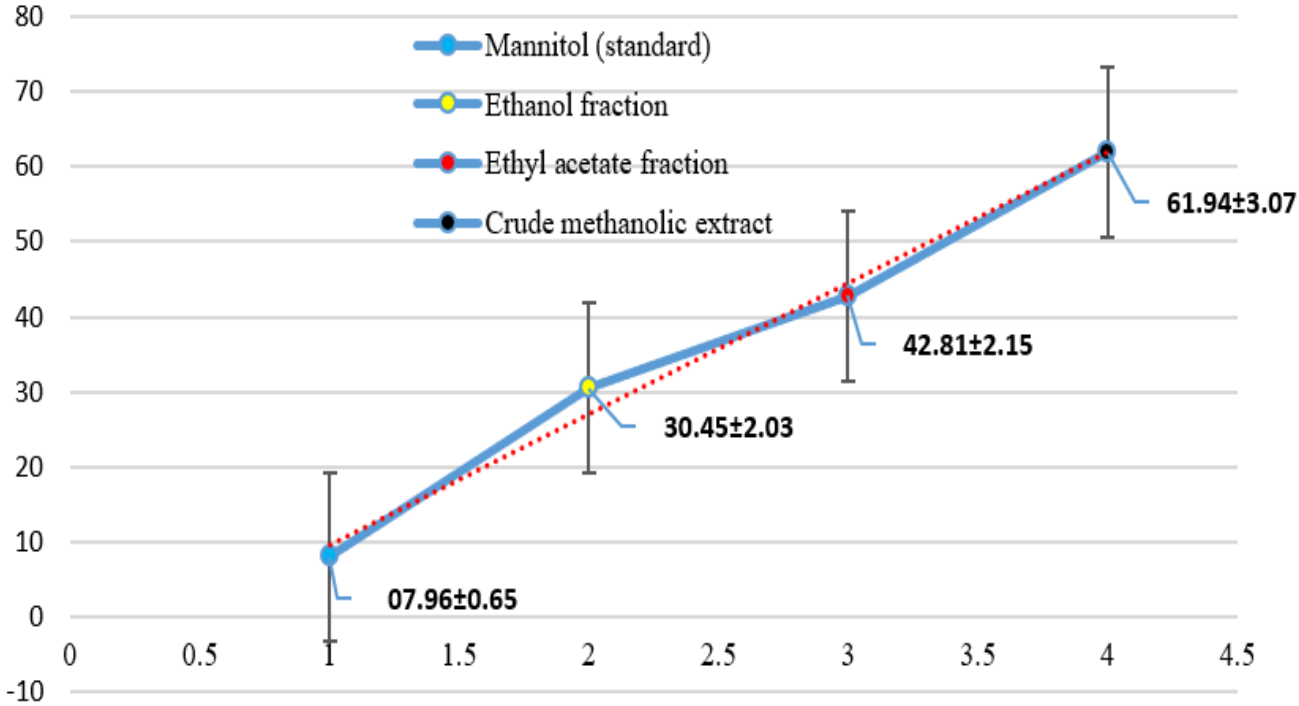


Figure 4. Antidiabetic potential (α-Glucosidase) of (Crude methanolic extract, ethyl acetate, ethanol fractions and acarbose (standard) of Celery (*Apium graveolens*)

Crude methanolic extract, ethyl acetate fraction, ethanol fraction, and celery (Apium graveolens) standards all demonstrated antioxidant potential, specifically superoxide radical scavenging, with values of 153.00±4.71, 109.45±5.53, 181.00±6.80, and 572.19±19.00, respectively. The hydroxyl radical scavenging results were obtained as follows: 21.08±1.04, 27.00±1.46, 25.65±1.40 Quercetin, and 572.19±19.00 respectively. Under examination, the most inhibitory effect was seen at the greatest dose, whereas the minimum effect was observed at the lowest dose. α-Amylase was found to be (87.01±4.06, 31.00±2.05, 49.68±2.75, and 16.00±1.03 respectively) depending on the

type of Celery (Apium graveolens) extract taken. α-Glucosidase activity was detected to be 61.94±3.07, 42.81±2.15, 30.45±2.03, and 07.96±0.65 correspondingly. Phenolic compounds such as flavonoids are abundant in plants, and the various studies have examined the various biological effect of the chemical, their antioxidant properties, in relation to various illnesses such as cancer and diabetes. Medicinal herbs are less toxic to chemical drugs due to their antioxidant properties and less adverse when compared to chemical drugs. The primary reason why herbal drugs are employed in preference to chemical drugs today is the fact that they contain a far lesser number of side effects [16–18]. A range of phytochemical compounds such as polyphenols are antioxidants and free radical collectors in plants. Polyphenols impact living things. The anti-oxidant action is one of the activities that is stimulated to restrain peroxidation and free radicals. The chemical properties of polyphenols are mostly very similar; this means that much of the phenolic groups have the ability to neutralize free radicals by reacting with hydrogen donors. Several researchers have conducted a lot of research on celery antioxidants. Many scientists have studied the phenolic and antioxidant constituents of celery. Being a marker of plant protection, celery leaves can counter the OH and DPPH radicals and also reduce the intensity of liposomal peroxidation. The sample mainly contained apigenin which is a flavonoid and p-coumaric acid which is the most common phenolic acid. Both the antioxidant capacity and the amounts of phenolic compounds were high in the plants that were studied. In this study, there was a positive correlation between the total flavonoid levels and antioxidant activity, total phenolic acids, and total phenolic. Another study examined the chemical constituents and antioxidant property of isolated essential oils of celery leaves [19]. The study on the necessary oils obtained after extracting the leaves of celery in their capacity to inhibit the DPPH radical was found to have the potential of inherent antioxidant effects of the given oil. Having read this, there should be no doubt that the key ingredients of celery can have a significant role to play in the role of an antioxidant. The methanol extract performed better than all other extracts that were tested in antioxidant activity. The seed showed the highest antioxidant activity when subjected to a complete concentration of the methanol extract of the plant. Other isolated luteolin and flavonoids also reduced reactive oxygen in contrast to the enhanced SOD enzymes which offered protection against damage [21, 22]. These chemicals could have been the cause of the antioxidant effect of celery leaves. Figures 3 and 4 indicate the antioxidant activities of the A. graveolens leaf extracts against acarbose, a control, on alpha-amylase and alpha-glucosidase. There was a marked inhibitory effect of the tested extract on both enzymes. The extract demonstrated more inhibitive effect on α-glucosidase, which is apparent. Further, the A. graveolens extract studied had a greater inhibitory effect on α-glucosidase compared to acarbose ($P<0.05$), and acarbose had a greater inhibitory effect on α-amylase. Plant extracts have become increasingly popular in the treatment of type 2 diabetes, one of the most prevalent disease types that need medication in the recent years. The hypoglycemic effect of A. graveolens aqueous extract should be further assessed because its analysis of acute toxicity indicates that it is safe to take without any side effects [23, 24]. The antidiabetic properties were determined through two steps which consisted of in vitro study and in vivo study. The rapid breakdown of carbohydrates to monosaccharides may cause the rise in the level of blood glucose, and it is facilitated by alpha-amyloase and alpha-glucosidases. The lowering of postprandial blood glucose levels is through the inhibition of the digestion of carbohydrates by blocking of α-amylase and alpha-glucosidases, which consequently decreases the amount of glucose released into the blood after eating [25]. Water is a good solvent to extract the bioactive compounds of A. graveolens seeds that had antidiabetic properties, as its aqueous extract suppressed the enzyme activity of the enzymes that were used. This finding supports the long-held opinion that A. graveolens has the potential

to reduce diabetes. Most of the bioactive chemicals found in plants particularly flavonoids have potent anti-alpha-glucosidase and anti-alpha-amylase enzymes. The extract phenolic chemicals, which are mainly tannins, are thought to have an antioxidant effect that results into a hypoglycemic effect. These chemicals influence the insulin activity at the molecular level by reducing insulin resistance, thereby showing their antidiabetic effect. Our results are also corroborated by this occurrence. There are also several phenolic compounds in A. graveolens, such as graveobiosides A and B. It is also possible to extract some flavonoids like apiin and luteolin by this plant [26]. In line with the findings of our study, these substances have demonstrated a greater capacity to inhibit α-glucosidase as opposed to α-amylose.

Conclusion

The numerous constituents in the plant, such as p-coumaric acid, luteolin, tannin and kaempferol, make celery a powerful antioxidant, with different amounts of the chemicals in different concentrations yielding different amounts of healing. Further studies on celery ought to be carried out on its other medicinal and commercial applications. The oil of Apium graveolens was studied by the GC-MS analysis. There were 45 components identified by the research. Cinnamaldehyde, butyric acid, phytol oleate, 1-Pyrenehexadecanoic acid, D-ascorbic acid, and 9,12-octadecadienoic acid ethyl ester are the main ingredients. Along with their culinary and medical purposes, herbs were present since the prehistoric times. The antidiabetic and antioxidant effect of herbs help in preserving them. Herbs can be added to dairy products in order to improve human health and medical conditions. Herbal products that demonstrate potential to have health benefit should meet regulatory standards on safety, effectiveness and quality testing. It should not have any adverse consequences. The scientific studies and documentation must be done in a systematic way.

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