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Biological activities of celery leaves, seeds and tuber parts collected from different regions

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Plants, with their natural and nutritional properties, are also important sources of medicine. The study aimed to determine total phenolic, total flavonoid, total oxidant, total antioxidant, antiproliferative and antimicrobial activities of parts of the celery (*Apium graveolens* L.) plant. Samples of celery were gathered in Turkey and Iraq. Celery parts (leaves, tuber, and seed) were extracted with ethanol and methanol using the Soxhlet apparatus. Total phenolic (Folin-Ciocalteu reagent), total flavonoid (aluminum chloride assay), total antioxidant and total oxidant (Rel Assay kits) and antimicrobial (agar dilution method) activities of parts of the celery plant were determined. Using the MTT assay, the antiproliferative activity against the human lung cancer cell line A549 was ascertained. The study's findings indicate that, in terms of biological activity, plant samples obtained in Turkey exhibited higher levels of total oxidant activity than those collected in Iraq; additionally, the antiproliferative activity of the plants were observed to be dose dependent. Plant samples also demonstrated high levels of activity against *Acinetobacter baumannii*. In conclusion, it has been established that the celery plant possesses natural antibacterial, antioxidant, and anticancer properties.

Keywords: Antitumour, *Apium graveolens*, Bioactive ompounds, Celery

Humans use plants for a variety of purposes. Numerous studies have demonstrated that plants possess high nutritional properties and are sufficient to meet the basic needs of individuals[1]. It has been stated that medicinal plants containing various phytochemicals such as vitamins, carotenoids, β-carbolines, phenolics and endogenous antioxidant metabolites have scavenging properties against free radicals[2]. Additionally, it has been reported that numerous plant species possess various biological benefits such as anticancer, antioxidant, hepatoprotective, antiageing, anti-inflammatory and DNA-protective properties[3-9]. Determining the medicinal potential of plants in addition to their nutritive properties is significant in terms of their applications. In our study, the biological activity of the Celery (*Apium graveolens* L.) plant has been determined.

A. graveolens, commonly known as celery, has been consumed as a vegetable since ancient times. Particularly, the leaves, stem, and tuber parts are included in the diet lists of many individuals. The leaves of celery, which are consumed as a vegetable in many regions of the world, possess a strong aroma. It is widely used as a flavouring agent in soups. Additionally, it is commonly used as a seasoning in various dishes, such as fish and meat dishes, as well as in salad. The seeds are commonly used in the cosmetic industry due to their aromatic properties. Additionally, its seeds are grinding and used as a spice. A fresh celery has 95% water, 3% carbohydrates, and 0.7% protein[10,11]. We determined the quantities of total phenolic, total flavonoid, total antioxidant, and total oxidant in celery gathered from Turkey and Iraq, along with its antibacterial and antiproliferative properties.

Materials and Methods

Samples of plants were gathered from Turkey (OKU210) and Iraq (OKU215). Herbarium samples are kept in Osmaniye Korkut Ata University, Biology Department. The leaves, seeds, and tubers of the plant were subjected to individual extraction processes using ethanol and methanol. In order to do this, 30g of dried plant samples were extracted for about 6h at 50°C using a Soxhlet equipment and 250mL of ethanol. The methanol extract was obtained using the same procedure. Crude extracts were subsequently prepared by evaporating the extracts' solvents in a rotary evaporator.

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Total phenolic and flavonoid tests

From the extracts of the plant parts, 1mL of stock solutions was obtained. The amalgamation of these solutions was executed by means of 1mL of Folin-Ciocalteu reagent in a volumetric ratio of 1:9 (v/v). Subsequently, a volume of 0.75mL of a 1% solution of Na_2CO_3 was introduced. The sample was subsequently subjected to incubation for a duration of 2h under ambient temperature conditions. Subsequently, the assessment was conducted at a wavelength of 760nm. The total phenolic content was quantified as mg/g using the calibration curve of the gallic acid standard solution[12]. The total flavonoid content of the plant extracts was determined by aluminium chloride analysis[13]. A mixture was prepared by combining 0.1mL of 10% $Al(NO_3)_3$, 0.1mL of 1M $NH_4CH_3CO_2$, 4.3mL of methanol, 0.5mL of quercetin, and 0.5mL of plant parts extract. Subsequently, the sample was subjected to an incubation period of 40 minutes. Following that, absorbance was measured at a wavelength of 415nm. The amount of flavonoid content was measured in mg/g.

Total antioxidant and oxidant status

Using the Rel Assay TAS (Total antioxidant status) and TOS (Total oxidant status) kits, the total antioxidant and oxidant levels of plant sections were determined. The TAS and TOS values were determined in accordance with the protocol established by the kit manufacturer. TAS values were calculated using Trolox as a calibrator. In mmol Trolox equivalent/L, the TAS value was stated. A calibrator known as hydrogen peroxide was employed to determine the TOS values. In μmol H_2O_2 equivalent/L, the TOS value was expressed. The percentage that resulted from dividing the TOS value by the TAS value was used to calculate the OSI (Oxidative stress index) (arbitrary unit) value[14].

Antiproliferative activity test

The viability of the A549 human lung adenocarcinoma cell line was tested against plant extracts using the MTT assay. After the cells reached 70–80% confluency, they were separated with a 3.0mL trypsin–EDTA solution. After plating, the samples were incubated for a full day. Plant extracts were used to create stock solutions, which were then applied to the plates at doses of 25, 50, 100, and 200μg/mL. Subsequently, it was incubated for 24h. The control cells were cultured without the use of fetal calf serum (FCS). Subsequently, the supernatants dissolved. The supernatants were incubated at 37°C until the formation of purple precipitate by adding 1mg/mL MTT. The MTT was then mixed with dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, USA) after the supernatants were removed. After that, an Epoch spectrophotometer (BioTek Instruments, Winooska, VT) was used to read the plates at 570 nm[15].

Antimicrobial activity tests

Plant extracts were tested against bacterial and fungal strains. The fungal strains as *Candida krusei* ATCC 34135, *C. albicans* ATCC 10231 and *C. glabrata* ATCC 90030 were utilised. The bacterial strains employed in this study were *Acinetobacter baumannii* ATCC 19606, *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922. Concentrations of plant extracts ranging from 12.5 to 800μg/mL were produced. The bacteria were cultured in Muller-Hinton broth medium. The culture media used for the fungus was RPMI 1640 broth. The minimum inhibitory concentration (MIC) value is the smallest inhibitory concentration that prevents the growth of microorganisms[16].

Statistical analysis

The analysis of TPC, TFC, TAS, TOS and OSI values of extracts was performed in triplicate. The statistical package for social sciences (SPSS version 23.0) was used to conduct a totally randomised analysis of the data, which were reported as means ± standard deviations. ANOVA was used to assess means of experimental results with statistically significant differences (P<0.05), and Duncan's multiple range tests were used for significance testing.

Results and Discussion

Total phenolic and flavonoid contents

Plants are very important natural products in terms of phenolic and flavonoid compounds[17]. We measured the amounts of total flavonoids (TFC) and total phenolics (TPC) in the leaves, seeds, and tuber sections of the celery plant (Table 1). The total flavonoid and phenolic contents of the celery plant, which was obtained from Turkey and Iraq, were measured in our study. It was found that, in comparison to the methanol extracts, the ethanol extracts generally showed higher total phenolic and

Table 1 — Total Phenolic and Flavonoid contents of Celery

Province	Used parts	TPC (mg/g)		TFC (mg/g)	
		Ethanol	Methanol	Ethanol	Methanol
Iraq	Leaves	62.32±2.03^{d*}	45.87±1.68^{c}	38.19±1.72^{c}	28.92±0.82^{c}
	Seeds	29.39±1.92^{b}	20.17±0.98^{b}	13.94±0.76^{b}	10.66±0.49^{b}
	Tuber	14.94±1.15^{a}	11.16±0.52^{a}	7.55±0.40^{a}	7.21±0.46^{a}
Turkey	Leaves	53.29±1.03^{c}	47.79±0.99^{c}	36.40±1.54^{c}	29.25±0.93^{c}
	Seeds	26.64±0.92^{b}	20.32±0.73^{b}	10.62±1.07^{ab}	9.24±0.42^{b}
	Tuber	15.97±0.76^{a}	9.40±0.48^{a}	7.43±0.83^{a}	7.19±0.18^{a}

[* Means followed by different letter(s) differ significantly at $P<0.05$ (Duncan's multiple range test)]

Table 2 — TAS, TOS, and OSI values of Celery

Province	Used parts	TAS (mmol Trolox equivalent/L)		TOS (μmol H_2O_2 equivalent/L)		OSI	
		Ethanol	Methanol	Ethanol	Methanol	Ethanol	Methanol
Iraq	Leaves	12.458±0.268^{c*}	10.874±0.277^{d}	7.866±0.184^{b}	9.616±0.238^{b}	0.063±0.003^{a}	0.088±0.001^{a}
	Seeds	9.857±0.288^{b}	9.564±0.274^{c}	6.526±0.198^{a}	8.577±0.158^{a}	0.066±0.004^{a}	0.090±0.004^{a}
	Tuber	7.374±0.246^{a}	6.708±0.133^{a}	11.382±0.224^{d}	13.105±0.233^{c}	0.155±0.008^{c}	0.196±0.007^{c}
Turkey	Leaves	12.722±0.194^{c}	11.292±0.167^{d}	14.551±0.282	15.319±0.108^{d}	0.114±0.002^{b}	0.136±0.001^{b}
	Seeds	9.880±0.365^{b}	9.205±0.227^{c}	10.552±0.230^{c}	12.721±0.115^{c}	0.107±0.004^{b}	0.138±0.004^{b}
	Tuber	8.141±0.108^{a}	7.397±0.087^{b}	15.482±0.251^{e}	17.614±0.196^{e}	0.190±0.001^{d}	0.238±0.005^{d}

[* Means followed by different letter(s) differ significantly at $P<0.05$ (Duncan's multiple range test)]

flavonoid concentrations. Moreover, it was found that the samples taken from Iraq had more leaves and seeds in them. Furthermore, it was observed that the tuber parts were higher in the samples collected from Turkey. It was found that the leaves had the highest total phenolic content. Celery leaves have been reported to have a total phenolic content of 30.03mg/g and a total flavonoid content of 18.5mg/g in the literature[18]. The plant samples we gathered from Turkey and Iraq in our investigation were found to have higher total phenolic and flavonoid contents in both ethanol and methanol extracts as compared to the previous study. The methanol extract of celery leaves contains 2.12mg/g of flavonoids and 51.09mg/g of total phenolic content, according to a different study[19]. It was found that the methanol extracts used in our investigation had a lower overall phenolic content in comparison to the previous report. However, it was observed that the methanol extracts utilised in our study exhibited a higher total flavonoid content. It is believed that the general reason for this is due to the region from which the plant is harvested.

Total antioxidant and oxidant status

Oxidising chemicals are produced by many living things as a by product of their metabolic processes. Oxidative substances like oxidants are suppressed in part by endogenous antioxidant molecules. However, due to the presence of high levels of oxidising compounds, the antioxidant defence system may prove inadequate[20,21]. In such situations, oxidative stress occurs. Numerous diseases such as cancer, multiple sclerosis, neurodegenerative disorders, cardiovascular diseases, Alzheimer's, and Parkinson's may arise in humans as a result of oxidative stress. Supplemental antioxidants are beneficial in reducing or suppressing the effects of oxidative stress[22,23]. In our study, the natural antioxidant properties of various parts of the celery plant have been identified (Table 2). TAS, TOS and OSI values of celery plant have not been reported before. This is the first time that this study has been located. The highest TAS value was detected in the leaves parts of samples collected from both Iraq and Turkey in our study followed by seed and tubers. In addition, it was found that the samples taken from Iraq had greater TAS values than the samples taken from Turkey. Additionally, it has been observed that plant parts exhibit higher TAS values in ethanol extracts. It has been observed that the samples collected from Iraq exhibit lower TOS and OSI values compared to the samples collected from Turkey. Furthermore, the highest TOS and OSI values of plant parts were determined in the tuber sections followed by seeds and leaves. Additionally, it has been observed that the TOS and OSI values of methanol extracts were higher compared to ethanol extracts. An indicator of the overall presence of antioxidant molecules in natural products is the total antioxidant status (TAS). Natural products with high TAS values demonstrate a high antioxidant potential[24]. Several techniques have been used to report in the literature that *A. graveolens* have

antioxidant potential[25,26]. Our study has shown that different parts of the celery plant exhibit high antioxidant activity. The literature has reports of the TAS, TOS, and OSI values for a variety of plant species. The reported total antioxidant status (TAS) values for *Mentha longifolia* subsp. *longifolia, Alcea kurdica, Rumex scutatus,* and *Ferulago platycarpa* were 3.628, 3.298, 8.656, and 5.688mmol/L, respectively. Furthermore, the TOS values were reported as 4.046, 8.312, 4.951, and 15.552μmol/L, while the OSI values were reported as 0.112, 0.252, 0.057, and 0.273, respectively[27-30]. Compared to these studies, it was observed that ethanol and methanol extracts of the leaves and seed parts of Celery plant collected from both Iraq and Turkey had higher TAS values than *M. longifolia* subsp. *longifolia, A. kurdica, R. scutatus* and *F. platycarpa*. Furthermore, it has been observed in our study that the ethanol and methanol extracts of the tuber parts of the celery plant were higher than those of *M. longifolia* subsp. *longifolia, A. kurdica,* and *F. platycarpa*, while lower than those of *R. scutatus.* It has been determined within this scope that the celery plant possesses a high antioxidant potential. The TOS value serves as a gauge for the overall oxidising power of the substances included in natural products[24]. The study reveals that the lowest total oxidant status (TOS) value of the celery plant used in our research was observed in the seed samples collected from Iraq, with a value of 6.526±0.198μmol/L. Compared to the studies reported in the literature, it has been determined that *M. longifolia* subsp. *longifolia* and *R. scutatus* have higher TOS values, while *A. kurdica* and *F. platycarpa* have lower TOS values of celery plant. In this regard, it has been noted that the celery plant's structure continues to have a normal capacity for creating oxidant chemicals. The OSI value is an indicator of the extent to which oxidising compounds produced within a natural product are suppressed by endogenous antioxidant compounds. The high OSI values indicate the insufficiency of natural product oxidants in suppressing oxidative compounds[24]. According to our research, the celery plant's lowest OSI value was found in leaves taken from Iraq, with a value of 0.063±0.003. Within this context, the OSI value of the Celery plant has been measured to be higher than that of *R. scutatus*, and lower than that of *M. longifolia* subsp. *longifolia, A. kurdica,* and *F. platycarpa*. This result demonstrates the ability of the celery plant to suppress oxidant compounds. The celery plant is said to be a good natural source of antioxidants.

Antiproliferative effect

Researchers' attention has shifted to the development of novel cancer medications in response to the rise in cancer diagnoses in recent years. Within this context, plants have been significant natural materials in supporting cancer treatments[31-34]. We investigated the effects of ethanol and methanol extracts from the celery plant's leaves, seeds, and tubers on the human lung adenocarcinoma cell line A549 (Fig. 1). Our investigation revealed that, depending on the rise in concentration, the methanol and ethanol extracts of the celery plant's leaves, seeds, and tubers demonstrated potent actions against the A549 human lung cancer cell line. The highest proliferation was exhibited by ethanol extracts of the seed portions of the plant. Subsequently, the leaves and tuber portions exhibited activity in sequence. It has been reported in the literature that the leaves of the celery plant are effective against the A549 human lung adenocarcinoma cell line[35]. It has been observed that the leaves of the celery plant used in our study exhibit strong activity. Furthermore, our study has determined that samples of the celery plant collected from both Iraq and Turkey exhibit similar activities. In addition, it has been found that ethanol extracts are more active than methanol extracts. Within the scope of our study, it has been observed that the seed part of the celery plant used in our research exhibits high antiproliferative activity. Within this context, it is believed that the leaves, seeds, and tuber parts of the plant may serve as natural anticancer agents.

Antimicrobial activity

Researchers' attention has recently been drawn to the development of novel antimicrobial drugs due to the rise in microbial illnesses. Unconscious use of medication leads to the emergence of resistant microorganisms[36]. Within this context, the antimicrobial drugs used are deemed insufficient. People typically favor the use of natural antibacterial agents because they may not like the adverse effects of synthetic medications[37]. Therefore, it is crucial to determine the antimicrobial potential of plants. We looked at how conventional bacterial and fungal strains responded to ethanol and methanol extracts made from the leaves, seeds, and tubers of the celery plant (Table 3 & Table 4). At different doses, ethanol and methanol extracts from the celery plant's leaves,

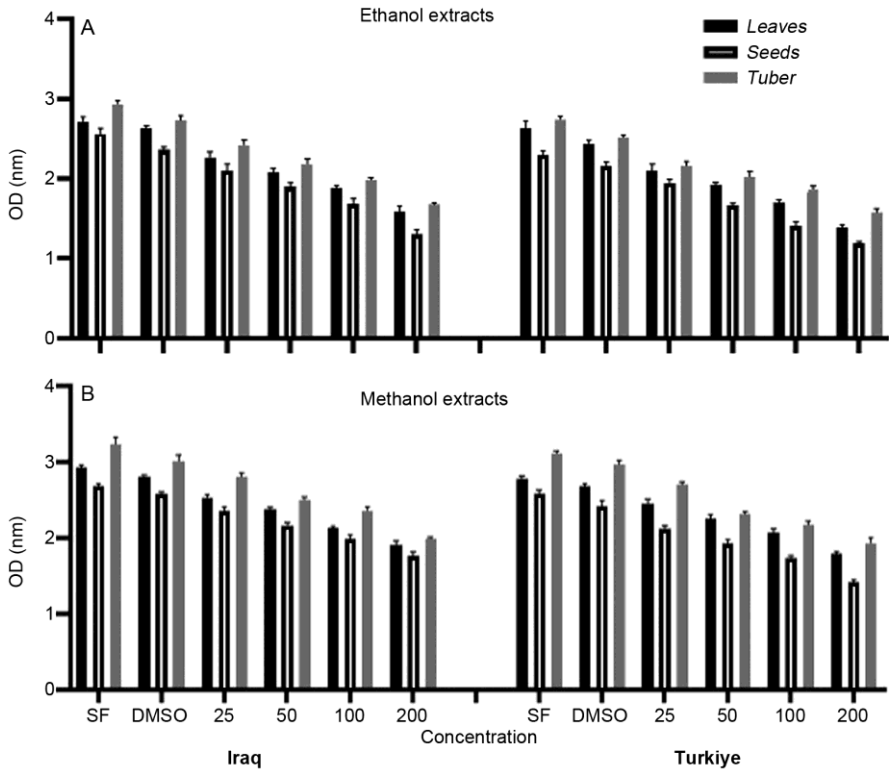

Fig. 1 — Antiproliferative effects of Celery (A) Ethanol extracts (B) Methanol extracts. (SF: The group not treated with chemicals, only kept in the medium; DMSO: The group in which the medium and DMSO were applied; the group in which the extract was applied at 25, 50, 100 and 200µg/mL concentrations)

Table 3 — MIC values of celery extracts against bacterial strains

Province	Used Parts	*S. aureus*		*S. aureus* MRSA		*E. faecalis*		*E. coli*		*P. aeruginosa*		*A. baumannii*	
		EtOH	MeOH	EtOH	MeOH	EtOH	MeOH	EtOH	MeOH	EtOH	MeOH	EtOH	MeOH
Iraq	Leaves	200	200	200	200	50	100	100	200	50	100	25	50
	Seeds	50	50	50	100	100	100	100	400	200	200	25	50
	Tuber	100	200	100	200	100	100	200	400	100	200	50	100
Turkey	Leaves	200	200	200	200	100	100	200	400	50	100	25	50
	Seeds	50	100	500	100	100	100	200	200	100	200	50	50
	Tuber	200	200	100	200	100	200	200	400	100	100	50	100

[*25, 50, 100, 200, 400µg/mL represents the lowest concentration that inhibits the growth of microorganisms]

Table 4 — MIC values of celery extracts against Fungal strains

Province	Used Parts	*C. glabrata*		*C. albicans*		*C. krusei*	
		Ethanol	Methanol	Ethanol	Methanol	Ethanol	Methanol
Iraq	Leaves	200	200	100	200	100	200
	Seeds	100	200	50	100	100	100
	Tuber	200	400	200	200	100	100
Turkey	Leaves	200	400	200	200	200	200
	Seeds	100	200	100	100	100	100
	Tuber	200	400	200	200	100	200

[*25, 50, 100, 200, 400µg/mL represents the lowest concentration that inhibits the growth of microorganisms]

seeds, and tubers were found to be efficient against bacterial and fungal strains in our investigation. It has been determined that ethanol extracts generally exhibit a higher efficacy compared to methanol extracts. Furthermore, the highest activity against bacteria was exhibited against *A. baumannii*. The highest activity was exhibited against *C. albicans* among the fungal strains. Additionally, it was observed that samples collected from Iraq exhibited superior efficacy against certain strains compared to

samples collected from Turkey. Additionally, it has been observed that extracts of seed parts generally exhibit higher efficacy against bacterial and fungal strains. According to literature, various parts of the celery plant have been reported to be effective against *P. aeroginosa, K. pheumoniae, B. aerogenes, B. coagulans, B. megatarium, B. subtilis, S. typhi, L. lichmani, Staph. aureus,* Shigella sp, *A. flavus, A. niger, C. neoformans, C. albicans* and *T. rubrum* at concentrations ranging from 250-500µg/mL[25]. The celery plant's leaves and seeds were found to be efficient against the test microorganisms utilised in our investigation at doses ranging from 25 to 400µg/mL. Additionally, it has been observed that the tuber portions are effective at concentrations ranging from 50-400µg/mL. As a result of our investigation, it has been established that the celery plant is a potential natural source of antibacterial agents.

Conclusion

This study assessed the biological activity of ethanol and methanol extracts from the edible portions of the celery plant, including the leaves, seeds, and tubers. The investigation revealed that several plant sections exhibited distinct antibacterial, antioxidant, and antiproliferative properties. Furthermore, it was found that the biological activity of the samples taken from various plant regions varied. It was concluded as a result that the plant might have antibacterial, antioxidant, and anticancer properties naturally.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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