

Full Length Research Paper

Nutraceutical potential and bioassay of *Apium graveolens* L. grown in Khyber Pakhtunkhwa-Pakistan

Anwar Ali Shad¹, Hamid Ullah Shah¹, Jehan Bakht^{2*}, Muhammad Iqbal Choudhary³
And Javid Ullah⁴

¹Department of Agricultural Chemistry, KKP Agricultural University, Peshawar, Pakistan.

²Institute of Biotechnology and Genetic Engineering, KKP Agricultural University, Peshawar, Pakistan.

³International Center for Chemical and Biological Sciences, HEJ Research Institute of Chemistry, University of Karachi, Pakistan.

⁴Department of Food Science and Technology KKP Agricultural University, Peshawar, Pakistan.

Accepted 22 July, 2011

The present study investigates the nutritive and pharmacological potential of *Apium graveolens* commonly available as wild plant in Peshawar and suburbs of Khyber Pakhtunkhwa region of Pakistan. For this purpose, plants of *A. graveolens* L. was collected from its natural habitat at mature stage (seedling stage) from Palusi, near KPK Agricultural University, Peshawar Pakistan. Similarly, celery seeds were obtained from 3 different ecological zone markets of Khyber Pakhtunkhwa including Peshawar, Swat and Dera Ismail Khan Regions of KPK Pakistan. Analysis of the data revealed that all the examined plants contained appreciable amount of moisture, ash, fat, protein and fiber. The wild celery had good level of vitamin C and β -carotene. The phytochemical screening of methanolic extracts of celery showed the presence of various groups of compounds including tannins, steroids, phenolic acids, terpenoids, flavonoids, volatile oils and saponins, whereas cardiac glycosides was absent in all the samples. Among the antioxidant parameters, a significant amount of total phenols and tannins were observed. Likewise elements analyzed were in the order of Na> P> K> Ca> Mg> Fe> Cu> Mn> Zn> Ni> Pb> Pt> Cd> Se> Cr. The results further revealed that Mg, Ca, P, K and Na were present in fairly high amount. The methanolic extract of all the examined celery showed positive antibacterial activity against different strains tested. Similarly, antifungal potential of the celery was determined against *Trichphyton longifuss*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glabrata* in concentration 200 $\mu\text{g ml}^{-1}$ of dimethyl sulfoxide (DMSO). The results revealed that all the organic solvents (hexane, chloroform, methanol) and water extracts showed no brine shrimp lethality and leishmanicidal activity.

Key words: Bioassay, proximate, antibacterial, antifungal, *Apium graveolens*.

INTRODUCTION

Khyber Pakhtunkhwa formerly known as North-West Frontier Province is situated in the north west of Pakistan (Coordinates: 34.00 °N and 71.32 °E). This province is located at the junction where the slopes of the Hindu Kush Mountains on the Iranian plateau and Eurasian land plate are located, while peripheral eastern regions are located near the Indian subcontinent, which give way to the Indus-watered hills approaching South Asia. Topography of this province varies from dry rocky areas

in the south to forests and green plains in the north. The province is spread over 74,521 km², 10% of which is cultivated. Peshawar (Provisional capital) covers a large area extending over 50 km from north to south and over 30 km from east to west. It is situated at an altitude of 347 m above the sea level. The Wild plants play a very important role in the livelihoods of local dwellers of Khyber Pakhtunkhwa as source of food and medicine which formulates an integral part of the subsistence strategy. The neglect of wild food plants, however, has been attributed to the inadequacy of information on their nutritional contents and potential to serve as food security (KP, 2010). *Apium graveolens* Linn belonging to the

*Corresponding author. E-mail: jehanbakht@yahoo.co.uk.

family Umbelliferae is an annual or biennial herb widely used as a spice and seasoning in food. Seeds of *A. graveolens* have wide commercial significance all over the world especially in Europe, North America, India, Iran and Pakistan (Spices Board, 2008). Celery has a long history of medicinal and food value. Celery seeds are used in the treatment of bronchitis, asthma, emmenagogue, galactagogue, nervine stimulant, liver and spleen diseases. The seeds are known for their carminative, diuretic and anti-inflammatory property (Jian et al., 1990; Wichtl, 1994). Tea prepared from celery seed is reputed to promote rest and sleep (Norman, 1990). Volatile oil obtained from seeds is used in the perfume and pharmaceutical industries (Lewis, 1984). An essential oil obtained from the plant has a calming effect on the central nervous system.

A broad range of biological activities have been attributed to *A. graveolens*. These include antimicrobial activity (Davidson and Naidu, 2000; Sipailiene et al., 2005), mosquito repellent potential (Tuetun et al., 2004; Choochote et al., 2004; Tuetun et al., 2009; Jain et al., 2009), larvicidal activities (Choochote et al., 2004), hepatoprotective activity (Ahmed et al., 2002), nematocidal, antifungal (Momin and Nair, 2001) and anti-hyperlipidemic property (Tsi et al., 1995, Tsi and Tsi, 2000). Phytochemical investigations on *A. graveolens* have also been shown to contain various groups of natural products (Kailan et al., 2009) including *n*-butylphalide derivatives. The discovery of sedanolide, sedanonic acid, and *n*-butyl phthalide from *A. graveolens* L. by Ciamician and Silber is one of the early classics of natural product chemistry. Researchers have identified 3-butylphthalides derivatives as constituents responsible for the aroma of celery and celeriac (Macload and Ames, 1989; Mookherjee and Wilson, 1990). Likewise, celery contains 1.5 to 2.0% of volatile oil, which is extracted by hydro-distillation. The characteristic odor is due to oxygenated compounds present in the oil, namely: sedanolide and sedanonic acid anhydride (Bjeldanes and Kim, 1977).

The local inhabitants of Khyber Pukhtun Khwa Province of Pakistan are using celery both for a number of food and medicinal purposes that are mostly imported from India, Afghanistan, Iran etc. A reasonable amount of celery is also cultivated in Punjab and Sindh (Pakistan). However, no information is available to the local people that this plant is widely available as weed. The present study was designed to investigate the nutritive and pharmacological potential of *A. graveolens* commonly available as wild plant in Peshawar and suburbs of Khyber Pakhtunkhwa region of Pakistan.

MATERIALS AND METHODS

Sample collection

A. graveolens L. was collected from its natural habitat at mature stage (seedling stage) from Palusi, near Khyber Pakhtunkhwa

Agricultural University, Peshawar. The plant sample was identified and a voucher specimen (Pesh-86375) was deposited in Herbarium of PCSIR Laboratory Peshawar. Likewise, the celery seeds were obtained from 3 different ecological zone markets of Khyber Pakhtunkhwa including Peshawar, Swat and Dera Ismail Khan (D.I. Khan). Collected celery seeds were dried in shade at ambient temperature for 5 to 7 days. The sample materials were pulverized by an electrical grinder and passed through 80 mesh sieve to obtain homogenizes powder and stored into an air-tight container.

Phytochemical screening

Chemical tests were carried out on the aqueous extract using standard protocol (Sofowara, 1993; Trease and Evans, 1989).

Biochemical analysis

All chemicals used in this study were of analytical grade and double distilled water was used for the preparation of reagents and other analysis. The sample material was analyzed for proximate composition, mineral profile, antioxidant potential and antimicrobial activity. Moisture was estimated by standard drying method based on the measurement of weight lost due to the evaporation of water ($\pm 95^{\circ}\text{C}$) using gravity oven model Uvi-85A (AOAC, 2000) (Method 3.003). Ash contents in the plant samples were determined using standard laboratory protocol (AOAC, 2000) (Method 7.009). The crude fat content was determined using soxhlet extraction method (AOAC, 2000) (Method 22.033) using SoxTec (Tecator, model NT 1043, Sweden).

The crude protein content was determined using Kjeldhal method (AOAC, 2000) (Method 2.055 to 2.058). The crude fiber determination involves (I) acid digestion of the sample to hydrolyze the carbohydrates and proteins and (II) alkali digestion to saponify fatty acids and related compounds (AOAC, 2000) (Method 22.38). Ascorbic acid was estimated by visual titration method of reduction of 2,6-dichlorophenol-indophenol dye.

The β -carotene contents were isolated by column chromatography and estimated spectrophotometrically (Noelle and Grivetti, 2002). The vanillin-HCl was used for the quantitative determination of tannins. Anti-oxidant activity was determined by using the 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay.

Mineral analysis

For determination of mineral contents, dried plant material, in triplicate, were subjected to wet digestion procedure in accordance with AOAC (2000). This digested solution aspirated directly onto a double beam atomic absorption spectrophotometer (Perkin Elmer, model Analyst 700) for the estimation of Ca, Mg, Fe, Zn, Cd, Cu, Cr, Mn, Se, Ni. Sodium and potassium were determined by flame photometry technique by standard method (AOAC, 2000).

Bioassays

Extraction

The powdered plant material (3.5 kg) was extracted with 85% methanol at room temperature (5 L \times 3) for 36 to 40 h. The solvent after extraction was evaporated at low temperature under reduced pressure in rotary vacuum evaporator to obtain crude extracts (ca 350 g). The extract was dissolved in water and partitioned into hexane (37 g), chloroform (41 g) and aqueous layers (19 g). The crude methanolic extract and fractions were subjected to biological screening/ bioassays.

Table 1. Proximate composition of wild and 3 different ecological region's celery (*Apium graveolens* Linn).

Celery source	Moisture	Ash	Fat	Protein	Fiber	NFE	Vitamin-C	β -carotene
	Percentage						(mg 100 ⁻¹ g)	
Wild	15.31	1.98	3.14	5.68	17.25	56.64	2.15	2.97
Peshawar	16.32	1.78	2.21	5.94	14.84	58.91	2.98	2.75
D.I. Khan	15.34	1.35	2.59	7.53	18.28	54.91	2.35	1.89
Swat	19.51	1.12	2.45	6.21	15.87	54.84	2.34	2.87
Mean	16.62±2.19	1.56	2.60	6.34	16.56	56.33	2.46	2.62

Antimicrobial assay

Anti-bacterial assay of the investigated extracts was determined by using Agar well diffusion method (Atta et al., 2001). Whereas, anti-fungal assay of the investigated extracts was determined by the agar tube dilution method (Choudhary et al., 1995). Growth in the culture medium containing different extracts was determined by linear growth in mm and % growth inhibition was calculated with reference to the negative control. The standard drug used was Miconazole 70 and Miconazole 98.4. Antileishmanial activity was determined using promastigotes for *in vitro* determination of leishmanicidal activity (Atta et al., 2001). Brine shrimp lethality bioassay of the crude extracts was performed using *Artemia salina* (brine-shrimp eggs) in three different concentrations (10, 100 and 1000 $\mu\text{g ml}^{-1}$). Etoposide ($\text{LD}_{50} = 7.4625 \mu\text{g ml}^{-1}$) was used as the standard reference cytotoxic drug. The data was analyzed with Finney computer program to determine LD_{50} values with 95% confidence interval. Insecticidal and cytotoxicity activity was determined the standard protocol of Isman (1987) and Carballo et al. (2002). The concentration of each sample was taken as 1019.10 $\mu\text{g cm}^{-2}$. The standard drug used was permethrin at concentration of 235.9 $\mu\text{g cm}^{-2}$.

RESULTS AND DISCUSSION

The proximate composition, vitamin C and β -carotene content (Table 1) revealed that all the examined plants contained appreciable amount of moisture (16.62±2.19%), ash (1.56±0.57%), Fat (2.60±1.09%), protein (6.34±1.41%) and fiber (16.56±31%). The wild celery had good level of vitamin C (2.15 mg^{-1} 100 g) and β -carotene (2.97 mg^{-1} 100 g). These results could explain that celery seeds are good source of proximate composition and vitamins. The phytochemical screening of methanolic extracts of celery (Table 2) showed the presence of various groups of compounds including tannins, steroids, phenolic acids, terpenoids, flavonoids, volatile oils and saponins, whereas cardiac glycosides was absent in all the samples. Plants and herbs contain secondary metabolites that have established medicinal activity. Also, they exhibit physiological activity (Atta et al., 2001). The presence of steroidal compounds is of great importance and interest in pharmacy due to their relationship with compounds such as sex hormones. This may be the reason that the leaves of *A. graveolens* Linn are used by the expected or breast feeding mothers to ensure their hormonal balance, since steroidal structure

could serve a potent starting material in the synthesis of these hormones (Okwu, 2001). Table 2 also presents tannins, phytic acid, total phenol contents and antioxidant potential of the celery samples examined. Among the antioxidant parameters, a good amount of total phenols (177.23 $\text{mg } 100^{-1}\text{g}$) and tannins (4.39 $\text{mg } 100^{-1}\text{g}$) were observed. This could explain that celery has a good radical-scavenging potential at various concentration levels irrespective of their origin. The methanolic extracts of the wild celery showed an important antioxidant effects at 50, 100 and 300 μg^{-1} ml (22.4, 29.3 and 32.5% respectively). Probably, seed/fruit parts of wild plants contained higher amounts of other phytochemicals in addition to the antioxidants quantified in this study. This explains that combination of antioxidant with phytochemicals is supportive to achieve the health benefits.

Likewise, results of the present study (Table 3) showed that the elements analyzed were in the order of Na> P> K> Ca> Mg> Fe> Cu> Mn> Zn> Ni> Pb> Pt> Cd> Se> Cr. The results of the current study further revealed that Mg, Ca, P, K and Na were present in high amount (556, 709, 4667, 2166 and 7000 $\mu\text{g g}^{-1}$ respectively) as compared to trace minerals. Zn, Fe and Ni were also found in all the celery samples in the range of 11.96 to 15.61, 2.01 to 5.5 and 2.01 to 5.5 $\mu\text{g g}^{-1}$, respectively. Zinc deficiency is of growing concern in the developing world because of the consumption of vegetarian foods that have inhibitory components for zinc absorption. Similarly, maximum value of $56.9 \pm 0.81 \mu\text{g g}^{-1}$ copper was found in all the four samples of wild celery, whereas minimum concentration of $39.98 \pm 1.18 \mu\text{g g}^{-1}$ copper was found in D.I. Khan celery. Copper is one of the essential micronutrients and its adequate supply for growing plants should be ensured through artificial or organic fertilizers (Itanna, 2002). Nickel is supposed to activate some enzyme systems, but its toxicity at higher levels is well established in certain areas of the world. However, nickel toxicity in humans is not a very common occurrence due to its low absorption (Shils, 1997). Trace amounts of iron, zinc selenium, copper, and manganese in foods are probably involved in antioxidant defence mechanisms (Colak et al., 2005), therefore, inadequate intake of these nutrients has been associated with ischemic heart disease, arthritis, stroke and cancer, where

Table 2. Antioxidant activity and phytochemical screening of wild and 3 different ecological regions celery (*Apium graveolens* Linn).

Celery source	Tannins	Phytic acid (mg 100 ⁻¹ g)	Total phenol	Antioxidant activity (50 µg ⁻¹ ml)			Phytochemical screening
				50	100	300	
Wild	4.39	22.05	177.23	22.44	29.37	32.54	Tannins, steroids, phenolic acids, terpenoids, flavonoids, volatile oils, saponins.
Peshawar	3.96	21.74	158.27	21.28	25.66	34.27	Tannins, steroids, phenolic acids, terpenoids, flavonoids, volatile oils, saponins.
D.I. Khan	3.89	19.85	155.41	23.37	26.45	37.33	Tannins, steroids, phenolic acids, terpenoids, flavonoids, saponins.
Swat	4.22	21.23	170.25	19.45	28.87	35.17	Tannins, steroids, phenolic acids, terpenoids, flavonoids, volatile oils, saponins.

Table 3. Analysis of different minerals in *Apium graveolens* Linn collected from different districts of Khyber Pakhtunkhwa Province.

Element	Wild	Peshawar	Swat	D.I. Khan
Cr	0.73 ± 0.04	1.37 ± 0.58	Traces	0.11 ± 0.01
Se	1.32 ± 0.46	0.28 ± 0.021	0.08 ± 0.021	0.31 ± 0.141
Cd	1.55 ± 0.42	1.66 ± 0.30	0.91 ± 0.12	0.31 ± 0.79
Pt	2.13 ± 0.44	2.63 ± 0.17	Not detected	1.83 ± 0.05
Pb	3.51 ± 0.74	2.37 ± 0.58	4.96 ± 0.82	5.12 ± 0.7
Ni	5.5 ± 0.31	3.76 ± 0.55	2.55 ± 0.02	2.01 ± 0.21
Zn	11.96 ± 1.28	14 ± 1.41	15.61 ± 1.84	14.5 ± 1.41
Mn	38.6 ± 1.13	39.3 ± 1.23	35.3 ± 0.91	32.13 ± 0.18
Cu	56.9 ± 0.81	42.4 ± 0.68	51.43 ± 1.29	39.98 ± 1.18
Fe	305.2 ± 8.87	101.4 ± 0.019	184.6 ± 1.33	201.5 ± 28.05
Mg	490 ± 209.9	556 ± 190.13	243 ± 89.81	316 ± 1.41
Ca	709 ± 35.65	513 ± 4.65	547 ± 77.18	403 ± 215
K	2166 ± 816	1400 ± 216	1426 ± 81.2	1235 ± 80.4
P	4667 ± 7.07	3243 ± 5.07	4543 ± 8.16	4623 ± 8.16
Na	5333 ± 3.29	7000 ± 2.60	4113 ± 8.58	4877 ± 3.77

*The results present an average of three replicates with SD.

Table 4. Antibacterial activities at 3 mg ml⁻¹ of crude extract of *Apium graveolens* Linn (mm zone of inhibition diameter).

Celery source	Fraction	Bacterial strains					
		<i>E. coli</i>	<i>B. subtilis</i>	<i>S. flexneri</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>
Wild	Hexane extract	ND	8	6	7	6.0	6
	Chloroform extract	ND	8	8	6	ND	6
	Methanol extract	10	12	8	9	7	9
	Water extract	ND	ND	7	6	6.0	8
Peshawar	Hexane extract	ND	6	6	7	ND	6
	Chloroform extract	ND	8	6	8	ND	7
	Methanol extract	9	10	8	10	6	8
	Water extract	ND	8	6	6	ND	8
D.I. Khan	Hexane extract	ND	6	6	6	ND	7
	Chloroform extract	ND	10	6	6	ND	6
	Methanol extract	9	10	9	9	ND	8
	Water extract	ND	12	6	6	ND	7
Swat	Hexane extract	ND	8	6	6	ND	6
	Chloroform extract	ND	14	6	8	ND	7
	Methanol extract	8	10	8	10	6	9
	Water extract	ND	8	6	8	ND	7
Standard drug (Imepinum)		26	26	25	19	18	22

ND: Not detected.

pathogenic role of free radicals is suggested (Lall et al., 1999). It is observed that the availability of various elements for growing plants is attributed to the composition of the soil, water and atmospheric condition as well as permissibility, selectivity and absorbability of plants for the uptake of different elements.

Wild plants play a vital role in fulfilling the basic health needs and offer a new source of antibacterial, antifungal and antiviral agents (Coelho de Souza et al., 2004). N-hexane, chloroform, methanol and aqueous extracts of celery was screened for their antibacterial, antifungal, lethality bioassay, insecticidal and antileishmanial studies (Tables 4 and 5).

The results revealed that these extracts showed various degrees of inhibition of antibacterial activity against some of the tested microorganisms, (*Escherichia coli*, *Bacillus subtilis*, *Shigella flexneri*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*) ranging between 6 to 14 mm (zone of inhibition). The methanolic extract of all the examined celery showed positive antibacterial activity against all strains. Similarly, antifungal potential of the celery was determined against *Trichphyton longifuss*, *Candida albicans*, *Aspergillus flavus*, *Microsporium canis*, *Fusarium solani* and *Candida glabrata* in concentration 200 µg ml⁻¹ of DMSO. The standard drug used was Miconazole 70 and

Miconazole 98.4. The result indicated that n-hexane and methanolic extract showed strong inhibitory activity against *Trichphyton longifuss* (70%) and *M. canis* (60 and 80%), respectively. Similar trends were also observed in other extracts of the said studied sample. *T. longifuss* is responsible for a pathogenic characteristic, cutaneous mycosis which causes a severe type of acute inflammatory infection of the hair and follicle known as Favus and results in permanent hair loss and sometime infects the nails and skin. *M. canis* is an animal pathogen responsible for cutaneous mycosis. It is the most common cause of ring worm infection of hair and skin in dogs and cats (Coelho de Souza et al., 2004). Human infection usually acquired by contact with infected animals, particularly cats.

The results revealed that all the organic solvents (hexane, chloroform, methanol) and water extracts showed no brine shrimp lethality. Similarly, the crude extracts were examined for their insecticidal activity by screening them against *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Callosbruchus analis* and *Trogoderma granarium*. It was observed that all the three organic solvents (hexane, chloroform, methanol) and water extracts showed no anti- insecticidal activity. The results showed that they exhibited no leishmanicidal activity with IC₅₀ value of >100 µgml⁻¹.

Table 5. *In vitro* antifungal bioassay of *Apium graveolens* Linn.

Celery source	Fraction	Name of fungi strain (% inhibition)					
		<i>T. longifusus</i>	<i>C. albicans</i>	<i>A. flavus</i>	<i>M. canis</i>	<i>F. solani</i>	<i>C. glabrata</i>
Wild	Hexane extract	0	0	0	0	0	0
	CHCl ₃ extract	0	0	0	0	0	20
	Methanol extract	70	0	0	80	0	50
	Water extract	30	0	0	0	0	0
Peshawar	Hexane extract	0	0	0	0	20	0
	CHCl ₃ extract	0	0	0	0	0	30
	Methanol extract	75	0	0	70	0	40
	Water extract	40	0	0	0	20	0
D.I. Khan	Hexane extract	0	0	0	0	0	0
	CHCl ₃ extract	0	0	0	0	0	20
	Methanol extract	80	0	0	75	0	40
	Water extract	35	0	0	0	20	0
Swat	Hexane extract	0	0	0	0	0	0
	CHCl ₃ extract	0	0	0	0	0	20
	Methanol extract	65	0	0	70	0	45
	Water extract	35	0	0	0	20	0

Conclusion

From the present study it can be concluded that plants under study contained moisture, ash, fat, protein and fiber, Mg, Ca, P, K and Na. The phytochemical screening of methanolic extracts of celery showed the presence of various groups of compounds including tannins, steroids, phenolic acids, terpenoids, flavonoids, volatile oils and saponins. The methanolic extract of all the examined celery showed positive antibacterial and antifungal activity against different strains tested. All the organic solvents (hexane, chloroform, methanol) and water extracts showed no brine shrimp lethality and leishmanicidal activity.

ACKNOWLEDGMENTS

The authors acknowledge the financial support provided by the HEC (Higher Education Commission, Pakistan) through HEC-BC link program Islamabad Pakistan and to the International Centre for Chemical and Biological Sciences HEJ-RIC, University of Karachi Pakistan for providing necessary space and facilities for bioassays.

REFERENCES

Ahmed B, Alam T, Varshnev M, Khan SA (2002). Hepatoprotective activity of two plant belonging to the Apiaceae and the Euphorbiaceae family. *J. Ethnopharmacol.*, 79: 313-316.

- AOAC (2000). Association of Official Methods of Analysis: Official Methods of Analysis, 17th edition (edited by Dr. William Horwitz); AOAC Int. Gaithersburg, Maryland, U.S.A.
- Atta UR, Choudhary MI, Thomsen WJ (2001). Bioassay Techniques for Drug Development; Harwood Academic Publishers, Amsterdam. The Netherlands.
- Bjeldanes LF, Kim IS (1977). Phthalide Components of Celery Essential Oil. *J. Org. Chem.*, 42: 2333-2335.
- Carballo LJ, Hernandez-India LZ, Perez P, Gravalos MD (2002). A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. *Biol. Med. Centra.*, 2: 1-10.
- Choochote W, Tuetun B, Kanjanapothi D, Rattanachanpichai E, Chaitong U, Chaiwong P, Jitpakdi A, Pongsri T, Doungtr R, Benjawan P (2004). Potential of crude seed extract of celery, *Apium graveolens* L., against the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae). *J. Vect. Ecol.*, 29: 340-346.
- Choudhary MI, Shahwar D, Perveen Z, Jabbar A, Ali I, Rahman AU (1995). Antifungal steroidal lactones from *Withania coagulance*. *Phytochem.*, 40: 1243-1246.
- Coelho de Souza G, Haas APS, Von Poser GL, Schapoval EES, Elisabetsky E (2004). Ethno-pharmacological studies of antimicrobial remedies in the south of Brazil. *J. Ethnopharmacol.*, 90: 135-143.
- Colak H, Soylak M, Turkoglu O (2005). Determination of trace metal content of various herbal and fruit teas produced and marketed from Turkey. *Trace Elements Elect.*, 22: 192-195.
- Davidson PM, Naidu AS (2000). Phyto-phenols. In: Naidu AS (Ed) *Natural Food Antimicrobial Systems*, CRC Press, Boca Raton, FL, pp. 265-294.
- Isman MB (1987). Insecticides of plant origin. Arnaen JT, Philogene BJR, Morand P (Eds.), *ACS Symposium Series 387: Am. Chem. Soc. Washington DC.*, p. 44.
- Itanna F (2002). Metals in leafy vegetables grown in Addis Ababa and toxicology implications. *Ethiopia J. Health Dev.*, 16: 295-302.
- Jian T, Yuangang Z, Thomas GH, Robert TR, Chi-Tang H (1990). Free and Glycosidically Bound Volatile Compounds in Fresh Celery (*Apium graveolens* L.) *J. Agric. Food Chem.*, 38: 1937-1940.
- Jain GC, Hemant P, Khajja BS, Kusum J, Jhalani S, Agarwal S, Sameer

- S Kailan Z, Feng Z, Zhihui L, Yulei Z, Lixia C, Feng Q (2009). Triterpenoids and Flavonoids from Celery (*Apium graveolens*). J. Nat. Prod., 72: 1563-1567.
- KP (2010). Khyber Pakhtunkhwa (Province, Pakistan): Geography-Britannica Online Encyclopedia. itannica.com.<http://www.britannica.com/EBchecked/topic/419493/Khyber-Pakhtunkhwa/Geography>. Retrieved.
- Lall SB, Singh B, Gulati K, Seth SD (1999). Role of nutrition in toxic injury. Indian J. Exp. Biol., 37: 109-116.
- Lewis DM (1984). Physiology and metabolism of alditols. In: Storage Carbohydrates in Vascular Plants (Ed. by DH Lewis), pp. 157-180. Society for Experimental Biology seminar series 19. Cambridge University Press, Cambridge.
- Momin RA, Nair MG (2001). Mosquitocidal, nematicidal and anti-fungal compounds from *Apium graveolens* L. seeds. J. Agric. Food Chem., 49: 142-145.
- Mookherjee BD, Wilson RA (1990). Tobacco Constituents. Their Importance in Flavor and Fragrance Chemistry. Perfum. Flavor, 15: 27-49.
- Noelle J, Grivetti LE (2002). Gathering practices of Karenwoem: questionable contribution to beta-carotene intake. J. Food Sci. Nutr. 53: 489-501.
- Norman J (1990). The Complete Book of Spices. Dorling Kindersley, London-UK.
- Okwu DE (2001). Evaluation of the chemical composition of indigenous spices and flavouring agents. Global J. Pure Appl. Sci., 7: 455-459.
- Shils ME (1997). Magnesium. In: O'Dell BL, Sunde RA, eds. Handbook of nutritionally essential minerals. New York: Marcel Dekker, Inc: pp. 117-152.
- Sipailiene A, Venskutonis PR, Sarkinas A, Cypiene V (2005). Composition and antimicrobial activity of celery (*apium graveolens*) leaf and root extracts obtained with liquid carbon dioxide. Acta Hortic., (ISHS) 677: 71-77.
- Sofowara A (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria, p. 289.
- Spices Board (2008). The Indian Spices Board: offers domestic and international prices for spices on its Internet site-<http://www.indianspices.com>.
- Trease GE, Evans WC (1989). Pharmacognosy. 11th edn. Brailliar Tiridel Can. Macmillian publishers.
- Tsi D, Das NP, Tan BK (1995). Effects of aqueous Celery (*A. graveolens*) extract on lipid parameters of rats fed a high fat diet. Planta Med., 6: 18-21.
- Tsi D, Tsi BKH (2000). The mechanism underlying the hypocholesterolaemic activity of aqueous celery extract, its butanol and aqueous fractions in genetically hypocholesterolaemic rick rats. J. Life Sci., 66: 755-776.
- Tuetun B, Choochote W, Rattanachanpichai E, Chaithong U, Jitpakdi A, Tippawangkosol P, Riyong D, Pitasawat B (2004). Mosquito repellency of the seeds of celery (*Apium graveolens*). Ann. Trop. Med. Parasitol., 98: 407-417.
- Tuetun B, Choochote W, Pongpaibul Y, Junkum A, Kanjanapothi D, Chaithong U, Jitpakdi A, Riyong D, Wannasan A, Pitasawat B (2009). Field evaluation of G10, a celery (*Apium graveolens*)-based topical repellent, against mosquitoes (Diptera: Culicidae) in Chiang Mai province, northern Thailand. Parasitol. Res., 104: 515-521.
- Wichtl M (1994). Herbal drugs and phytopharmaceuticals. Medpharm Scientific Publishers, Stuttgart, pp. 81-82.