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Chemical Composition, Phytochemical Constituents and Antioxidant Activities of the Seeds Extract of *Apiumgraveoleus L* from Yemen

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ABSTRACT

The traditional medicine still plays an important role in the primary health care in Yemen. Celery seeds are known to have carminative, stimulant, stomachic, emmenagogue, diuretic, antirheumatic, anti-inflammatory, and laxative properties. The antioxidant activities of ethanol, methanol, water, hexane and ethyl acetate of *Apiumgraveoleus L* were investigated spectrophotometrically against 1,1- diphenyl-2-picrlhdrazyl, hydrogen peroxide, and by ferric reducing power , total antioxidant capacity methods .Total phenols ,flavonoids, tannins, alkaloids were also determined using the standard methods. The five extracts showed the beset of total phenol in water extracts (2549.9 mg/100g dw) but the lowest was in hexane extract (166.19 mg/100g). Methanol extracts exhibited the strongest activity against DPPH radicals,but the lowest was in hexane extract.The ferric reducing activities of the extracts were significantly lower than the standard drugs used in this order : vitamin C > water > ethyl acetate > methanol > ethanol > hexane extract

Keywords: celery. antioxidant. total phenol, total flavonoids.

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INTRODUCTION

The use of Medicinal and Aromatic plant species in Yemen goes back thousands of years and form an important part of the culture, Herbal medicine represents one of the most important fields of traditional medicine in Yemen especially in rural areas.[1] Free radicals are defined as molecules having an unpaired electron in the outer orbit.[2] They are generally unstable and very reactive. Examples of oxygen free radicals are superoxide, hydroxyl, peroxyl ($RO^{\bullet}_2$), alkoxyl ($RO^{\bullet}$), and hydroperoxyl ($HO^{\bullet}_2$) radicals. Nitric oxide and nitrogen dioxide ($NO^{\bullet}2$) are two nitrogen free radicals .[3] They occur continuously in the cells as a result of enzymatic and non-enzymatic reactions to different molecules in the body .[4] Free radicals represent a class of highly reactive intermediate chemical entities whose reactivity is derived from the presence of unpaired electron in their structure, which are capable of independent existence for very brief interval of time .[5] Free radicals and other reactive species are derived either from normal essential metabolic processes or from external sources, such as exposure to x-rays, ozone, cigarette,smoking, air pollutants, industrial chemicals .[6] Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function, Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction .[7] Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases[8]. Antioxidants are beneficial components that neutralize free radicals before they can attack cell proteins, lipids and carbohydrates[9].Synthetic drugs such as butylatedhydroxytoluene (BHT), rutin, and butylated hydroxyl anisole (BHA) are commonly used. However, they have been reported to cause adverse side effects such as toxicity, cell damage, inflammations, and atherosclerosis in animals and humans[10].

Medicinal plants are in important source of antioxidants. The secondary metabolites like phenolic and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark[11]

Celery seeds are known to have carminative, stimulant, stomachic, emmenagogue, diuretic, antirheumatic, anti-inflammatory, and laxative properties, it is prescribed for epilepsy or psychiatric problems due to its tranquilizing effect. The oil is used to treat asthma, flatulence, and bronchitis. Leaves and petioles are used for skin problems in addition to the above-mentioned uses[12].

MATERIALS AND METHODS

Plant sources and preparation

Celery seeds collected also in 2010 from (Lahj –Yemen). Plant samples were collected and transferred in separated sterilized plastic sacks. The seeds were ground into fine powder using a hammer mill and stored at 4◦C until analysis.

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Chemical analysis

Determination of the lipid content:

Weigh 3-5 g of sample into a completely dried thimble and Place thimble in the Soxhlet's apparatus and fill the flask ¾ with ether. Start the water in condenser and heat the flask and set on 5-6 drops per second for four hours, Take out the thimble. Keep it at room temperature for evaporation of ether and then keep overnight in the oven at 105C°, Remove the thimble from oven, cool it in a desiccator and weigh.[10]

Calculation Wt. of sample = (Wt. of thimble + sample) – wt. of thimble

Wt. of fat = (Wt. of thimble + sample) - (Wt. of thimble + sample after extraction)

Ether extract (%) = $\frac{\mathbf{wt.of fat}}{\mathbf{wtofsample}} * \mathbf{100}$.[13]

Determination of crude fibre content:

Defatted sample (1g) was placed in a glass crucible and attached to the extraction unit (InKjel, D-40599, behr Labor-Technik GmbH, Dusseldorf, Germany). 150 ml boiling 1.25% sulphuric acid solution was added. The sample was digested for 30 min and then the acid was drained out and the sample was washed with boiling distilled water. After this, 1.25% sodium hydroxide solution (150 ml) was added. The sample was digested for 30 min, thereafter, the alkali was drained out and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was allowed to cool in a desiccator and weighed (W1). The sample was then ashed at 550°C in a muffle furnace (MF-1- 02, PCSIR Labs., Lahore, Pakistan) for 2 h, cooled in a desiccator and reweighed (W2). Extracted fiber was expressed as percentage of the original undefatted sample and calculated according to the formula:

$$\text{Crude fibre (\%)} = \frac{\text{Digested sample (W1)} - \text{Ashed sample(W2)}}{\text{Weight of sample}} \times 100 .^{[14]}$$

Determination of crude protein

The crude protein was determined using micro Kjeldahl method [15]. Two grams of oven-dried material was taken in a Kjeldahl flask and 30 ml conc. H2SO4 was added followed by the addition of 10 g copper sulphate. The mixture was heated first gently and potassium sulphate and 1 g then strongly once the frothing had ceased. When the solution became colorless or clear, it was heated for another hour, allowed to cool, diluted with distilled water and transferred to a 800 ml Kjeldahl flask, washing the digestion flask. Three or four pieces of granulated zinc, and100 ml of 40% caustic soda were added and the flask was connected with the splash heads of the distillation apparatus. Next 25 ml of 0.1 N receiving flask and distilled. When two-thirds of the liquid sulphuric acid was taken in the had been distilled, it was tested for completion of reaction. The flask was removed and

titrated against 0.1 N caustic soda solution using methyl red indicator for determination of Kjeldahl nitrogen, which in turn gave the protein content.

Determination of moisture

Approximately 2 g of the material under test is accurately weighed (to 0.001 g) into a small dish. This is then placed in the oven for 1 hour, removed from the oven and put in the desiccator to cool. It is then weighed. The dish is replaced in the oven for 30 minutes and the process repeated to constant weight[16].The moisture content is found using the following formula

$$\% \text{ moisture} = \frac{\text{inital weight} - \text{ final weight}}{\text{inital weigh}} \times 100$$

Determination of total ash

For determination of ash content, 10 g of each sample was weighed in a silica crucible. The crucible was heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about3–5 h at 600°C. It was cooled in a desiccator and weighed to ensure completion of ashing. To ensure completion of ashing, it was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently till the weight became constant (ash became white or greyish white). Weight of ash gave the ash content[15].

Determination of minerals

Weigh 2.0000 g of the dried and milled (to 1 mm) sample into a silica crucible and place in a cold muffle furnace with the chimney vent open, and allow to heat up to 450°C. Close the vent and maintain at this temperature overnight. Remove from the furnace and allow to cool, then add15 drops HCl from a polythene Pasteur pipette, being careful to moisten all the sample. Using a fume cupboard, gently evaporate off all the HCl on a hotplate at moderate heat, then remove and cool. Dissolve the residue in 0.1 M HCl, and transfer quantitatively to a 10-ml volumetric flask. Make up trace element standards in 0.1 M HCl covering the expected ranges in the sample solutions and analyse by (spectrophotomètre d' absorptim atomique.AI 1200.Aurora. Canada) according to the instrument manufacturer's instructions Calculation. The sample solution is of 2 g in 10 ml, therefore the concentrations in µg ml–1 of the trace element should be multiplied by 5 to give the concentration in µg g–1 of the trace element in the dried sample [17].

Determination of total carbohydrate:

Percentage carbohydrate was given by: 100 – (percentage of ash + percentage of moisture + percentage of fat + percentage of protein)[18].

Determination of alkaloids:

Alkaloids contents of the plants were determined using the method that described byHarborne[19].using soxhlet,ten grammes of the powdered sample was extracted with 250 mL of ethanol period five hours, extracted of ethanol was evaporated to dryness with a rotary evaporator,under reduced pressure at 40 ◦C. dry residue repeat by 150 mL of chloroform and acidify by HCl 5% pH 3 ,it let pillow during 30 minutes in the room temperature, the phase acid aqueous were extracted by 150 ml of chloroform , basify by the NaHCO3 5% pH 9 and lit it during 15 minutes in the room temperature . the chloroform phase was evaporated to dryness with a rotary evaporator under reduced pressure. The dry residue are the total alkaloids.

Determination of Tannin

Five grams of each part (leaves, stems) was milled into powder. The powder was extracted with 100 ml acetone–water (70/30, V/V), and the mixture was stirred continuously for 72 h at room temperature. Then, the mixture was filtrated and evaporated under vacuum at 40 8C to remove acetone. The washed with 30 ml dichloromethane to remove lipid soluble remaining solution was substances. After that, the solution was further extracted with ethyl acetate at a ratio of 30/30 (V/V). The water layer was separated and extracted twice more similarly. Then the resulting water layer was evaporated to dryness, and the resulting substance was weighed [20].

Determination of total phenols:

The powdered plant material (2g) was extracted with methanol, at room temperature overnight. The methanol extracts were combined and concentrated under reduced pressure on a rotary evaporator.Total phenolic content of each plants extract was determined with the Folin–Ciocalteu's reagent (FCR) according to the published method [21]. Each sample (0.5 ml) was mixed with2.5 ml FCR (diluted 1:10, v/v) followed by 2 ml of Na2CO3 (7.5%, v/v) solution. The absorbance was then measured at 765 nm after incubation at30C^{o} for 90 min. Results were expressed as Gallic acid equivalent (mg Gallic acid/100g dried extract).

Determination of Total flavonoids

The total flavonoid content of celery extract was determined by a colorimetric method as described in the literature [22]. Each sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a NaNO2 solution (15%). After 6 min, 0.15 ml of aluminum chloride (AlCl3) solution (10%) was added and allowed to stand for 6 min, then 2 ml of NaOH solution (4%) was added to the mixture. Immediately, water was added to bring the final volume to5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at510 nm versus prepared water blank. Results were expressed as Catechin equivalent (mg Catechin/100g dried extract).

Determination of ferric reducing power of the extracts

The reducing power of the seeds extract of celery was evaluated according to the method described by [23]. The mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 ml of the extracts and standards prepared in distilled water. The resulting mixture was incubated for 20 min at 50°C, followed by the addition of 2.5 ml of TCA (10% w/v), which was then centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v). The absorbance was then measured at 700 nm against blank sample. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract.

DPPH scavenging assay

The hydrogen atom donation ability of chemical compounds in leaves and stems was measured on the basis to scavenge the 2,2-diphenyl-1-picrylhydrazil free radical [24]. Fifty microliter of various concentrations of the extracts in methanol were added to 1950 ml of a 0.025 g/l methanol solution DPPH. After a 30-min incubation period at room temperature, the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Ablanc} \_ \text{Asample}}{\text{Ablanc}} \times 100$$

Where A blank is the absorbance of the control reaction(containing all reagents except the test compound), A sample is the absorbance of the test compound.

Measurement of hydrogen peroxide scavenging activity

1 ml of sample was mixed with 2.4 ml of 0.1 M phosphate buffer (pH 7.4), and then 0.6 ml of 43 mM solution of H_2O_2 in the same buffer were added. The mixture was incubated at room temperature for 40 min and the absorbance values of the reaction mixtures at 230 nm were recorded against a blank solution containing phosphate buffer without H2O2 for each sample[25]. The hydrogen peroxide scavenging activity was measured according to the following

$$H_2O_2 \text{ scavenging activity(\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Determination of total antioxidant capacity

The total antioxidant capacity (TAOC) of hexane, ethyl acetate, methanol,ethanol and water extract of celery seeds was evaluated by the method of Prieto[26]. An aliquot of 0.1 ml of sample solution (1 mg/ml) was combined with 1 ml of reagent solution (600 mMsulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was

measured at 695 nm against a blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions. The antioxidant capacity was expressed as the number of equivalents of α-tocopherol (mg/g of extract).

RESULTS

Results show that celery seeds contains a highest percentages of Carbohydrate and protein and lowest percentage of moisture and lipid. table (1).

Table1 : Nutritive values of experiment's plants parts

Plant Sample	Moisture %	Total Ash%	Protein% N×6.25	Lipid (%)	CrudeFiber%	Carbohydrate %	Nutritive value (Cal/100 g)
Celery	7.60	13.2	16.37	9.61	14.77	38.63	306.49

The concentration of Ca, Na, Zn, Ag, Cu, Fe and Pb.in Celery samples is given in Table (2).The highest concentrations of Ca, Na,. And the lowest concentrations of Ag, Pb, and Zn

Table 2:Mineral composition of plants samples

Plant Sample	Ca(ppm)	Na(ppm)	Zn(ppm)	Ag(ppm)	Cu(ppm)	Fe(ppm)	Pb(ppm)
Celery	6425	1425	45.5	0.025	18.75	162,25	6,25

The yields of the extracts were calaulated as percent by weight of the celery seeds. According to the chemical composition and polar or no polar nature of phenolic compounds, celery contains a relatively high percentage yield in Ethyl acetate ,ethanol,methanol and water while lower in hexane .yeild ,total phenolic and total flavoinods shown in table 3

Table 3:Yeild , Total phenolic content (TPC), flavonoid content (FC) and chelating activity of organic solvent extracts of fenugreek expressed as gallicacid,andcachain and t, respectively.

sample	yeild	TPC mg GAC/g dw	TFC mg cach/g dw
methanol Extraction	13.67±1.44	437.64±41.96	209.9±32.8
ethanol Extraction	16.81±2.23	245.75±11.6	120.08±11.5
Ethyl acetate Extraction	17.30±3	388.16±32.2	312.8±25.4
water Extraction	13.6±1.18	2549.9±234.16	590.37±54.34
hexane Extraction	12.95±0.68	166.19±44.27	46.34±3.6

The total phenolic content was determined by following a modified Folin-Ciocalteu reagent method. Using a standard curve of Gallic acid (R2 = 0.9964).In table 3 the results

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were expressed as Gallic acid equivalent.TPC was in the range of 2549.9 - 166.19 mg/100g of celery seeds extract. The amounts of total phenolic compounds were higher in water extract 2549.9mg/100g while lowest for hexane 166.19mg/100g.

Using the ALCl3 reagent and Catechin as standard (R2=0.9991), the total flavonoids are in the range from 590.37- 46.34 mg/100g of Catechin equivalent (Table 3).The highest value for the water was 590.37 mg/100g of celery with the following decreasing order of the extract water> Ethyl acetate > methanol > ethanol > hexane.

The potential of the plant extracts Fe3+ to Fe2+ by electron transfer is an in dictation of their antioxidant ability. The reducing power of the extracts in comparison with the standards (vitamin C) is presented in Figure 1. The observed change of yellow color of test solution to various shades of blue and green, depending on the concentration of the extract, is an indication of its antioxidant activity. The ferric reducing activities of the extracts were significantly lower than the standard drugs used in this order : vitamin C > water > ethyl acetate > methanol > ethanol > hexane extract.

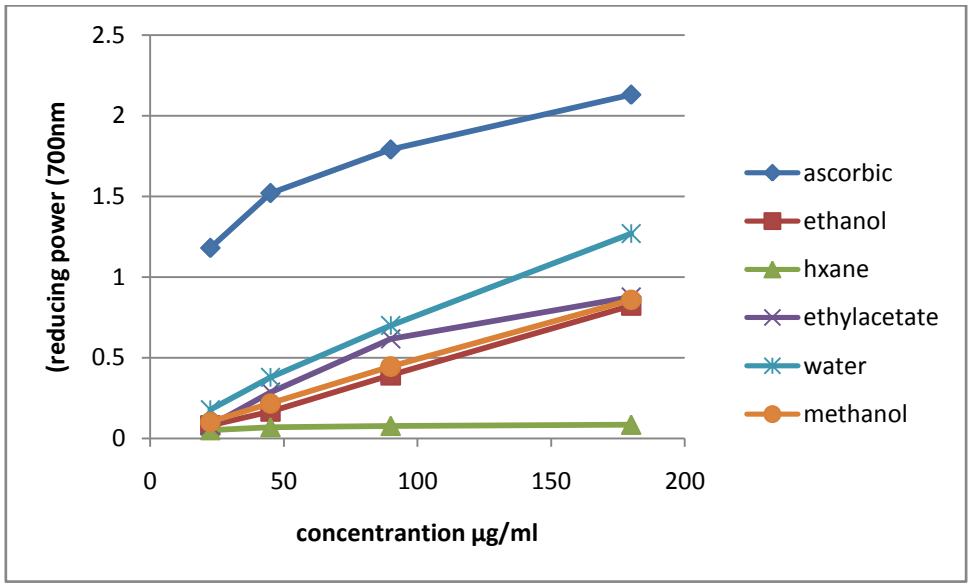

Figure 1: Reducing power of different solvent extracts of celery

The DPPH scavenging potential of the seeds extracts of celery concentration dependent as shown in Figure 2 .The IC50 of DPPH by the various solvent extracts and the standard drugs was recorded in decreasing order :BHA> methanol> vitamin C > ethyl acetate > water > ethanol > hexane, it was 71.61 ± 5.39 µg/ml, 83.825 ±1.03 µg/ml, 90.495 ± 22.8µg/ml, 101.415 ± 9.53µg/ml, 191.855 ± 3.23 µg/ml, 191.855 ± 3.23 µg/ml, 257.567±4.84µg/ml, 5.5±0.028 mg/ml respectively.

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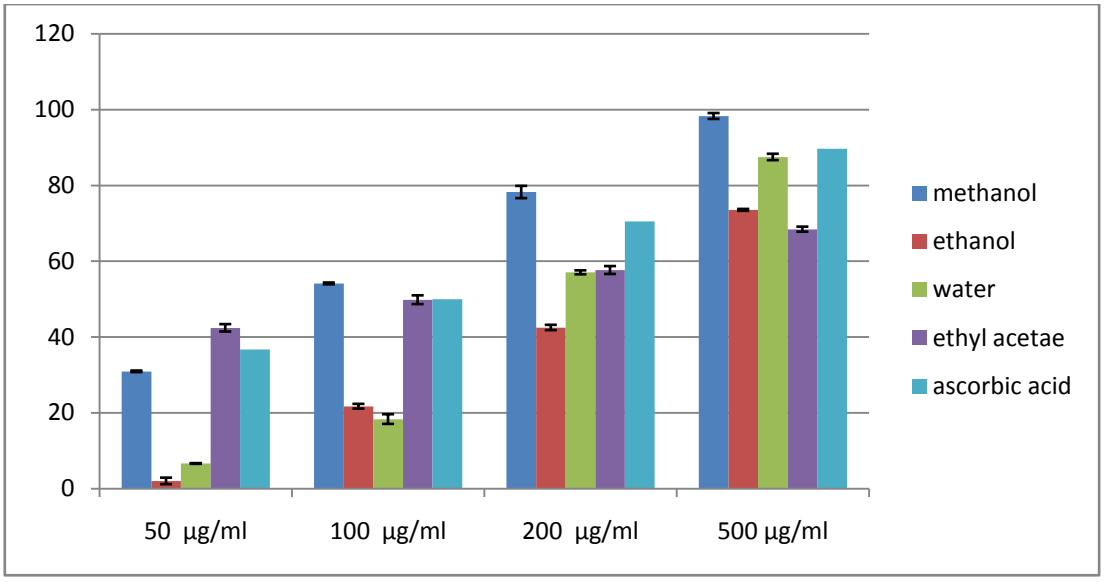

Figure 2: DPPH radical scavenging activity of the seeds extract of celery obtained using different solvents

The scavenging activity of the seeds extract of celery against hydrogen peroxide is shown in Figure3. The scavenging activities of the plant in various solvent were in the order: ethyl acetate > methanol >water > ethanol > hexane

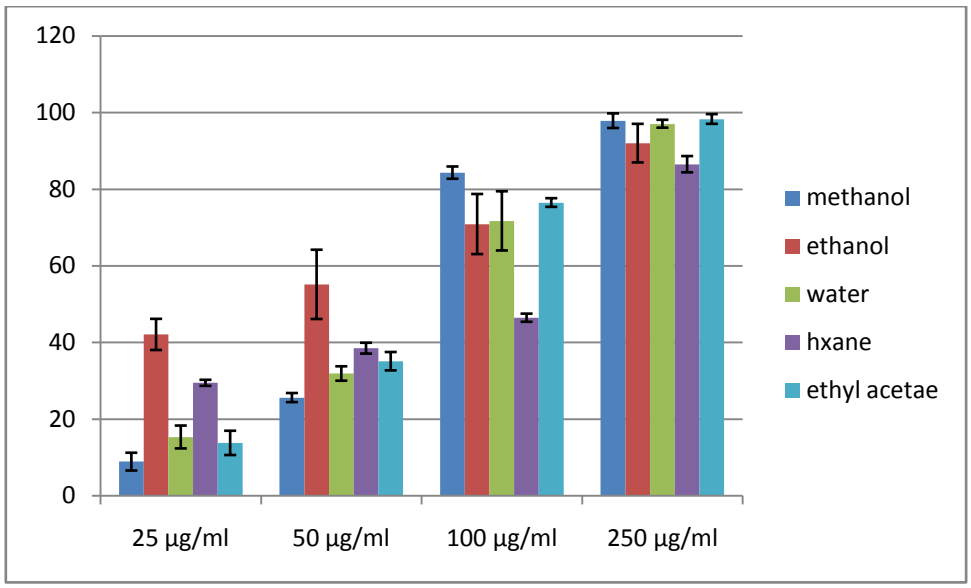

Figure 3:Hydrogen peroxide radical scavenging activity of the celery extract obtained using different solvents

The antioxidant capacity of seeds extracts of celery was found to decrease in the following order :methanol> ethyl acetate > hexane > water > ethanol as showed in (Figure 4)

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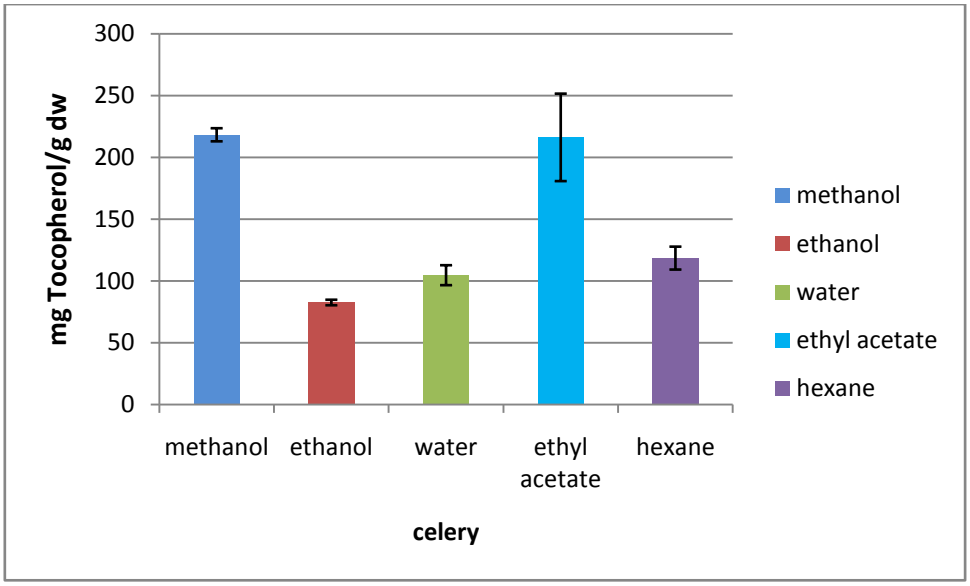

Figure 4: Total antioxidant capacity (TAOC) activity of the celery extract obtained using different solvents

DISCUSSION

Proteins play a central role in biological systems, the various biological function of protein can be categorized as enzyme catalysts, structural protein, contractile proteins, hormones, transfer proteins, antibodies,storage proteins, and protective proteins[27][. Our results showed that the concentration of protein and carbohydrate are good but they were low relatively to that reported byKhare[12].

The mineral are essential for human body ,they are basic content of many of body tissues such as calcium and phosphor for bone and iron for blood and muscles [28].

These dietary fiber in food have been shown to be useful in reducing blood glucose levels in diabetes, in reducing blood cholesterol levels, for treatment of cardiovascular disease and also in preventing bowel cancer [29].

The phenolic compounds may contribute directly to the antioxidant action [30]. And they are known to exhibit strong antioxidant activities, which have direct antioxidant properties due to the presence of hydroxyl groups, which act as hydrogen donor [31].Additionally, they are found to be effective in scavenging free radicals as a result of their redox properties that allow them to act as reducing agents [32]. In this study TPC was in the range of 2549.9-166. 19 mg/100 g of celery seeds. They amounts of total phenolic compounds were higher in water extract 2549.9 mg/100g while lowest for hexane. studies show that celery seeds, leaves and roots have total phenolic range 5100-1637. 1mg/100g in leaves [33], 233.1 in roots [34]While total phenolic content was 2486mg/100g in seeds [35].

The ability of a substance to act as an antioxidant depends on its strength to reduce ROS by donating hydrogen atom [36]. The reducing power ability of the plant extract was found out by measuring the transformation of Fe^{3+} into Fe^{2+}. The reducing power ability of a compound usually depends on the existence of reductions, which mainly act by braking the free radical chain reaction by donating a proton [37]. The results of reducing power in our

study were 0.21645 nm, 0.4454 nm, 0.85825 nm at concentration 45µg/ml, 90µg/ml, 180µg/ml respectively were higher in compare with that of Muthuswamy[37]. who studied reducing power ability of methanolic extract for celery seeds, and obtained that the reducing power was 0.0279 nm , 0.0946 nm, 0.1440 nm, 0.1971 nm at the concentrations 50µg/ml, 100µg/ml, 200µg/ml, 400µg/ml respectively. The reducing power of water extract in this study was the higher among the other extracts, these results are in relative with that of total phenols and total flavonoids where water extract had five fold that of the other extracts.

DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom, this method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolourizes the DPPH the DPPH solution [38]. The weak activity observed in the DPPH radical scavenging assay in hexane extract may be as a result of the low level of total phenolic and flavonoid content in the plant extract of hexane, statistic result of each TPC and TFC with DPPH by T test showed that they are negative correlation between each of TPC and TFC with DPPH of all extract, but there no significant different.

Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects [39].

Celery activity against hydrogen peroxide was good in all of plant extracts, and there were correlation between these results and the results of reducing power of plant extracts, that may refer to the similarly in reaction mediums and the conditions of these methods, where the water is the main solvent in both reactions and the pH was ranged from 6.6-7.4.

The antioxidant capacity of the fractions was measured spectrophotometrically throughphosphomolybdenum method, which was based on the reduction of Mo (VI) to Mo (V) by thesample analyte and the subsequent formation of green phosphate/Mo (V) compounds with amaximum absorption at 695 nm [40].

With regard to the result of the phytochemical structure of celery and each of reducing power, DPPH, Hydrogen peroxide in comparing with total capacity of celery we found that there are accordance with all the extracts in particularly methanol, ethyl acetate, and ethanol extract which showed the high activity in all methods of antioxidant activity that used in this study.

CONCLUSION

Based on our study we can conclude that Celery seeds is a potent as source of nutrients as protein, lipids, sugars, ash and fiber. It also contains important quantities of polyphenols, flavonoids, and vital trace minerals, in addition Celery seeds seems to be respectable antioxidant, therefor Celery seeds might be used as food supplement, and to contribute in health improvement and maintenance, it will be useful in particularly with the disease that related with the free radicals such as diabetes mellitus, cancer, and cardiovascular disease.

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