

## ELEMENTAL ANALYSIS OF *CALENDULA OFFICINALIS* PLANT AND ITS PROBABLE THERAPEUTIC ROLE IN HEALTH

Shahnaz Ahmed\*, Atiq-ur-Rahman, M Qadiruddin and Shamim Qureshi

Pharmaceutical and Fine Chemical Research Centre, PCSIR Laboratories Complex, Karachi-75280, Pakistan

(Received 30 January 2001; accepted 10 December 2002)

Elemental analysis of crude ethanolic and aqueous extracts of *Calendula officinalis* plant and its individual parts i.e. flowers, leaves and roots reveal the presence of major, minor and trace elements in appreciable quantities. Elements detected in crude ethanolic and aqueous extracts of whole *Calendula* plant are calcium 5319, 2464 ppm, sodium 16990, 14745 ppm, potassium 79782, 71617 ppm, magnesium 6059, 9060 ppm, iron 74.459, 22.94 ppm, copper 8.05, 1.27 ppm, manganese 37.22, 36.96 ppm, zinc 51.50, 34.00 ppm, nickel 5.10, 2.45 ppm, cobalt 1.39, 0.98 ppm and cadmium 0.40, 0.50 ppm. While chromium and lead were found to be present in 1.01 ppm in ethanolic and in traces in aqueous extracts.

Highest percentages of iron 125, 28.16 and copper 80.59, 10.19 ppm were found to be present in ethanolic and aqueous extracts of root portion respectively. The highest quantity of nickel 8.46 ppm was found in aqueous extracts of roots while in ethanolic extract it was 7.10 ppm in flowers. *Calendula officinalis* and its individual parts were found to be rich in calcium, sodium, potassium and magnesium contents which are not only essential but are also required for the haemostatic control of the body.

**Key words:** Herbal plant, Elemental analysis, *Calendula* plant.

### Introduction

*Calendula officinalis* Linn, Compositae, commonly known as Genda, Zargul or Marigold (Kirtikar and Basu 1933; Chopra *et al* 1985) is not only a spring board for monasteries and garden but has an array of uses in drugs, foods, feed, beverages, dyes, culinary, cosmetics, perfumery industries and at ceremonial religious occasion. (Kirtikar and Basu 1933; Robert 1968; Chopra *et al* 1985; Mamchur *et al* 1987; Loggia *et al* 1994; Kalvathev *et al* 1997; Vasudevan *et al* 1997).

Medicinal uses of *Calendula* plant and contemporary pharmacological studies have proved that the effectiveness of *Calendula* plant mostly depends upon its organic constituents (Mamchur *et al* 1987; Jakupovic *et al* 1988; Loggia *et al* 1994; Vasudevan *et al* 1997) but the effectiveness of inorganic constituents of *Calendula* plant has been neglected despite of the fact that many states of health impairments and disease are linked to elemental imbalance (Under-Wood 1977; Shahnaz *et al* 1994; Liu Longhim and Cushiu 1997). Efforts have been made to develop health protective products based on trace elements and traditional plants (Wang Chang Chung and Waugh 1997). The survey of literature on *Calendula* plant reveal that it is an effective remedy against epileptic fits, fever, pain, kidney trouble, muscular pain, hypertension, skin diseases, cuts, wounds, ulcers, leucorrhoea,

cancer and bleeding piles. The plant is also effective as hypocholesterolemic, antimicrobial anti-inflammatory, haemostatic and against urinary tract infection (Kirtikar and Basu 1933; Chopra *et al* 1985; Mamchur *et al* 1987; Krazhan and Garazha 1999; Klossner and Axid 2000).

Present work, therefore, is an attempt to provide information on elemental contents of *Calendula officinalis* plant in order to have an unbiased scientific look along with the realization of munificent role of elements present in the plants as remedial agents. These elements could act as an adjuvant or as an alternate effective therapy of controlling, combating or treating certain diseases/disorders along with the organic constituent in the plants.

### Materials and Methods

**Experimental procedure. Collection of plants:** The *Calendula officinalis* plant was collected from the floral beds of PCSIR Laboratories Complex, Karachi in the month of April, washed thoroughly and dried in air before use.

**Estimation of moisture.** Samples (2.0g), each of whole *Calendula* plant, flowers and leaves, were taken in a weighed china dish. It was dried in an oven at  $130^{\circ}\text{C} \pm 1^{\circ}\text{C}$  till the weights were constant. The loss in weight was taken as moisture content. Estimation was carried out by the method of (AOAC 1984).

\*Author for correspondence

**Estimation of ash.** Weighed materials, each of dried whole *Calendula* plant, flowers and leaves were ignited in a muffle furnace at 550°C, until whitish grey ash was obtained. The resultant masses were cooled and treated with 1.0ml concentrated nitric acid and reignited for 1.5h to render them completely free from carbon. Samples were cooled, reweighed and percentage ash content of each samples was calculated.

**Extraction.** Fully grown mature plants were taken, washed and dried in air. Whole plant and its individual parts i.e. flowers and leaves 1.0 kg each and roots 0.25 kg were chopped into small bits and soaked in 95% ethyl alcohol for 96 h with continuous agitation for 10 h day. The solvent was then decanted and concentrated *in vacuo*. The resultant gel like mass was called as ethanolic extract. A part of the ethanolic extract was then partitioned with water and petroleum ether (2:1, v/v). Aqueous layer was then separated and concentrated under reduced pressure at room temperature into semi-solid mass to obtain the aqueous extract.

**Reagents.** All reagents, used in present study, were of Analar grade; triple glass distilled water was used in the preparation of all solutions and standards.

**Digestion of samples.** Test materials (1.0g) each of alcoholic and aqueous extract of whole *Calendula* plant and their individual parts (i.e. flowers, leaves and roots) were digested with concentrated nitric acid (HNO<sub>3</sub>), in acid washed pyrex tubes at 120°C, till the solution was clear and the volume was reduced to about 1.0ml and then was made up to 10.0 ml with distilled water. A sample blank was also prepared similarly. Estimations were made using standard addition technique (Price 1978). The dilutions were made such as to keep the concentration of different elements within the linear range of absorbance.

**Estimation of elements.** Sodium and potassium estimations were made using Corning Flame Photometer, Model No.410. HITACHI Z-8000 Atomic Spectrophotometer with Zeeman background corrector and microprocessor control was used in the flame version for other elemental analyses using standard addition technique (Price 1978).

## Results and Discussion

Moisture contents of whole *Calendula* plant, leaves and flowers were 81.83, 85.39 and 85.94% while ash contents on dry basis were 11.62, 14.78 and 7.21% respectively. The reported values are average of replicates.

Elemental analysis of the ethanolic and aqueous extracts of whole *Calendula* plant and its individual parts, i.e. flowers, leaves and roots reveal the presence of major, minor and trace

elements in appreciable quantities (Tables 1 and 2). Thirteen elements i.e. Na, K, Ca, Mg, Fe, Zn, Mn Cu, Ni, Co, Pb, Cr and Cd were found to be present in ethanolic extract of whole *Calendula* plant and leaves. Cobalt and cadmium were not detected in flowers while nickel, cobalt and chromium were not present in root part. Lead and cadmium were found to be present in traces in the alcoholic extract of the root part. On the other hand, all the thirteen elements were found to be present in the aqueous extract of whole *Calendula* plant and leaves. Chromium and lead were detected in traces in whole plant. While chromium was not found in roots. Iron and copper were found to be present in higher concentration in root portion of both ethanolic and aqueous extract (Tables 1 and 2) as compared to other elements while nickel was high only in the aqueous extract of the roots. High percentage of sodium, and potassium were found to be present in root part while calcium and magnesium in leaf portions of both ethanolic and aqueous extract.

Data obtained on the elemental analysis of both ethanolic and aqueous extracts of whole *Calendula* plant and its individual parts lie in conformity with the available elemental data obtained on the basis of their percent concentration of total human body weigh which indicate Ca as major with average concentration of 1% or more, Na, K and Mg as minor from 1 to 0.01% and Fe, Zn, Cu, Cd, Pb, Mn, Co, Ni, and Cr below 0.01% as trace elements, (Table 3) (Heydron 1987).

Furthermore, all these detected elements have a well-documented record of their therapeutic, preventive, medicinal and biological role in many pathophysiological conditions and functions through various mechanisms (Under Wood 1977; Liu-Longhim and Cuishu 1997) Moreover, Ca, Mg, Na, K and Fe and taken specially as essential part of die (Chattergee and Rana 1997).

All the trace elements detected in ethanolic and aqueous extract of the whole *Calendula* plant and its individual parts confirm their uses in different ailments.

Calcium, sodium, potassium and magnesium are nor only the chief electrolytes but are also essential for the haemostatic control of the body. They are essential for the nervous system, maintenance of fluid volume in the body, contractile mechanism of muscles, maintenance of correct rhythm of heartbeat, clotting of blood etc. (Fleck 1971; William 1971; Shahnaz *et al* 1994; Edward *et al* 1995; David 1996; Liu-Longhim and Cushu 1997). Calcium is also a constituent of liposusin and age pigment (Fleck 1971; William 1971). Calcium and magnesium deficiencies result in disease of respiratory system (Hou-Xiaolin *et al* 1995).

Chromium, Co, Zn, Mg, and Cu play an important role in controlling elevated insulin level, impaired glucose tolerance and

**Table 1**  
Elemental analysis of *Calendula* plant (alcoholic extract)

S.No.	Name of elements	Whole plant (ppm)	S.D.±	Flowers (ppm)	S.D. ±	Leaves (ppm)	S.D. ±	Roots (ppm)	S.D.±
1.	Ca	5319.00	18.19	886.00	15.00	1930.00	17.32	284.50	3.24
2.	Na	16990.00	13.58	3447.00	16.70	5409.00	15.62	42764.00	26.48
3.	K	79782.00	19.15	14772.00	18.02	27775.00	19.22	49344.00	25.55
4.	Mg	6059.00	9.94	468.00	6.55	2631.00	4.40	2467.00	6.92
5.	Fe	74.45	3.93	72.30	0.53	39.50	0.25	125.00	2.25
6.	Cu	8.05	0.70	7.49	0.05	5.50	0.09	80.59	0.25
7.	Mn	37.22	0.52	3.26	0.01	119.50	0.74	8.22	0.04
8.	Zn	51.50	2.62	5.15	0.02	54.50	0.52	34.54	0.34
9.	Ni	5.10	0.10	7.10	0.08	4.00	0.01	-	-
10.	Co	1.39	0.06	-	-	2.00	-	-	-
11.	Cr	1.01	-	0.23	-	0.65	-	-	-
12.	Pb	1.01	-	0.77	-	3.00	0.01	Traces	-
13.	Cd	0.40	-	-	-	0.40	-	Traces	-

S.D. = Standard Deviation.

**Table 2**  
Elemental analysis of *Calendula* plant (aqueous extract)

S.No.	Name of elements	Whole plant (ppm)	S.D.±	Flowers (ppm)	S.D. ±	Leaves (ppm)	S.D. ±	Roots (ppm)	S.D.±
1.	Ca	2464.00	17.350	5892.00	26.22	6982.00	59.778	195.22	5.570
2.	Na	14745.00	29.460	8793.00	29.086	20030.00	98.173	34549.00	65.390
3.	K	71617.00	20.290	40889.00	69.555	95797.00	98.658	101919.00	71.330
4.	Mg	9060.00	26.162	1759.00	15.710	6532.00	38.736	1002.00	18.350
5.	Fe	22.94	1.048	13.13	0.205	11.00	0.467	28.16	0.030
6.	Cu	1.27	0.059	0.77	0.039	2.00	0.176	10.19	0.070
7.	Mn	36.96	0.210	29.13	0.726	163.00	2.810	8.64	0.040
8.	Zn	34.00	0.075	34.00	0.262	29.50	0.700	50.10	0.220
9.	Ni	2.45	0.027	3.17	0.153	6.50	0.050	8.46	0.045
10.	Co	0.98	0.025	-	-	2.56	0.010	-	-
11.	Cr	Traces	-	0.08	0.030	0.10	-	-	-
12.	Pb	Traces	-	0.77	0.036	5.50	0.050	4.66	0.026
13.	Cd	0.50	0.036	0.05	0.011	0.45	-	1.21	0.036

elevated serum cholesterol concentration, a risk factor for cardio vascular disease (Mertz 1969; Kelevey 1975; Under-Wood 1977). Clinical studies made on preparations rich in Cr, Co and Zn have proved their therapeutic role in depigmentation hypothermia, hypotonia, central nervous system degeneration, apnea, blood vessel rupture, hypocholesteremia, anaemia and diarrhea (Kelevey 1975). Nickle is also an important trace element. It plays an important role in structure of membranes, lipid metabolism, regulation of RNA and DNA of some enzyme systems. Furthermore, its deficiency can effect iron absorption (Mertz 1974; Vohora 1983). Magnesium inhibits calcium phosphate precipitation in tissues

and thus protects against nephrocalcinosis and formation of renal stones (Cramer 1932). Lower physiological doses of Cr appears to decrease serum cholesterol, atherosclerosis, aortic plaque and increases glucose uptake (Kelevey 1975). Cobalt is an essential co-factor for vitamin-B<sub>12</sub>, while Fe, Cu, and Co are required for the production of red blood cells and prevention of pernicious anaemia. Most research studies indicate the iron deficiency leads not only to behavioral changes but also to biochemical changes in brain. Iron also plays a pivotal role in erythropoiesis and in many intracellular reactions of oxygen transport (Edward 1995 and David Chappal 1996).

**Table 3**  
Elements in reference of man/human body

S.No	Elements	Symbol	Percent of total body wt.	Overall body content mg/kg
1.	Calcium	Ca	1.40%	-
2.	Potassium	K	0.20%	-
3.	Sodium	Na	0.14%	-
4.	Magnesium	Mg	0.03%	-
5.	Iron	Fe	-	10-100
6.	Zinc	Zn	-	10-100
7.	Copper	Cu	-	1-10
8.	Lead	Pb	-	1-10
9.	Cadmium	Cd	-	1-10
10.	Manganese	Mn	-	0.1-1
11.	Cobalt	Co	-	0.01-0.1
12.	Chromium	Cr	-	0.01-0.1
13.	Nickle	Ni	-	0.01-0.1

A perusal of literature on elemental composition of herbal drugs indicates that Ca, Cu, Fe, K, Mg, Cr and Zn have important therapeutic role in diabetes mellitus, specially Cr, Mg, Mn and Zn. Manganese and Zinc are very effective for the treatment of male sterility with low sperm count (Han and Mingsong 1997). The metallic compounds are also valued for their cosmetic and skin therapeutic effects (Berne *et al* 1985; Dumas and Bonte 2000). British National Formulary listed 11 elements for dermatological use. Among the known essential elements, only Zn seems to have received sufficient attention. It is used in powder, paste, ointments, creams, shampoos, lotions and antidandruff preparations. Zinc is also used as a life saving agent in cases of acrodermatitis enteropathica, acne, alopecia aerata, dissecting cellulitis of scalp, wound, ulcer, eczema and dissecting superficial malignant skin lesions known as Mohs surgery (William 1971; Stromberg and Agren 1984; Klossner and Axel 2000). Furthermore, trace elements like K, Zn, Mg, Fe and Cu are also used in the formulation of antibacterial insecticides along with plant extracts (Yang 2000).

It can be easily concluded that the elements detected in *Calendula* plant have a well-documented record of their curative nature and fully support the curative nature and fully support the curative and therapeutic role claimed to cure many ailment. Efforts are needed to cull out the full therapeutic role of *Calendula officinalis* plant based on its elemental composition for better healing and cure.

## References

AOAC 1984 *Methods of Analysis*. 4<sup>th</sup> ed, edited by Sidney Williams Arlington, Virginia, USA, p 152.

- Berne B, Venge P, Ihman S 1985 Complete healing associated with oral zinc therapy. *Arch Dermatol* **21** 1208-1209.
- Chatterjee M N, Rana S 1997 *Text Book of Medical Biochemistry*. 2<sup>nd</sup> ed Jaypee Brothers Medical Publisher (P) Ltd, Bangalore, India, pp 811-846.
- Chopra R N, Chopra I D, Handa K D 1985 *Indigenous Drugs of India*. UN Dhar and Sons Pvt. Ltd., Calcutta, India, p 470.
- Cramer W 1932 Experimental production of kidney lesion by diet. *Lancet* **2** 174.
- David C 1996 *Biochemistry, a Case Oriented Approach*. 6<sup>th</sup> ed, Mosby year book St. Louis p 20.
- Dumas M, Bonte F 2000 *Cosmetic or Dermatological Composition Containing Substances for Increasing the Functionality and/or Expression of CD44 Membrane Receptors of Skin*. PCT Patent Int. Appl. WO 0057836.
- Edward CRW, Bouchier IAD, Haslett C, Chilvers EC 1995 *Davidson's Principles and Practice of Medicine* 7<sup>th</sup> ed, Churchill Livingstone, UK, p 705.
- Fleck H 1971 *Introduction to Nutrition* 2<sup>nd</sup> ed, Macmillan Co. London, UK, pp 167-200.
- Han K, Mingsong LU 1997 Relationship between sterility with insufficient sperm and zinc and manganese. *Guang Weil yuansu kaxue*, **4**(7) 57-58.
- Heydron K 1987 The distribution of some trace elements in health and disease. In: *Proceedings of 1<sup>st</sup> International Conference on Elements in Health and Disease*. Edited by Hakim M S, Hamdard Foundation, Karachi, Pakistan, pp 3-29.
- Hou Xiaolin, Wu Lanlan, Yuan L, Huang L, Jin Zhang X, Chen Y 1995 Study on the relationship between trace elements and respiratory system disease. *Hejishu* **18** (2) 121-128.
- Jakupovic J, Greny M, Bohlmann F, Rustaiyan A, Koussari S 1988 Sesquiterpene glycosides from *Calendula persica*. *Planta Medica* **54**(3) 254-256.
- Kalvatchev Z, Walder R, Garzaro D 1997 Anti HIV activity of extracts from *Calendula officinalis* flowers. *Biomed and Pharmacother* **51** 176-180.
- Kelevey L M 1975 Coronary heart disease, the zinc copper hypothesis. *AMJ Clin Nutr* **28** 764-774.
- Kirtikar K R, Basu B D 1933 *Indian Medicinal Plants*. 1<sup>st</sup> ed. Lalit Mohan Basu, Allahabad India, **II** p 470.
- Klossner, Axel 2000 Agents for Topical Treatment of Skin Diseases. Germany, German Patent Offen, DE, 19, 904, 801, (Cl, A61 K33/04).
- Krazhan I A, Garazha N N 1999 *Calendula* infusion for treating inflammatory periodontal diseases. *Izobreteniya* **22** 276-77.
- Liu-Longhim, Cuishu 1997 Studies of the relationship between trace elements with mental illness. *Guang Weiliang Yuansu Kexue* **4** (12) 49.

- Loggia R, Tubaro A, Sosa S, Backer H, Sarr S T 1994 The role of triterpenoids in topical anti-inflammatory activity of *Calendula officinalis* flowers. *Planta Medica* **60** (6) 516-520.
- Mamchur F I, Bakin S A, Zuzuk B M, Kvarostyanoi K, Karchenko I V 1987 *Calendula* as a medicinal plant. *Farm Zh (Kiev)* **3** 68-69.
- Mendlein J D, Thompson J E, Fine A, Kinsella K J 2000 Beverages for Glucose Metabolism Disorders. Patent PCT. Int Wo. 0057, 729. (C1A23L2/00).
- Mertz W 1969 Chromium occurrence and function in biological system. *Physiol Rev* **49** 163-169.
- Mertz W 1974 The newer essential trace elements-chromium, nickel, vanadium and silicon. *Proc Nutr Soc* **33**(3) 307.
- Price W J 1978 *Analytical Atomic Absorption Spectrometry*. W & J. Mackay Ltd., Lordswood Chatham, Kent, UK.
- Robert T N 1968 The ethanobotany of tagetes. *Economic Botany* **22** (4) 317-325.
- Shahnaz A, Atiq-ur-Rehman, Mohammad Qadeer-ud-din, Yasmeen B 1994 Elemental Analysis of a Herbal Drug "Intellan", a neuro-energizer. *Jour Fac Pharm Gazi* **11** (1) 83-90.
- Stromberg H E, Agren M S 1984 Topical zinc oxide treatment improves arterial and venous leg ulcers. *Brit Jour Derm* **111** 641-643.
- Under-Wood E J 1977 *Trace Elements in Human and Animal Nutrition*. 4<sup>th</sup> ed, Academic Press, New York, USA.
- Vasudevan P, Kashyap S, Sharma S W 1997 Tagetes a multi-purpose plant. *Bioresour Technol* **62** (1-2) 29-35.
- Vohora S B 1983 *Medical Elementology Institute of History of Medicine and Medical Research*. Printing Press, New Delhi, India, p 26.
- Wang Chang-Chung, Waugh X 1997 Application of traditional Chinese herbs to develop health protective products of trace elements. *Guang Weil Yuansu Kexue* **4** (9) 61-64.
- William D R 1971 *The Metals of Life*. Von Nostrand London, UK, p 4 and 5.
- Yang-Xianglin 2000 Preparation of antibacterial insecticide, C. A. Vol. **133** (15) 204193q.