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Standardization of Crude Extracts Derived from Selected Medicinal Plants of Pakistan for Elemental Composition Using SEM-EDX

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Abstract: The elemental analysis of crude extracts derived from selected medicinal plants of Pakistan, including *Trichodesma indicum* (whole plant), *Paeonia emodi* (aerial parts), *Aconitum laeve* (roots) and *Sauromatum guttatum* (corms, berries and leaves) was carried out using SEM-EDX technique. Various elements such as C, O, Cl and K were present in all tested plants' extracts, Al was observed in *P. emodi* and *A. laeve*, S was detected in *T. indicum* and *S. guttatum* (corms and leaves) while Mg and P were found only in *S. guttatum* (corms). The data showed that C was present in highest quantity in *T. indicum* and *S. guttatum* (leaves) (>80%) followed by O while in the rest of extracts these two elements were found in approximately equal quantities. The other detected elements were present in comparatively trace amounts. These results were also evaluated in correlations with previously reported biological activities of the tested extracts. This study would provide a new and alterative technique for the standardizations of crude extracts.

Key words: Medicinal plants, standardization, crude extracts, elemental analysis, SEM-EDX

INTRODUCTION

Plants have been used extensively as medicine for the treatment of various ailments since pre-historical times. Several hundred-plant species, in the form of whole plant, crude extracts or purified constituents, are used in indigenous system of medicines, which have ultimately evoluted into the modern therapeutic sciences (Hamayun *et al*., 2004; Zaman and Khan, 1970). Pakistan has lot of God gifted wealth of medicinal plants, has a rich tradition of herbal remedies and, like most developing countries; its rural population still relies mainly on the indigenous system of medicine for their health related matters (Khattak *et al*., 1985). Crude medicinal plant materials worth more than Rs. 150 million (~3 million US$) are used in Pakistan per year while large quantities of these materials are also exported to the international market (~6 million US$) although at cheaper prices (Atta-ur-Rahman and Choudhary, 2005). Therefore, the value-addition into these currently valueless but potentially worthy products is the utmost need of the time. There are several ways for the economic value addition to such products including physical, chemical and biological standardizations. In continuation to our biological standardization of some selected medicinal plants of Pakistan (Khan *et al*., 2005a; b), we have now tested crude ethanolic extracts of those plants for their elemental compositions using scanning electron microscopy attached with energy dispersive X-ray (SEM-EDX) technique.

SEM-EDX, among the various analytical techniques used for elemental analysis, is highly qualified for the identification and the quantification of different elements in various samples of biological and environmental importance. Beside, a powerful tool for such analysis, the method is non-destructive and is more advantageous in multi-elementary analysis than the other existing methods (such as ICP-AES, ICP-MS, AAS and NAA which require sample homogenization and dissolution) in the ease of sample preparations and analysis without any chemical treatment or separations of the constituent elements of the samples (Williamson *et al*., 2004; Salvador *et al*., 2003). Although, this technique has been extensively used for elemental analysis in samples of diverse nature (Williamson *et al*., 2004; Ayo-Yusuf *et al*., 2005; Rahid *et al*., 2001; Klose *et al*.,

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2003) but only few studies are available in literature where this modern and rubusted analytical technique has been employed for the determination of elemental composition in samples from vegetative sources especially of medicinal importance (Sagner *et al*., 1998).

In the present study, we have applied this analytical technique, probably for the first time, to determine the elemental composition of crude extracts derived from the selected medicinal plants (Table 1). This investigation will provide a reliable step towards the standardization of the crude extracts from medicinal plants.

MATERIALS AND METHODS

Plants materials: The plants *T. indicum* (whole plant), *P. emodi* (aerial parts) and *A. laeve* (roots) were collected from Swat, Pakistan. All these plants were identified by Mehboob-ur-Rehman, Plant Taxonomist, Department of Botany, Government Degree College Matta, Swat, Pakistan. *S. guttatum* (corms, leaves and berries) was collected from Mohmund Agency, Pakistan and its identification was confirmed by Prof. Abdur Rashid, Department of Botany, University of Peshawar, Peshawar, Pakistan.

Preparation of extracts: In each case, the plant material was first shade dried and then pulverized into fine powder (*T. indicum*; 4.8 kg, *P. emodi*; 2.1 kg, *A. laeve*; 1 kg and *S. guttatum*: tubers; 5.5 Kg, leaves; 40 g, berries; 30 g). All the powdered plant materials except *A. laeve* were macerated with ethanol. The powder of *A. laeve* was first defatted with *n*-hexane and then the residue was extracted with ethanol. The crude extracts obtained were filtered and evaporated in *vacuu* to dryness (*T. indicum*; 300 g, *P. emodi*; 355 g, *A. laeve*; 80 g and *S. guttatum*: tubers; 315 g, leaves; 6 g, berries; 4 g).

Elemental analysis: The crude extracts derived from the selected medicinal plants were subjected to the elemental analysis using Scanning Electron Microscope (SEM) (Jeol 5910, Japan) with an energy dispersive X-ray spectrometer (EDX) (Oxford Instruments, UK).

RESULTS AND DISCUSSION

The selected plants, *T. indicum*, *P. emodi, A. laeve* and *S. guttatum* grow indigenously in fair quantities and have been reported traditionally for the treatment of various ailments. The chemical and biological profiles of these plants are presented in Table 1. More recently, we screened the crude extracts derived of these plants for various *in vitro* biological activities and their standardizations (Khan *et al*., 2005a; b). Neither these plants nor their extracts have, however, ever been tested for their elemental composition. Here, we present, for the first time, the results of elemental composition of crude extracts derived from these plants using SEM-EDX technique. The SEM-EDX spectra obtained for these extracts are given in Fig. 1 while their elemental compositions are listed in Table 2.

As can be seen from Fig. 1a, the crude extract derived from *T. indicum* showed the presence of various elements such as C, O, S, Cl and K, in which C was in the highest percentage (>80%) followed by O while small quantities of S, Cl and K were also detected (Table 2).

Similarly, C, O, Cl, K and Al were detected in the crude extracts derived from *P. emodi* and *A. laeve* as depicted in Fig. 1b and c. As shown in Table 2, C was found in highest percentage in *P. emodi* extract followed by O while O was detected in highest concentration in the extract from *A. laeve* followed by C. Small amounts of Cl, K and Al were also present in these extracts.

The crude extracts derived from various parts (corms, berries and leaves) of *S. guttatum* showed some variations in their elemental constituents and composition as shown in Fig. 1d-f. C, O, Mg, P, S, Cl and K were detected in the crude extract derived from corms (Fig. 1d), while that derived from berries displayed the presence of C, O, Cl and K only (Fig. 1e). As shown in Fig. 1f, elements such as C, O, S, Cl and K were detected in the crude extract derived from the leaves of *S. guttatum*. The percentage composition of all these elements is presented in Table 2. As can be seen in Table 2, O was found as the major element in the corm followed by C while in berries C and O were present in almost equal amounts. In leaves, C was found to be the most dominant element (>80%), which was followed by O. The rest of the detected elements were, however, present in small quantities in all these extracts. Mg, P and S were detected only in the corms extract while S was found in the leaves extract only (Table 2).

As shown in Table 2 and Fig. 1, all the extracts possessed C and O in the highest percentage. Both these elements are more or less present in equal ratio except *S. guttatum* (leaves) and *T. indicum* where these are present in almost 4:1 ratio, respectively. These two elements along with Cl and K were the common elements to all the extracts. The Cl was most abundant (0.88 wt. %) in *S. guttatum* (berries) among the tested plants. The Cl contents of the extracts derived from *P. emodi* and *S. guttatum* (corms and leaves) were in the range of 0.3-0.4 wt.% while *A. laeve* and *T. indicum* possess the lowest concentration of Cl (0.14 and 0.15 wt.%, respectively). Highest concentration of K was detected in various parts

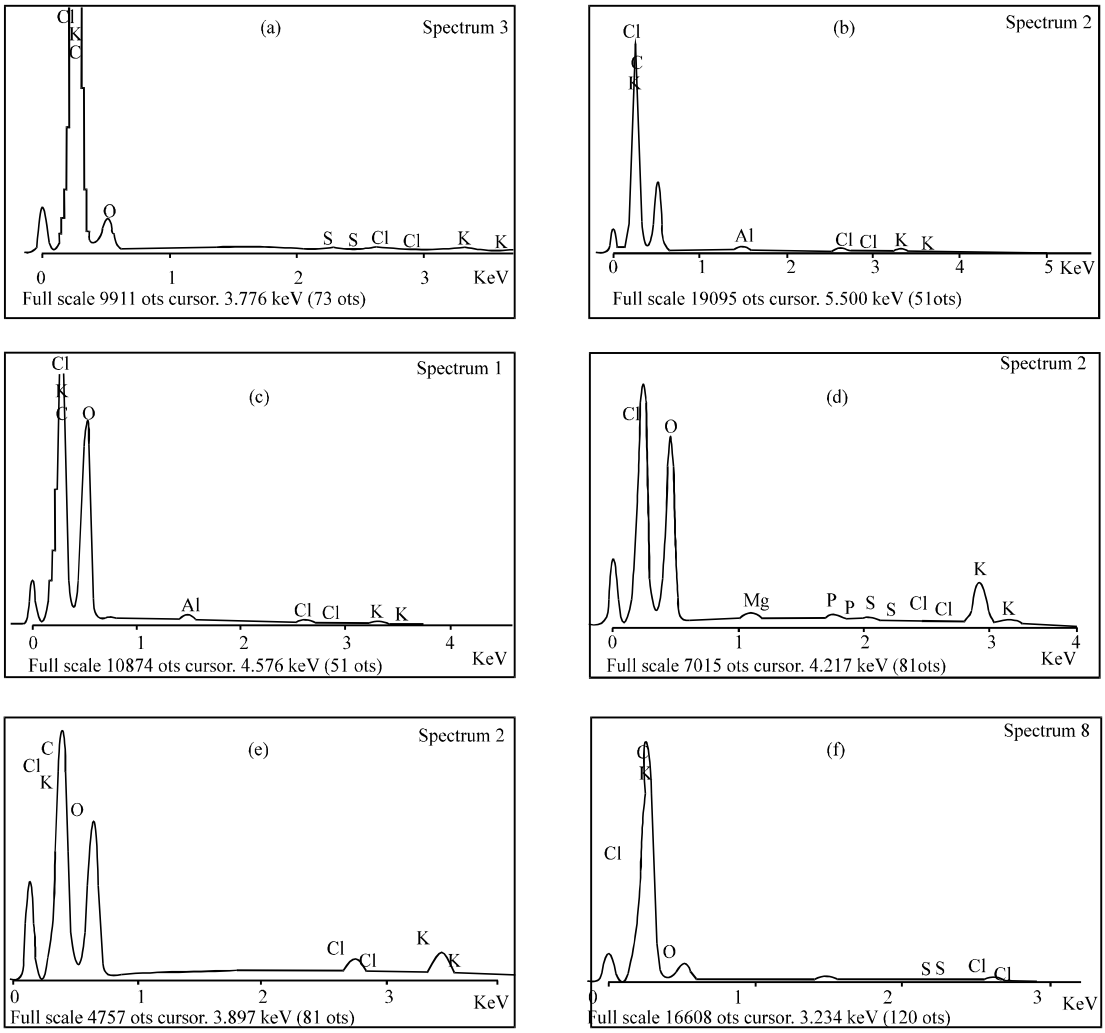

Fig. 1: SEM-EDX spectra for elemental analysis of the crude extracts derived from the selected plants of Pakistan, *T. indicum* (a); *P. emodi* (b); *A. laeve* (c); *S. guttatum* (corms); (d); *S. guttatum* (berries) (e); *S. guttatum* (leaves) (f)

of *S. guttatum* especially the corms and the berries containing 2.29 and 1.67 wt.%, respectively. The leaves contained 0.39 wt. % of this element, which was still higher than the other plants used in the present investigation. *T. Indicum*, *P. emodi* and *A. laeve* possessed K in 0.17, 0.32 and 0.15 wt. %, respectively. Al was only common to *P. emodi* (0.25 wt. %) and *A. laeve* (0.24 wt. %) while the other plants extracts were devoid of this element. Similarly, S was detected only in *S. guttatum* (corms and leaves) and *T. indicum* in 0.15, 0.1 and 0.09 wt. %, respectively. Sulphur containing compounds are already known from the inflorescence of *S. guttatum* (Borg-Karlson *et al.*, 1994) and thus on the basis of these results of elemental analysis it is assumed that these or related constituents may also be present in the corms and leaves of this plant. Similarly, *T. indicum* may also possess some sulphur containing compounds. Uniquely in these plants, *S. guttatum* (corms) only contain Mg and P (0.38 and 0.30 wt. % respectively).

In contrast to the other nutrients, living organisms cannot synthesize minerals elements. Only small fraction of the Ca, Mg and P and most of the Na, K and Cl are present as electrolytes in the body fluids and soft tissues. Electrolytes present in blood or cerebrospinal fluid maintain acid-base and water balance and osmotic pressure. They regulate membrane permeability and exert characteristic effects on the excitability of muscles and nerves (Nielsen, 1987; Bukhari *et al.*, 1987). Therefore, the tested extracts may also be beneficial in the deficiency disorders of these elements along with their core pharmacological activities.

Table 1: Details (name, family, synonyms, local names, uses, reported constituents and part(s) studied) of the selected plants of Pakistan

Plant	Family	Synonyms	Local name(s)	Uses	Reported constituents	Part(s) studied
T. indicum	Boraginaceae	*Trichodesma hirsutum* *Borago indica*	Kulang kallen Nile karaji	Snakebite, diuretic, urinary diseases, depuratives, dysentery, expulsion of dead fetus, fever, sudorific, pectoral, sedative, cough and veterinary uses (Shinwari and Khan, 2000; Perianayagam *et al*., 2004; Ambasta Shri,1986; Chadha, 1976; Srikanth *et al*., 2002; Ali, 1999; http://www.ayurvedavillage.org/ AyurvedicTOMonth.html).	Fatty acids and non-steroidal compounds (Hassan *et al*., 1982).	Whole plant
P. emodi	Paeoniaceae		Mamekh (Urdu) Peony rose Himalayan (English)	Epileptic attacks, cholera, whooping cough, uterine diseases, colic, bilious obstructions, convulsions, dropsy, hysteria, diarrhea, emetic, cathartic, backache, tonic, blood purifier, purgative, headache, dizziness, vomiting and to aid pregnancy (Hamayun *et al*., 2004; Shinwari *et al*., 2003; Kirtikar, 1918; Watt, 1892; Ahmad and Sher, 2005).	1β,3β,5α,23,24-pentahydroxy -30-12,20 (29)-dien-28-oic acid (triterpene), oleanolic acid, betulinic acid, ethyl gallate, methyl grevillate 1,5-dihydroxy -3-methylanthra-quinone, wurdin benzoyl-wurdin (monoterpene glycosides), paeoniflorin, lactiflorin oxypaeoniflorin, emodinol (triterpene), benzoic acid, 3-hydroxybenzoic acids, paeonins A and B (monoterpene galactosides) (Nawaz *et al*., 2000; Muhammad *et al*., 1999; Riaz *et al*., 2003a, b).	Whole plant
A. laeve	Ranunculaceae	-	Jungli atis Maniree	Medicine (Saqib and Sultan, 2005).	8-methyl lycaconitine, 14-demethyl-lycaconitine, *N*-deethyllycaconitine-*N*-aldehyde, lappaconitine, lycaconitine, lapaconidine, lycoctonine, swatinine, delphatine, puberanine and N-acetylsepaconitine (Bisset, 1981; Shaheen *et al*., 2005).	Roots
S. guttatum	Araceae	*Sauromatum venosum* *Typhonium venosum* *Arum cornutum* *Typhonium venosum*	Zahar moora and Marjanay (Pushto) Sanp ki bhooti (Urdu) Voodoo lily (English)	Stimulating poultice in snakebite (Shinwari and Khan, 2000).	Lectins, Dimethyl sulphides, *p*-caryophyllene, indole, skatole, ammonia, trimethylamine, primary amines (Smith and Meeuse, 1966; Borg-Karlson *et al*., 1994; Chen and Meeuse, 1971).	Corms, berries and leaves

Table 2: Elemental analysis of crude extract derived from the selected medicinal plants of Pakistan

	Elemental composition (%)															
	C		O		Mg		P		S		Cl		K		Al	
Extracts	Weight	Atomic	Weight	Atomic	Weight	Atomic	Weight	Atomic	Weight	Atomic	Weight	Atomic	Weight	Atomic	Weight	Atomic
T. indicum	81.80	85.84	17.78	14.01	nd	nd	nd	nd	0.09	0.04	0.15	0.06	0.17	0.06	nd	nd
P. emodi	58.62	65.63	40.44	33.99	nd	nd	nd	nd	nd	nd	0.36	0.14	0.32	0.11	0.25	0.13
A. laeve	47.38	54.65	52.09	45.11	nd	nd	nd	nd	nd	nd	0.14	0.05	0.15	0.05	0.24	0.13
S. guttatum (corms)	44.44	52.62	51.45	45.74	0.38	0.22	0.30	0.14	0.16	0.07	0.35	0.14	2.29	1.06	nd	nd
S. guttatum (berries)	49.24	57.09	48.21	41.96	nd	nd	nd	nd	nd	nd	0.88	0.35	1.67	0.60	nd	nd
S. guttatum (leaves)	80.28	84.76	18.84	14.93	nd	nd	nd	nd	0.10	0.04	0.40	0.14	0.39	0.13	nd	nd

nd: not detected

In our previous studies on the screening of these extracts for various biological activities, *P. emodi* and its subsequent fractions were found to be the most promising especially in enzyme inhibition and antioxidant activities with high phenolic contents (Khan *et al*., 2005a). This extract also displayed excellent herbicidal, moderate insecticidal (Khan *et al*., 2005b) and antispasmodic activities. *S. guttatum* (corms) was found to be the only

extract to have antifungal and antibacterial activities along with brine shrimp cytotoxicity, phytotoxicity and moderate insecticidal and antispasmodic activities. However this extract did not display any enzyme inhibition activities (unpublished data). The rest of the plants found in those investigations were in the overall order of biological importance as; *T. indicum* > *S. guttatum* (berries) > *S. guttatum* (leaves) > *A. laeve* (unpublished data).

The significant biological activities of *P. emodi* may be due the presence of balance amount of C and O in combination with other elements especially Al as shown in Table 2. Al was also found in *A. laeve* but this plant showed least biological activities, which may be due to the absence of the potential constituents for those bioactivities. Antifungal and antibacterial activities of *S. guttatum* (corms) may be due to the presence of S in combination with Mg and P (Table 2). However, it is extremely difficult to draw any correlation between the elemental contents of these crude extracts and their biological activities, which may be due to their complex nature. Detailed research will be required in order to draw any relationship among these parameters along with possible road map for their effectiveness for the treatment of various diseases. However, the current study gives a new, alternative, easy and reliable method for the standardization of the crude extracts derived from various medicinal plants.

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