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RESEARCH ARTICLE

DETECTION OF TRACE ELEMENTS IN MEDICINAL FLOWERS OF PAKISTAN

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Abstract

In classical literature of *Unani* Medicine many plants have been mentioned for different disorders but *Materia Medica* failed to exhibit significant progress in terms of medicinal plants included in indigenous formulations with reference to diseases. Pakistan is rich in flora due to diverse ecological zones. Only 12% of the flora is being used as medicinal plants. Elements (*Arkan*) are of prime importance and essential for good health. Iron metabolism is dependent upon copper. Iron deficiency has been associated with decreased immune function. Cu is essential for the production of RNA. Nickel deficiency results in decreased growth. The elemental composition of their parts such as roots, leaves, barks, flowers, seeds and gums must be detected. Flowers have always been natural reservoir of bioflavonoids, elements, vitamins and their reputed effects. Pakistani population is unaware of this natural healing effect of flowers. Fourteen species of flower were analyzed for their composition of seven elements such as Co, Cu, Cr, Fe, Ni, Mn and Pb with the help of Atomic Absorption Spectrophotometer. The results showed the following order of analyzed elements Fe>Mn>Ni>Cu>Pb>Cr and Co. It shall be a great help in understanding their role in particular diseases, knowledge about their inorganic component and their synergistic role in herbal formulations such as decoctions.

Introduction

The Flora of Pakistan is very rich due to diverse climatic conditions as well as different ecological zones. It has more than 6000 of flowering species and at least 12% of the Flora of Pakistan is being used as Medicinal Plants (Ali, 2011). In Pakistan, most of the Flora remains virtually unexplored from point of view of the medicinal utilization through *Unani* System of Medicine (Rizvi, 2007). Ancient Greco-Arab masters of medicine carried out research on many flowers and panned down their observations in their manuscripts for therapeutic purposes. Al-Razi (865-925 A.D.) recommended Chamomile and Red Rose for Cancer diseases. Al-Biruni (973-1040 A.D.) described in his book *Kitab al Saydanah* that Saffron is very effective in liver complaints. Ibn Sina (980-1073 A.D.) recommended Violet in lung troubles. Ibn al-Baitar (1197-1248 A.D.) believed to use Saffron as a tonic. Daud al-Antaki (1599 A.D.) described the flower of Jasmine is beneficial for aphrodisiac and arthritis (Adly, 1982; Ahmad et al., 1989). In *Unani System of Medicine* elements have been given the prime importance. It starts at very micro level and they are vital part of our body. If they are not available to our bodies in the right balance form and amount, our health suffers

(Said, 1996). Cu is essential for the production of RNA. Chromium is used to regulate insulin signaling for biological activities and control sugar metabolism. Iron plays an important role in the production of hemoglobin (Hb) and supply oxygen to the red blood cells. Fe deficiency is associated with myocardial infarction (Dastagir et al., 2014). Ni is essential for the synthesis of insulin and its deficiency may lead to anemia. Mn improves memory or activates nervous system and acts as a catalyst for oxidation (Chen, 1993). The Neem flowers revealed the presence of Cu 8.925, Fe 878.75, Ni 8.05, Pb 16.55 and Zn 30.55 µg/g (ppm).They are used in skin diseases (Rizvi, 2007). The flowers of *Nepata hindostana* (Roth.) contain Ca, 2.12, Mg 0.086, K 33.83, Na 6.90 and Zn 0.0057 mg/g ash. They are useful in cardio vascular complaints like angina pectoris (Ahmad and Siddiqui, 1985). Bibi et al., (2006) have determined the micro-macro elements i.e. Al, Ca, Fe, Na, Mg, K, Si in different parts of Gul-e-Banafsha (*Viola odorata*). In the present study seven elements viz., Co, Cu, Cr, Fe, Ni, Mn and Pb are analyzed in fourteen medicinal flowers. Although, the value of medicinal flowers has been recognized worldwide, utilizing of species in Pakistan has not received much attention. It shall be a great help in understanding their role in particular diseases, knowledge about their inorganic component and their synergistic role in herbal formulations.

Materials and Methods

Collection of Medicinal Flowers

Initially the fresh and mature specimens of fourteen different species of medicinal flowers were collected (1kg each) from different areas of Karachi, Pakistan between April to October, 2013 (**Table 1)**. The best time to collect medicinal flowers is at midday, when they are fully open and in dry weather. The collected flowers separately were brought to the laboratory, where dust were eliminated and washed thoroughly with distilled water then rinsed thrice with deionized water to remove environmental pollutants from the surface of flowers.

Wet Digestion of Flowers

The different beautiful flowers were initially dried under shade at room temperature. Samples of each flower were precisely weighed and placed in beakers. They were prepared as suggested by Allen and co workers (Allen et al., 1986) with little substantiation in minutiae 2.0±0.01g powdered sample was taken in 12mL of acid mixture HNO_3:$HClO_4$ (5:1) and refluxed overnight, refluxed samples were digested on hot stirrer plate at 80^oC until honey white yellow clear solution was obtained then 1-2mL of $HClO_4$ added in solution and digested again until the homogeneous as well as clear solution obtained (**Fig.1**). The resulting solution filtered through Whatman filter paper No.42 (England) and further diluted into a 50mL using deionized water.

Elemental Assay

The Atomic Absorption Spectrophotometer (AAS, Model Perkin-Elmer AAnalyst-700, USA) was used to estimate Co, Cu, Cr, Fe, Ni, Mn and Pb at the Department of Chemistry, Federal Urdu University, Karachi. The various instrument parameters have been presented in (**Table 2**). Instructions for instrument setting, calibration, sensitivity for specific elements as laid down in the operational manual were strictly followed.

Results and Discussion

Fourteen medicinal flowers have been analyzed for their composition of seven elements Co, Cu, Cr, Fe, Ni, Mn and Pb (**Table-3**, **Fig. 2**). It seems that the flowers are rich in nutrients and tend to accumulate more Fe than Mn, Ni, Pb, and Co etc. Among investigated flower Fe was found in highest quantities in *Tricodesma indica* (12.74µg/g) and lowest in *Aloe barbadensis* (1.255µg/g). Mn was also found in large amount in *Tricodesma indica* (1.509µg/g) and in lowest in *Aloe barbadensis* (0.132µg/g). The flowers (*Rosa damascena*) contain Fe 215.232 µg/g and Mn 227.65 µg/g (Arora & Ansari, 1986). Ni was detected in highest quantity in *Acacia nilotica* (0.626µg/g) and and lowest in *Aloe barbadensis* (0.004µg/g). Cu was observed in highest amount in *Adenium obesum* (0.499µg/g) and lowest in *Aloe barbadensis* (0.050µg/g). Pb was found in *Tamarix aphylla* (0.611µg/g) and lowest in *Rosa damascena* (0.002µg/g). Cr was detected in highest amount in *Tricodesma indica* (0.188µg/g) and lowest in *Aloe barbadensis* (0.013µg/g).

Table-1: Investigated Medicinal Flowers & the Area of Collection

Botanical Name	Family	Code # used	Distribution & Area of Collection	Date of Collection	Remedies
Acacia nilotica L. Del. Gul-e-Babul	Mimosaceae	AN-01	Sindh, Bolan, Lasbela, Sibi, Khyber Pakhtun Kwa, Hamdard University	10.6.2013	Recommended in jaundice and palpitation. Reported as anticancer activity (Rizvi, 2001; Meena et al., 2009).
Adenium obesum (Forrsk.) Roem & Schult. Gul-e-Sehrai Gulab	Apocynaceae	AO-02	Cultivated in Karachi, Gardens, Hamdard University	28.6.2013	The extracts possessed antiviral activity (99.3 inhibitions at the concentration of 10 µg/mL (Kiyohara et al., 2012).
Allamanda cathartica Schard. Gul-e-Har kakra	Apocynaceae	AC-03	Cultivated in Karachi, Gardens	20.9.2013	The flowers act as a laxative (Joselin et al., 2012).
Aloe barbadensis L. Gul -e-Ghikuar	Asphodeloideae =Liliaceae	AB-04	Mangopir, cultivated at Hamdard University	7.10.2013	Phenolic constituents of flowers include *in vitro* antioxidative capacity (Keyhanian & Stahl-Biskup, 2007).
Capparris decidua (Forrsk) Edgew Gul-e-Karir	Capparaceae	CD-05	Sindh, Mangopir, Northern by Pass Karachi, Chitral, Gilgit, Swat	15.7.2013	Regarded as antihemorrhagic, antioxidant (Tlili et al., 2010)
Ioxra coccinea L. Gul-e-Guta	Rubiaceae	IC-06	Cultivated in Karachi, Gardens, Urdu University	2.10.2013	Relieve blood shot eyes, cure sores, dysentery, dysmenorrhoea, ulcers Antimutagenecity. (Bhattacharjee & De, 2005; Wongwattanasathien et al., 2010)
Lawsonia inermis L. Gul-e-Mehndi	Lythraceae	LI-07	Sindh, Baluchistan, Punjab, cultivated at Hamdard University	16.7.2013	Act as refrigerant, sedative, soporific. Infusion of flowers cure headache (Rizvi, 2001; Anonymous 2003; Bhattacharjee & De, 2005)
Plumeria acutifolia (Poiret) L. Gul-e-Champa	Apocynaceae	PA-08	Cultivated in University of Karachi	4.7.2013	Possess significant anxiolytic potential (Chatterjee et al., 2013).

Rosa damascena Mill. Gul-e-Surkh	Rosaceae	RD-09	Cultivated at Hamdard University, Thatta, Multan, Ziarat, Mirpus Khas	16.7.2013	Active against *Candida albicans*, *Bacillus cereus*. Cardio and liver protective (Ahmad,1985, Talib & Mahasneh, 2010).
Sesamum indicum L. Gul-e-Til	Pedaliaceae	SI-10	Cultivated in Dadu, Tharparkar, Sialkot, Kasur, Muzafargarh, Gujranwala, Sibi & Lasbela, Hamdard University.	7.10 2013	Alcoholic extract showed antitumor effect on tumor growth (Xu et al., 2003).
Tamarindus indica L. Gul-e-Imli	Caesalpiniaceae	TI (a)-11	Planted Sindh, Jehlum , Hamdard University	12.6.2013 .	Showed antibacterial activity, useful in jaundice, urinary discharge, bleeding piles, conjunctivitis & appetizer (Al-Fatimi et al., 2007).
Tamarix aphylla L. Gul-e-Farash	Tamaricaceae	TA-12	Found in Sindh, Bund Murad Khan	3.7.2013	Flowers are used for removal of kidney stone and retention of urine (ICCBS, 2010).
Thespesia populnea (L.) Sol. ex Correa Gul-e-Pararspipal	Malvaceae	TP-13	Cultivated in Road Sides, Karachi, Hamdard University	10.5.2013	The MeOH of flowers showed antibacterial activities against *Shigella flexneri* , *Rhodococcus terrae* , *Escherichiae coli Streptococcus faecalis, Klebsiella pneumonia, Brevibacterium luteum, Micrococcus flavum, Proteus mirabilis, Bacillus licheniformis Micrococcus luteus, Flavobacterium devorans* , *Shigella sonei, S. dysentriae* (Saravanakumar et al., 2009).
Tricodesma indicum (L).Sm Gul-e-Andusi	Boraginaceae	TI(b)-14	Found throughout Pakistan up to 1400meters Deh Bund Murad Khan, Hamdard University	15.6.2013	Regarded as emollient and diuretic (Rizvi, 2007).

----- Underline means the area of collection of flowers.

Table-2: Instrument Parameters (PE-A Analyst 700)

Elements	Symbol	Wave Length (nm)	Slit Width (nm)
Cobalt	Co	240.7	0.7
Chromium	Cr	357.9	0.7
Copper	Cu	324.8	0.7
Iron	Fe	248.3	0.2
Manganese	Mn	403.1	0.7
Nickle	Ni	341.5	0.7
Lead	Pb	283.3	0.7

Recommended flame: Air-acetylene

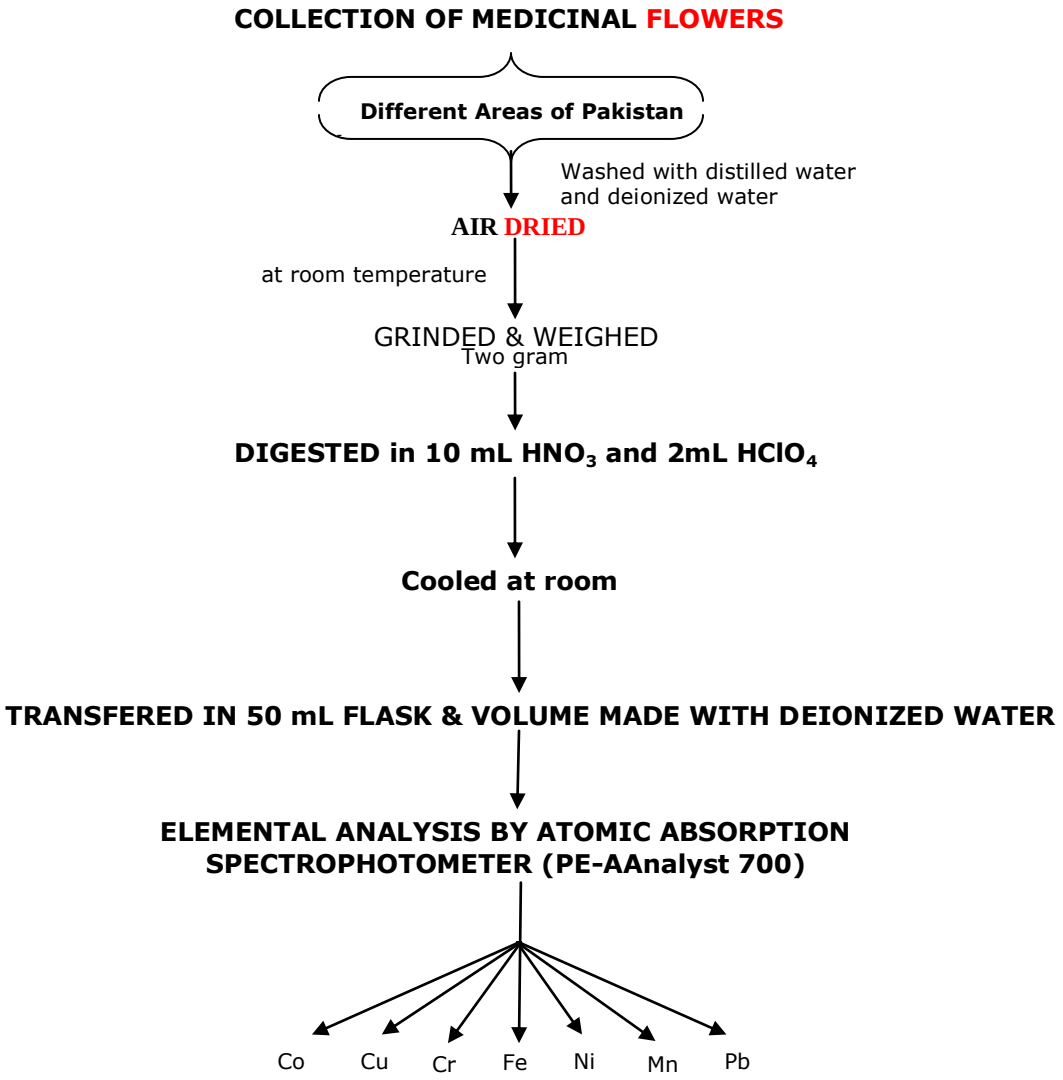

Figure 1. Scheme of Study for Elemental Composition of Medicinal Flowers

Table- 3: Elements in Medicinal Plants[X] (µg/g)

Code#	Values	Co	Cu	Cr	Fe	Ni	Mn	Pb
AN-01	Mean ±SD	0.078±0.018	0.227±0.004	0.124±0.049	11.15±0.015	0.626±0.094	0.909±0.178	0.349±0.006
AO-02	Mean ±SD	0.049±0.024	0.499±0.004	0.035±0.031	8.979±0.102	0.073±0.071	0.665±0.056	BDL
AC-03	Mean ±SD	0.089±0.010	0.348±0.006	0.181±0.032	12.21±0.038	0.593±0.014	1.338±0.049	0.104±0.024
AB-04	Mean ±SD	0.012±0.032	0.050±0.002	0.013±0.047	1.255±0.006	0.004±0.017	0.132±0.032	0.016±0.043
CD-05	Mean ±SD	0.082±0.035	0.263±0.007	0.070±0.040	8.917±0.031	0.083±0.049	0.588±0.138	BDL
IC-06	Mean ±SD	0.004±0.002	0.117±0.001	0.068±0.032	2.483±0.020	0.023±0.012	0.235±0.028	0.061±0.060
LI-07	Mean ±SD	0.051±0.022	0.157±0.008	BDL	5.003±0.029	0.127±0.032	0.752±0.059	0.022±0.050
PA -08	Mean ±SD	0.075±0.017	0.354±0.006	0.091±0.038	9.266±0.093	0.614±0.048	0.544±0.099	0.109±0.019
RD-09	Mean ±SD	0.036±0.021	0.179±0.005	0.042±0.082	6.833±0.021	0.532±0.117	1.095±0.2552	0.002±0.040
SI-10	Mean ±SD	0.005±0.006	0.242±0.002	0.084±0.005	3.246±0.004	0.048±0.030	0.331±0.033	0.027±0.042
TI (a)11	Mean ±SD	0.038±0.016	0.251±0.001	0.057±0.007	5.609±0.044	0.436±0.064	0.333±0.018	0.166±0.048
TA-12	Mean ±SD	0.064±0.005	0.144±0.001	0.150±0.024	11.49±0.024	0.396±0.099	1.282±0.041	0.611±0.017
TP-13	Mean ±SD	0.097±0.011	0.147±0.012	0.130±0.010	10.15±0.080	0.577±0.042	1.332±0.068	0.082±0.013
TI (b)14	Mean ±SD	0.089±0.019	0.304±0.004	0.188±0.017	12.74±0.019	0.447±0.055	1.509±0.126	BDL
Average amount		**0.769**	**3.282**	**1.233**	**109.331**	**4.579**	**11.045**	**1.549**

[X] indicates n=3 replicates, SD = Standard Deviation, BDL = Below Detection Limit

Co was found in highest value in *Thespesia populnea* (0.097µg/g) and lowest in *Ioxra coccinea* (0.004µg/g). Gul-e-Madar (*Calotropis procera*) contains 80 µg/g Ni, 100 µg/g Cu, 130 µg/g Pb, 130 µg/g Cr and 170 µg/g Co (Khan et al., 1989).

Among investigated these species *Adenium obesum*, *Capparis deciduas* revealed the presence of trace metal order Fe> Mn> Cu> Ni> Co> Cr> Pb as well as *Rosa damascena*, *Tricodesma indica* Fe>Mn>Ni>Cu> Cr>Co> Pb and *Plumeria acutifolia*, *Tamarindus indica* Fe> Ni> Mn> Cu> Pb> Cr>Co. While in other species such as *Acacia nilotica, Allamanda cathartica, Aloe barbadensis Ioxra coccinea, Lawsonia inermis, Sesamum indicum, Tamarix aphylla, Thespesia populnea* were found the following trace elements order Fe> Mn>Ni> Pb> Cu> Cr> Co; Fe>Mn>Ni>Cu>Cr >Pb >Co; Fe>Mn> Cu> Pb> Cr>Co >Ni; Fe>Mn> Cu> Cr> Pb> Ni> Co; Fe>Mn> Cu>Ni> Co > Pb >Cr; Fe>Mn> Cu> Cr> Ni> Pb > Co; Fe>Mn> Pb > Ni> Cr> Cu> Co; and Fe>Mn> Ni> Cu>Cr >Co > Pb respectively. Sahito et al., (2001) have analyzed trace elements i.e. Co, Cr Cu, Ni, Pb from *Catharanthus roseus* and *Vinca roseae*, It showed high level of Fe 2.49 µg/g. in flowers of pink variety. Chouhan et al., 2002 have investigated the nutritive element of *Capparis decidua*. It revealed high amount of Iron (Fe) 268 µg/g in flowers.

In over all, the results showed the following order of elements Fe>Mn>Ni>Cu>Pb>Cr>Co in beautiful flowers of Karachi, Pakistan. Iron deficiency is associated with myocardial infarction. Iron is administered to young anemic persons; due to the presence of tested elements; medicinal flowers or other parts of plant could act on liver, portal system and heart. Anemia which is known as *Faqqruddam* in *Unani* Medicine has been meticulously managed with stepwise approach considering all the causative factors (Parmar et al., 1993; Javed et al., 2009). This study confirmed that many flowers contain a good source of Fe and other trace elements. These flowers may be directly or indirectly helpful in the management of health care.

Conclusion

A number of fruits, vegetables and medicinal plants are growing in Pakistan. Aim of our study was to know the proportion of elements in leaves, seeds, roots and flowers which are beneficial for human body such as Al, Ca, Cr, Cd, Fe, I, Mg, Mo, Mn, Na, Pb, K, Zn etc and to draw the complete picture of elementology of medicinal plants found in different regions of Pakistan. The results of elemental composition in flowers shall be helpful in understanding their role in particular diseases, knowledge about their inorganic component and their synergistic role in formulations.

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