

Research Article

Quantification of minor, trace and toxic elements in stems of *Santalum album* (L.), *Mangifera indica* (L.) and *Tinospora cordifolia* by instrumental neutron activation analysis

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Abstract

Stems of *Santalum album* (Sandalwood), *Mangifera indica* (Mango wood), and *Tinospora cordifolia* (Giloy) are widely used in the preparation of herbal medicines and formulations in the traditional Indian health care system called Ayurveda. These were analyzed for 4 minor (K, Ca, Cl, Mg) and 13 traces (As, Ce, Co, Cr, Cu, Fe, Hg, La, Mn, Na, Se, V, and Zn) including toxic elements by instrumental neutron activation analysis (INAA). Samples in powder form along with reference materials (NIST SRM 1547 and INCT MPH-2) as comparators were irradiated for 1 min/6 h in Dhruva/CIRUS reactors at BARC, Mumbai. Gamma activity was measured by high-resolution gamma-ray spectrometry. In general, K, Ca, Fe, Mn, and Zn contents are very high in all the samples but *Santalum album*, widely used as a perfume, is more enriched in K, Ca, Cr, Zn, and Se. The concentration of Ca is always high as a major constituent (> 10 mg/g) in all the stem/bark of plant species. A strong inverse correlation ($R^2 = 0.9999$) was observed between Fe and Zn in all three samples and that may be useful in drug manufacturing.

Introduction

Many civilizations around the world have extensively used the plant kingdom as the source of herbal medicines. *Santalum album* L. (Sandalwood) (Figure 1A), *Mangifera indica* L. (mango wood) (Figure 1B), and stems of *Tinospora cordifolia* (giloy) (Figure 1C) are essential ingredients of many herbal formulations used in the traditional Indian health care system called Ayurveda known since 2500 BC. Sandalwood and mango

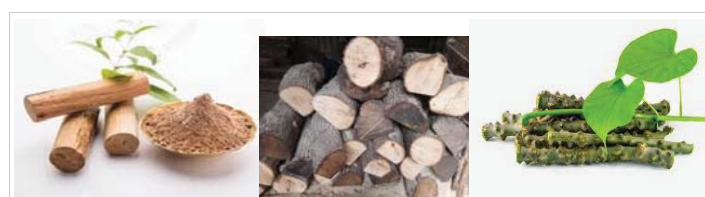


Figure 1: Typical pictures of stem pieces of (A) *Santalum album*, (B) *Mangifera indica*, (C) *Tinospora cordifolia*.

wood are also used in *yagya* (holy fire) and sandalwood is widely used to spread fragrance [1]. All these plants have been known for ages and are widely found in India, Sri Lanka, and Myanmar. Sandalwood is primarily used as a coolant and its oil is prepared by steam distillation with an average content of 4.5% - 2.5%. It is extensively used in perfumes, cosmetics, and its aqueous paste is a sacred unguent. It exhibits significant antipyretic effects, sedative effects, and astringent activity, making it useful as a disinfectant in genitourinary and bronchial tracts, diuretic expectorant, and stimulant. Essential oil of *S album* contains more than 90% sesquiterpene alcohols of which 50% - 60% is the tricyclic α -santalol and 20% - 25% is β -santalol [2]. Also, it has been tested for *in vitro* activity against the Herpes simplex virus [3]. Sandalwood is commonly known as *Chandana* in India and its powder has antiseptic properties that prevent pimples, acne, sores and reduce wrinkles. Aqueous extract of mango wood stem bark is proposed as a nutritional supplement (antioxidant) and used

More Information

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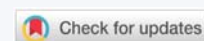
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Keywords: Elemental analysis; INAA; Micronutrients; *Santalum album*; *Mangifera indica*; *Tinospora cordifolia*; Ca content





as an anti-inflammatory, analgesic, and immunomodulatory including dermatological disorders [4].

Giloy (*T cordifolia*) or Guduchi is a glabrous, woody climbing shrub belonging to the family of Menispermaceae. Though all its parts are useful stem is particularly used in the treatment of fever, jaundice, emaciation, urinary problem, diabetes, skin diseases, heart disease, diarrhea and dysentery, leprosy, and helminthiasis [5]. Sharma, et al. [6] have reviewed its diverse pharmacological importance in terms of its chemical constituents such as glycosides, alkaloids, steroids, diterpenoid lactones, sesquiterpenoid, and lignans that establish the phytochemistry and pharmacological activity. It exhibits antifungal activity, antioxidant activity, antiallergic, antiviral, antimicrobial, and antibacterial activity, hypolipidemic effect, anti-HIV potential, antitoxic effect, and wound healing activity. Kapil and Sharma [7] have found *T cordifolia* to possess immune potentiating compounds exhibiting anti-complementary and immunomodulatory activities. Recently Khan and Rathi [8] have reviewed attempts of *T cordifolia* being used for enhancing the immune system, prevention, management, and development of new therapeutic activity to treat SARS-CoV-19. An exhaustive compendium on medicinal plants by Ross [9] deals with chemical constituents such as essential oils, antioxidants, antimicrobial properties, and curative agents against various diseases. Besides pharmaceuticals, these plants have found extensive use in cosmetics, agriculture, and food industries [10]. It has been suggested that the decomposition and transformation of medicinal herbs into the vapor phase during wood burning (*Yagya*) releases many volatile phytochemicals thus spreading fragrance and helping in the purification of the environment [11].

It has been reported that medicinal plants, in general, contain a high concentration of polyphenols, flavonoids, tannins, alkaloids, and other bioactive molecules having high metal binding capacity [12,13]. Also, medicinal plants are known to accumulate many mineral (inorganic) elements derived from the local soil depending on its characteristics and geo-environmental factors [12,14]. Many elements such as Fe, Mn, Co, Cu, Zn, etc, present in soil are likely to be assimilated by plants and the same may be supplied to the human body in bioavailable form without causing any deleterious effects [12]. Only adequate amounts of these micronutrient elements are essential to maintain metabolic functions in our body because its excess or deficiency may cause serious harmful effects resulting in diseases. In the last few decades, the importance of micronutrients and trace elements has been greatly realized in human diseases and their curative effects, Therefore, many workers from different countries have extensively analyzed medicinal herbs and herbal formulations using different techniques such as atomic absorption spectrometry (AAS) [15], energy dispersive X-ray fluorescence (EDXRF) [16], inductively coupled plasma mass spectrometry (ICP-MS) [17], particle-induced X-ray emission (PIXE) [18,19] and

instrumental neutron activation analysis (INAA) [12,20-22]. During the last few decades, we have extensively used INAA for the quantification of elemental contents in a variety of medicinal herbs and herbal formulations [23-26].

It is believed that the materials such as sandalwood, mango wood, and giloy stem used in performing *Yagya* contain many volatile organic compounds (VOCs) which enter the atmosphere when they are burnt in holy fire. Further, these VOCs are likely of medicinal importance when inhaled by the persons performing *Yagya*. Thus they are likely to become immune to or get cured of various diseases. This has been referred to as smoke therapy as it was practiced in ancient India when the smoke of many herbs was found to be effective as having antibacterial with disinfectant properties [27]. In an earlier study, we have identified three new phytoconstituents in methanolic and hexane extracts of *M indicia* (mango wood) [28]. GC-MS studies have been used to identify phenol, myristic acid, diethyl phthalate, ethyl vanillin and cyclotene in the fumes extract of *T cordifolia* [29]. Similarly, for *S album*, compounds identified are dibutyl phthalate, 2, 4-ditert butyl phenol, cyclotene, phenol, guaiacol, diethylene glycol, and hydroquinone [29]. *T cordifolia* extract is widely marketed in tablet/powder form in India and is also used in many herbal formulations.

In the present study, we have used INAA for the determination of 4 minor and 13 trace and ultra-trace elements in three stem samples of two trees and a climber using short and long reactor irradiation with neutrons followed by assaying of gamma activity at different intervals by high-resolution gamma-ray spectrometry. Reference Materials (RMs) from the National Institute of Standards and Technology (NIST), USA, and the Institute of Nuclear Chemistry and Technology (INCT), Poland were used as comparator standards. An attempt has been made to interpret elemental contents in terms of curative effects of medicinal plants.

Experimental

Sample collection and preparation

The dried stem samples of *S album*, *M indica* and *T cordifolia* were procured from Shanti Kunj Pharmacy, Haridwar. These were carefully washed with doubly distilled water to remove any dirt or other surface contaminants. Finally, these were dried at 80 °C in an oven for 24 h, crushed to fine homogeneous powder, and passed through a 100 mesh sieve. Reference Materials NIST SRM 1547 (Peach leaves) [30] and INCT MPH-2 (Mixed Polish Herbs) [31], as comparator standards, were used as received from the respective agencies. All the samples and RMs were dried in an oven at 80 °C for 2 h before packing for irradiation.

Irradiation and counting

About 20 mg each of the samples and RMs were packed



in high-density polypropylene rabbits and irradiated at $5 \times 10^{13} \text{ ncm}^{-2}\text{s}^{-1}$ for 1 min in a pneumatic carrier facility (PCF) of Dhruva reactor at the Bhabha Atomic Research Centre (BARC), Mumbai. Approximately 100 mg each of samples and RMs were packed in aluminum foil and irradiated at $\sim 10^{12} \text{ n cm}^{-2}\text{s}^{-1}$ for 6 h in the CIRUS reactor of BARC. The gamma activity of the activation products of the samples and RMs was assayed after suitable cooling time using a Compton suppressed gamma-ray spectrometer with 40% efficiency HPGe detector (EG & G ORTEC) having a resolution of 2 keV at 1332 keV of ^{60}Co and coupled to a PC based 8k MCA [12]. All the spectral data were recorded and analyzed using PHAST software in the Radiochemistry Division of BARC. The peak areas under characteristic photo peaks of gamma rays of various radionuclides were used for calculating elemental concentrations using the comparator method of INAA [12].

Results and discussion

First, elemental concentrations for the two RMs were calculated using each other as comparator standards at various intervals of cooling. Mean elemental concentrations so obtained for the two RMs, NIST SRM 1547 (Peach Leaves) and INCT MPH-2 (Mixed Polish Herbs) along with their certified/information values [30,31] are listed in Table 1. Standard deviations were calculated based on triplicate analyses. Also listed in Table 1 are Z scores for both the RMs. It is observed that a comparison of our data with the certified values is in good agreement within $\pm 5\%$ - 10% in all the cases. Further, standard deviations, in all cases, are small and well within $\pm 10\%$. Z score values for most elements are within ± 3 except for a few cases. Afterward, elemental concentrations in three samples were calculated using each of the two RMs as comparator standard. Mean elemental concentrations along with standard deviations calculated on the basis of replicate analyses, counting at different intervals, different photo peaks, and using two RMs are listed in Table 2. On

Table 2: Elemental concentrations in stems of three medicinal plants.

Elements	<i>Tinospora cordifolia</i>	<i>Santalum album</i>	<i>Mangifera indica</i>
As (ng/g)	400 ± 30	630 ± 50	230 ± 20
Ca (mg/g)	12.6 ± 1.1	22.7 ± 1.6	17.2 ± 1.2
Ce (µg/g)	1.97 ± 0.06	2.45 ± 0.09	1.10 ± 0.05
Cl (mg/g)	4.89 ± 0.14	1.77 ± 0.04	2.07 ± 0.12
Co (ng/g)	54.0 ± 3.0	56.0 ± 3.0	57.0 ± 4.0
Cr (µg/g)	1.14 ± 0.12	1.21 ± 0.09	0.97 ± 0.07
Cu (µg/g)	6.33 ± 0.51	4.01 ± 0.42	2.39 ± 0.39
Fe (µg/g)	229 ± 17	129 ± 9	237 ± 21
Hg (ng/g)	66.1 ± 1.9	71.2 ± 2.3	59.7 ± 1.3
K (mg/g)	24.6 ± 0.3	29.3 ± 0.5	26.8 ± 0.5
La (µg/g)	8.42 ± 0.59	8.49 ± 0.64	4.66 ± 0.31
Mg (mg/g)	2.15 ± 0.22	1.48 ± 0.16	0.52 ± 0.08
Mn (µg/g)	56.7 ± 1.5	46.9 ± 1.2	38.9 ± 1.1
Na (mg/g)	0.09 ± 0.01	0.12 ± 0.01	0.31 ± 0.02
Se (ng/g)	180 ± 17	290 ± 30	90 ± 9
V (µg/g)	0.92 ± 0.05	0.61 ± 0.04	1.72 ± 0.11
Zn (µg/g)	34.6 ± 2.1	44.6 ± 2.4	33.9 ± 2.1

*These mean ± SD values are on the basis of three independent analyses of samples.

the basis of data in Table 1, we can presume that elemental concentrations in samples should also be reliable within $\pm 5\%$ - 10% limits. A perusal of elemental data in Table 2 shows that some elements are at mg/g level whereas others are at µg/g or ng/g level which may be called minor, trace, and ultra-trace elements respectively. These are discussed separately in the following paragraphs.

Minor elements

A perusal of data in Table 2 shows that concentrations of K, Ca, Mg and Cl, all electrolytic elements are found at mg/g level and decrease in the order $K > Ca > Cl > Mg$. The case of Fe is on the borderline as it is found in the range 0.13 - 0.24 mg/g being highest for *M indica* and lowest for *S album*. Surprisingly, the concentration of K is highest and varies in a close range of 24.6 - 29.3 mg/g with a mean concentration of $26.9 \pm 2.3 \text{ mg/g}$. It suggests that even though all three stems are of different plant species but their K concentration is almost of the same level.

Table 1: Comparison of our data on elemental concentrations in Standard Reference Materials for data validation [30,31].

Elements	NIST SRM -1547 Peach Leaves			INCT-MPH-2 Mixed Polish Herbs		
	Measured	Certified	Z-score	Measured	Certified	Z-score
As (ng/g)	53 ± 8	60 ± 18	-3.33	209 ± 9	191 ± 23	0.78
Ca (mg/g)	14.8 ± 1.1	15.6 ± 0.2	-4	10.3 ± 0.8	10.8 ± 0.7	-0.71
Ce (µg/g)	7.99 ± 0.79	[10]	-	1.31 ± 0.2	1.12 ± 0.10	-
Cl (mg/g)	0.30 ± 0.01	0.36 ± 0.02	-2.8	2.36 ± 0.17	2.84 ± 0.20	-2.4
Co (µg/g)	0.07 ± 0.06	[0.07]	-	0.18 ± 0.02	0.210 ± 0.025	-1.16
Cr (µg/g)	1.06 ± 0.03	[1.0]	-	1.74 ± 0.12	1.69 ± 0.13	0.38
Cu (µg/g)	3.66 ± 0.32	3.71 ± 0.4	-0.125	6.72 ± 0.48	7.77 ± 0.53	-1.98
Fe (µg/g)	229 ± 11	218 ± 14	0.78	489 ± 26	[460]	-
Hg (ng/g)	34.7 ± 2.8	31 ± 7	0.53	20.3 ± 1.7	17.6 ± 1.6	1.69
K (mg/g)	24.7 ± 0.2	24.3 ± 0.1	0.40	17.4 ± 1.1	19.1 ± 1.2	-1.42
La (µg/g)	7.33 ± 0.51	[9]	-	0.60 ± 0.03	0.571 ± 0.046	0.58
Mg (mg/g)	4.72 ± 0.19	4.32 ± 0.08	3.1	2.36 ± 0.21	2.92 ± 0.18	-3.05
Mn (µg/g)	87.3 ± 7.2	98 ± 3	-3.6	183 ± 12	191 ± 12	-0.67
Na (µg/g)	23.1 ± 1.4	24 ± 2	-0.45	381 ± 19	[350]	-
Se (ng/g)	117 ± 7	120 ± 9	-0.33	141 ± 9	NA	-
V (µg/g)	0.34 ± 0.02	0.37 ± 0.03	-1	1.03 ± 0.08	0.95 ± 0.16	0.5
Zn (µg/g)	18.7 ± 0.3	17.9 ± 0.4	2	37.7 ± 2.3	33.5 ± 2.1	2

Values in parenthesis [] are for information only.

However, this is not the case with other minor constituents of Ca, Cl, and Mg which all occur in a much wider range with mean concentrations of 17.5 ± 4.9 , 2.91 ± 1.14 , and 1.38 ± 0.86 mg/g respectively. A comparison of elemental contents in bar diagram of minor constituents (Na, K, Mg, Ca) including that of Fe is shown in Figure 2, wherefrom it is observed that concentrations of K and Ca are particularly high in all the samples but a decrease in the order *S album* > *M indica* > *T cordifolia* being highest in sandalwood. On the contrary Na content, at mg/g level, varies in a large range of 0.09-0.31 mg/g with a mean value of ~12 mg/g. However, Mg content is highest in *T cordifolia* (2.15 ± 0.22 mg/g) and lowest in *M indica* (0.52 ± 0.08 mg/g). Similarly, concentrations of Cl, an anionic electrolytic element that remains associated primarily with Na and K, show a wider range with the highest content in *T cordifolia* (4.89 ± 0.14 mg/g) and lowest in *S album* (1.77 ± 0.04 mg/g). Thus, all three stem samples are enriched in electrolytic elements, irrespective of being cationic or anionic. These play an important role in the transport of liquids along with nutrient elements from root to stem to leaves and flowers.

A comparison of Ca contents in bark/stem of three samples of this study with that of *A indica* [26] and *T arjuna* [32] from our earlier work along with those of other trees from Brazil [33], India [34,35] and Kenya [22] are listed in Table 3. In all

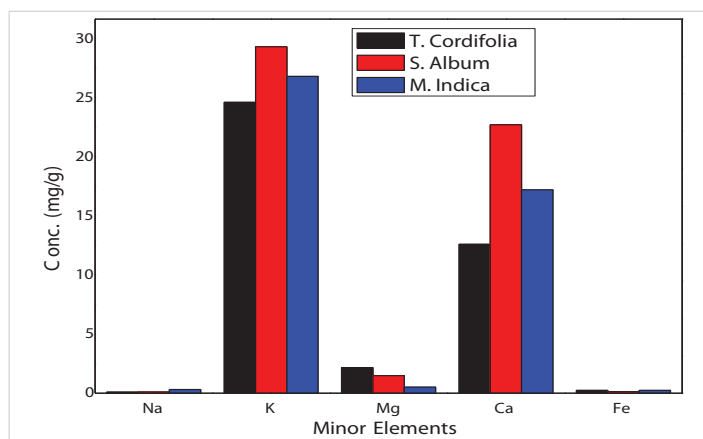


Figure 2: Bar diagram showing comparative contents for minor constituent elements in *M indica*, *S album* and *T cordifolia*.

Table 3: Comparison of Ca content (in mg/g) in stem/bark of some medicinal plant species from India, Kenya, and Brazil.

Name of Plant species	Concentration of Ca (mg/g)	Reference
<i>Santalum album</i> (Chandana)	22.7 ± 1.6	This work
<i>Mangifera indica</i> (Mango)	17.2 ± 1.2	This work
<i>Tinospora cordifolia</i> (Giloy)	$16.3 \pm 0.1^*$ 10.22	Garg, et al. [23] Mahima, et al. [35]
<i>Azadirachta indica</i> (Neem)	$14.4 \pm 5.9^*$	Garg & Verma, [26]
<i>Terminalia arjuna</i> (Arjuna)		
<i>Erythrina velutina</i> (Mulungu)	$34.1 \pm 11.6^*$	Garg, et al. [32]
<i>Salvadora persicawall</i> (Kharajal)	21.15 \pm 0.92	Leal, et al. [33]
<i>Glycyrrhiza glabra</i> (Yashtimadhu, Mulethi)	35.10	Benson, [22]
	12.97 \pm 0.85	Ghosh & Singh, [34]

*These values are from our own work reported earlier [23,26, and 32]. In parenthesis () are given local names of the plants.

of our studies as well as of others on the analysis of bark/stem of trees [22,31-35], Ca content is found to be high in the range 12.6 - 34.1 mg/g or > 1% by weight. Hence K and Ca are in fact major constituents. Lowest Ca content in *T cordifolia* stem may be attributed to the fact that it is not a tree but a creeper and hence not so strong. Earlier in a separate study, stems of *T cordifolia* were analyzed in our laboratory [23] whence Ca content was found to be 16.1 ± 0.1 mg/g. Mahima, et al. [35] have reported Ca content of 10.22 mg/g by atomic absorption spectrophotometry (AAS). Thus an overall range of Ca content in *T cordifolia* is found in a range of 10.2 - 16.1 mg/g with a mean value of 13.0 mg/g. These are quite comparable (within ~20%) considering that all three samples were derived from different sources and analyzed by different workers using different techniques. Ca is a unique essential nutrient linked to transpiration, playing a vital role in plant structure and signaling that regulates water flow [36]. In humans, Ca builds healthy bones and teeth as it is located in the skeleton by virtue of its phosphate salts. It also assists in blood clotting because of its presence in extracellular fluid as a carrier of cellular signals [37]. It may impair the absorption of other elements such as Fe, Mg and Zn. In case of Mg, however, variation is in a much wider range of 0.52 - 2.15 mg/g with a mean concentration of 1.38 ± 0.85 mg/g and decreasing in the order *T cordifolia* > *S album* > *M indica*. It has been suggested that proper Mg^{2+} homeostasis is compulsory and its deficiency or excess amount may manifest many diseases [38]. As all three stem samples analyzed in this study are enriched in Ca content, these may be used as natural supplements for Ca along with other trace nutrients in bioavailable form.

Trace elements

Manganese, zinc, copper, chromium, cobalt, vanadium, and selenium are all considered micronutrients and are found in trace amounts at $\mu\text{g/g}$ or ng/g level. It is observed that the concentration of Mn is highest in the range of 40 - 60 $\mu\text{g/g}$ followed by Zn (30 - 45 $\mu\text{g/g}$), Cu (< 10 $\mu\text{g/g}$), V (< 2 $\mu\text{g/g}$), Cr (~1 $\mu\text{g/g}$), Co (> 50 ng/g) and Se (100 - 300 ng/g). Some of these elements (Se, Cu, Cr, Zn, As, Mn, Co, and Hg) have been plotted as a bar diagram showing a comparison of elemental contents in Figure 3. It is observed that concentrations of Mn, Zn, and Cu, all nutrient elements are particularly high in all three samples but a decrease in the order Mn > Zn > Cu. All three elements are nutritionally important and play a vital role in various biochemical processes in the human body [38-40]. Besides, higher Mn and Zn contents may also be associated with antioxidant properties justifying their use in traditional herbal medicines [4]. Zinc is an essential component of a large number of enzymes such as carboxypeptidase participating in the metabolism of other micronutrients. Out of three plant species, *S album* is particularly enriched in Zn, Se, and Cr whereas *T cordifolia* is enriched in Mn and Cu. Gowrishankar, et al. [18] have also analyzed *T cordifolia* by PIXE and our results are comparable for most elements except Cr and Cu. Mahima, et al. [35] have also reported a few trace elements (Fe, Mn, Cu,

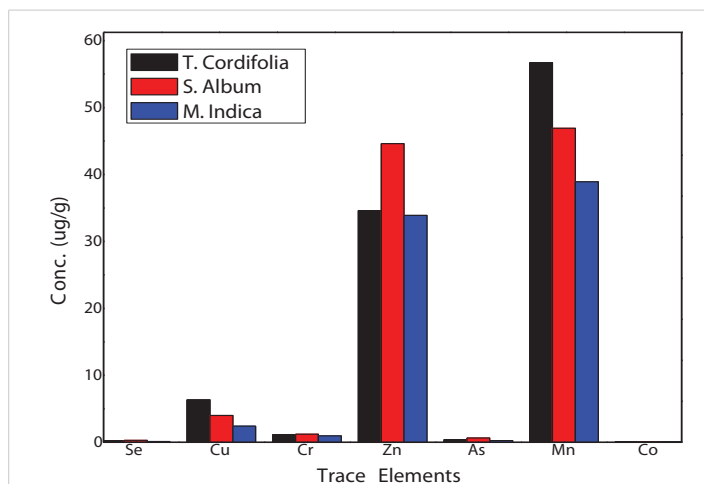


Figure 3: Bar diagram showing variation of trace element contents in stem samples of *S. album*, *M. indica*, and *T. cordifolia*.

and Zn) in *T. cordifolia* by AAS that are somewhat lower. This may probably be attributed to the destructive nature of AAS and the sample being from a different location. Higher contents of Mn and Cu in *T. cordifolia* may be attributed to the extensive medicinal use of this herb in the Ayurvedic health care system. Mn is an essential nutrient because it serves as a cofactor for numerous cellular processes. It activates enzymes such as pyruvate carboxylase and superoxide dismutase essential for the synthesis of polysaccharides and glycoprotein [39]. Concentration of Co, however, is at the ultra-trace level (56 ± 2 ng/g) in all three samples. The concentration of V is highest in *M. indica* (1.72 ± 0.11 µg/g) and lowest in *S. album* (0.61 ± 0.04 µg/g). It is considered as a close blueprint of phosphate and is likely to take over a regulatory function in metabolic processes depending on phosphate [37]. Selenium considered an essential element as a part of selenoenzyme glutathione peroxidase is also found at ultra-trace (ng/g) level. It is now considered an essential micronutrient for mammals though it is also proven to be toxic in excess amounts leading to selenosis [38]. It has been proposed that the minor and trace elements remain bound with phytoconstituents such as polyphenols, steroids thus making them bioavailable/bioaccessible to our body system [14,24,40].

Arsenic and mercury, primarily considered toxic elements, are found at 200 - 600 ng/g and 60 - 70 ng/g levels respectively. Arsenic is linked with toxicity and its ability to cause diseases as a potential health hazard. However, there is evidence that it has to sustain a role in living organisms [39]. Mercury is considered highly toxic and poisonous affecting the body's nervous system. It is observed that as and Hg both are within permissible levels [41] and may have been associated with the stem/bark of respective trees as environmental contaminants due to vehicular emissions and spray of insecticides/pesticides. Because of increasing industrial and vehicular emissions, Chen [42] has suggested following good agricultural practices (GAP) and good manufacturing practices (GMP) while preparing herbal medicines and also continuously monitoring these for toxic elemental contents.

Elemental correlations

In biological systems, many elemental correlations have been attempted as their interrelations have been correlated with human diseases [37]. Earlier in our laboratory, Choudhury, et al. [43] have studied the interaction of several elements in medicinal herbs used in the treatment of diabetes and observed a statistically significant relationship between Cu and Zn. It has been shown that synergistic interactions occur between essential micronutrient elements showing participation in the same biochemical processes. Recently Konieczynski and Wesolowski [44] have particularly emphasized statistically significant correlation pairs; Zn-Mn, Fe-Zn, Mn-Fe, and Cu-Zn in medicinal plants being used as raw materials. Because of this, we examined several correlations and observed an excellent inverse correlation between Fe and Zn with a high correlation coefficient, $R^2 = 0.9999$ as shown in Figure 4. In the same Figure 4, another correlation between Fe and Mn is attempted but these do not seem to correlate ($R^2 = 0.7571$) at all. Correlation between Fe and Zn indicates that these elements may participate in mutual biochemical reactions in an antagonistic manner. Derkach and Khomenko [45] have also reported a similar correlation between Fe and Zn in three Ukrainian medicinal plants. This may be useful while manufacturing drugs.

Conclusion

Three stem samples of *S. album*, *M. indica* and *T. cordifolia*, used as herbal medicines or in the preparations of herbal formulations were analyzed for 4 minor (K, Ca, Cl, Mg) and 13 trace elements (Fe, Zn, Mn, Cu, Cr, Na, La, As, Hg, V, Co, Se, Ce) by INAA. All the minor constituents at mg/g level were found in decreasing order $K > Ca > Cl > Mg$. Calcium content in all three samples was found comparable with those of stem/bark samples of medicinal plants reported in the literature. Contents of Fe, Mn, Zn, and Cu were in significant amounts with a strong inverse correlation between Fe and Zn ($R^2 = 0.9999$) indicating antagonistic interaction between the two elements. Toxic elements as and Hg were found below the permissible

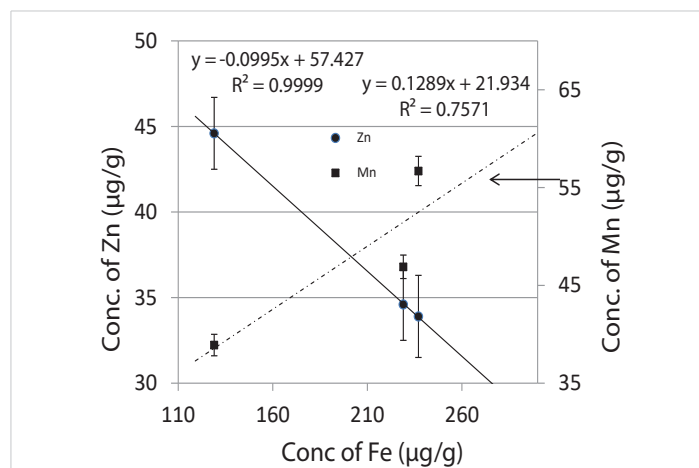


Figure 4: Correlation of Fe with Zn and Mn in *S. album*, *M. indica* and *T. cordifolia* showing antagonistic interaction between Fe and Zn.

level. Present data on elemental concentrations will help in understanding the role of elements in the medicinal stem samples used in the cure of diseases and the development of new formulations used as natural drugs.

Declarations

Consent for publication: We certify that the research work presented in this paper is our own work and original. Further, it is not submitted anywhere else for publication and has the consent of all the authors.

Availability of data and material: All the data generated or analyzed in this study are contained within this article.

Author's contribution: A N Garg- Conceptualisation, planning, supervision, writing, reviewing, and editing the manuscript.

Ruchi Singh- Visualisation, sample collection, preparation, data analysis, compilation, and drafting.

R S Maharia- All experimental work at BARC, Mumbai, and data collection.

R K Dutta- Data checking, supervision, and reviewing.

A Datta- Correlation of data, statistical analysis, Figure drawing, and checking.

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