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# TRACE ELEMENT ANALYSIS OF SOME MEDICINAL HERBS BY PROTON INDUCED X-RAY EMISSION (PIXE)

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Proton induced X-ray Emission (PIXE) has been used to investigate the concentration of nineteen trace elements in eleven medicinal herbs commonly used for the treatment of different diseases in Pakistan. Mg, Si, K, Ca and Fe were detected in all the medicinal herbs studied. Germanium has been detected only in violet flower. Indian Lilac, field marigold and violet flower have the highest number of trace elements with maximum concentration. No toxic elements such as Pb, Hg etc. were detected in these medicinal herbs. The accuracy of our results was validated by analyzing standard reference material Citrus Leaf (NIST- SRM -1572). Results indicate that medicinal herbs are a rich source of trace elements which may be useful for the cure of certain ailments.

**Keywords:** Trace element analysis, Medicinal herbs, PIXE.

## 1. Introduction

Trace elements present in very small amounts in the human body play an important role in controlling the metabolic functions [1]. Any deviation of these elements from normal limits can cause severe disorders in the biological functioning of various organs. Deficiency of iodine and iron, for example, can result in goiter and anemia respectively.

The role of herbal medicines for the treatment of various diseases and ailments has been established in the subcontinent since ancient times. Medicinal plants have generally attracted attention for the investigation of organic ingredients like oils, alkaloids, vitamins and other similar compounds. Not much attention has been paid to study their inorganic contents, especially, the trace element contents of these plants. Some metals are known to play an important role in curing a number of diseases such as asthma, syphilis diarrhea dysentery etc. [2, 3].

Hikmat the traditional and popular method of treatment in Pakistan is based on the use of different parts of the medicinal plants and extracts derived from them. Hikmat is cost effective and is affordable by a very high percentage of the population.

Trace elements play an important role in the production of many bioactive chemicals in plants and therefore determine their toxic and medicinal effectiveness. Herbal medicinal plants are found to be rich in one or the other individual element which could possibly be a link for the therapeutic action of the medicine. In this context, therefore, determination of trace elements content of medicinal herbs could provide useful information leading to understanding on the effectiveness of the herbal medicine for the treatment of diseases.

Several techniques such as atomic absorption spectrometry, inductively coupled plasma mass spectrometry and neutron activation analysis are employed for elemental analysis but they all require complex sample preparation methods. In the present study proton induced X-ray emission (PIXE) has been employed to investigate the trace element contents of eleven herbal medicines commonly used in Pakistan. PIXE is based on the emission of characteristic X-rays by different elements present in the sample when bombarded by protons of few MeV energy from an accelerator. The characteristic X-rays emitted by each element has specific energy which bears its signature. These X-rays are detected by an energy dispersive Si(Li) detector and displayed as counts versus channels. PIXE is a very versatile, nondestructive, fast, multielemental analysis technique and requires very little sample preparation [4-6].

## 2. Experimental

Eleven commonly used medicinal herbs were purchased in powder form from the local market. Their vernacular names, English names, and botanical names alongwith the therapeutic uses are given in Table 1 [7]. Five mg of each medicinal herb was mixed with 10 µl of Y $(NO_3)_3$ solution in double distilled water containing 1.16 mg of Y as internal standard and deposited on a triangular

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Table1. Botanical classification and nomenclature of medicinal herbs alongwith their therapeutic uses.

| Vernacular | English Name | Botanical Name | Family | Uses |
|---|---|---|---|---|
| Saunf | Fennel , sweet fennel | *Foeniculum Vulgare* | Umbelliferae | Abdominal pain, flatulence, colic, gastric indigestion, stomach debility, phlegmatic disorders |
| Kalonji | Black cumin seeds | *Nigella Sativa* | Ranunculaceae | Inflammation, allergie, cancer, viral infections, asthma, eczema, boils, cold symptoms, baldness, facial paralysis, kidney and bladder stones, earache |
| Podina | Field mint, pepper mint | *Menthe arvensis* | Labiatae | Bronchitis, chlorella, chronic indigestion, fever, hiccup, liver pain, menstrual irregularity, sinusitis |
| Ajwain | Omum ajowan seeds | *Trachyspermum ammi* | Umbelliferae | Acidity, dyspepsia, flatulence, neuralgic pain, rheumatic pain, atonics dyspepsia, bronchitis, cholera, chronic fever, colic, diarrhea, flatulence, hysteria, indigestion, liver stiffness ,profuse expectoration |
| Mulathi | Liquorice, sweet wood | *Glycyrrhiza glabra* | Papilionaceae | Addison disease, isthmian, catarrhal affections of pulmonary mucus membrane, catarrhal affections of throat, cough |
| Banafsha | Violet flower | *Viola odorata* | Violaceae | Biliousness, blood heat, catarrhal affection, conjunctivitis, constipation, fever, headache, infantile disorders, kidney disorders, lung troubles, pleurisy, pneumonia, uterus prolapsus. |
| Goond | Tragacanth | *Cochlospermum religiosum* | Cochlospermaceae | Isthmian, dysentery, eye irritation, eye troubles, gonorrhea, hemoptysis, syphilis, thoracic region complaints. |
| Gul Ashrafi | Field marigold | *Calendula arvensis* | Asteraceae | Inflammation, acne, abdominal cramps, constipation |
| Gaindaa | Pot marigold | *Calendula officinalis* | Asteraceae | Inflammation, constipation |
| Neem | Indian Lilac, Margosa | *Melia azadirachta* | Meliaceae | Ulcers, atomic dyspepsia, general debility, leprosy, skin diseases, stomatitis, syphilitic sores, wounds, leprosy |
| Gulab | Rose, Damask rose | *Rosa damascena* | Rosaceae | Eye irritation, burning sensation, chronic fever, toothache, enlarged tonsils, urogenital complaints, urticaria. |

piece of 100 µm thick Mylar. The deposited sample covered an area of 1.5 $cm^2$. The samples so prepared were dried under a lamp and placed inside a desiccator. The samples were mounted on a pyramid shaped nuclear grade graphite sample holder which can take four samples at a time. The sample holder was introduced into the vacuum chamber for irradiation by a collimated proton beam from Pelletron Accelerator Model 6SDH-2 installed at the Accelerator Laboratory CASP, Government College University, Lahore. The beam current was 10nA and the beam size at the target was 2.5mm. The characteristic X-rays produced by the interaction of 3.8 MeV protons with elements present in the sample were detected by energy dispersive Si(Li) detector with 30 $mm^2$ area and having an energy resolution of 138 eV. The Si(Li) detector had an aluminum coated 0.4 µm thick NORVAR window. The signal generated by the detector was processed by the usual PIXE electronics and analyzed by a multichannel analyser (MCA). The spectrum was displayed by MCA as counts (concentration) versus channel number (energy). The system was energy calibrated using L X-rays from a gold target.

For the determination of concentration of elements present in the sample, the system was calibrated using a known mixture of 12 salts containing elements with atomic number between 11 and 40. Known amount of Y $(NO_3)_3$ dissolved in double distilled water was added as internal

Table 2. PIXE analysis of Citrus Leaf (NIST-SRM-1572).

| Elements | Our value (µg/g) | | NIST (µg/g) |
|---|---|---|---|
| | Average | Standard Deviation | |
| Mg | 6242 | 1052 | 5800 ± 153 |
| S | 3805 | 663 | 4070±46 |
| Cl | 417 | 159 | (414) |
| K | 20638 | 2318 | 18200±306 |
| Ca | 41541 | 6987 | 31500±514 |
| Mn | 74.5 | 18.1 | 23.0±1.0 |
| Fe | 137.6 | 19 | 90.0±5 |
| Ni | 38.8 | 16.1 | 0.6±0.2 |
| Cu | 22.2 | 4.2 | 16.5±0.5 |
| Zn | 12.7 | 8.7 | 29±1.0 |

Value in parenthesis is not certified

standard. Sensitivity of the system for the twelve elements relative to Y was determined from the ratio of the real counts for the K X-ray peaks for the elements and Y. Sensitivity curve was obtained by plotting sensitivity for an element versus its K X-rays energy. Sensitivity for other elements not present in the mixture for the K X-ray peaks was deduced from this curve. Similarly the sensitivity for heavy elements like Ba, Hg and Pb was experimentally measured by using L X-rays peaks of these elements and the K X-ray peak of Yttrium as described above.

To test the calibration of our system and validate our results, a reference material Citrus Leaf (NIST- SRM- 1572) was analyzed. The target of the reference material for irradiation was prepared exactly in the manner described above. A few mg of the reference material was mixed with known amount of Y $(NO_3)_3$ as internal standard. Elemental concentrations of the elements contained in the reference material analyzed in our laboratory alongwith their certified values are presented in Table 2. The agreement between the two results for the elements Mg, S, Cl and K is within the limits of error. For Ca, Fe, Cu and Zn the difference between the two results is from 5 percent for Ca to 23 percent for Fe, however, for Mn and Ni the agreement is very poor.

## 3. Results and Discussion

The analysis of 11 commonly used medicinal herbs for 19 major and minor trace elements was carried out in our lab. Typical PIXE spectrum of a medicinal herb is shown in Figure 1. In this figure the peaks due to Yttrium used as internal standard are clearly visible. One can also see the K X-ray peaks of Mg, Si, Cl, K, Ca, V, Fe, Zn and Sr. The results as average of 3-4 measurements with standard deviation are presented in Table 3. The concentrations have been measured on dry weight basis. Mg, Si, K, Ca and Fe are present in all the medicinal herbs. Other elements like Al, S, Cl, Ti, V, Cr, Mn, Ni, Cu, Zn, Ge, Br and Sr have only been detected in some of the medicinal herbs. Germanium has been detected only in violet flower and Br is present in sweet wood and tragacanth which also contains fairly large amounts of K, Ca and Sc.

Magnesium plays an important role for the growth of human body, formation and function of bones and muscles [8]. Magnesium also helps the body to absorb K and Ca besides controlling the insulin level. All of the medicinal herbs contain Mg ranging in concentration from 1455 to 8526 µg/g. highest being in Neem (Indian Lilac). This supports its use as chewing stick for cleaning teeth in the subcontinent because of its role in the maintenance of bones. Aluminum is present in all medicinal herbs except fennel, black cumin seeds and tragacanth. The concentration of Al varies between 151 to 2677 µg/ g. Neem again has the highest concentration of Al which is involved in the action of few enzymes in the body. Silicon is present in large quantity in all the herbs under investigation. The concentration of silicon is lowest for tragacanth (2454µg/g) and highest for peppermint (24130µg/g). Silicon alongwith Ca plays an important role for the growth and maintenance of bones, ligaments, tendons, fingernails, skin and hair [8]. Sulphur is important for proper functioning of liver, digestion of food and its conversion to energy. The concentration of S ranges between 173 µg/g for sweet wood to 3308 µg/g for Neem which makes it very useful for skin disease, syphilitic sores and wounds. Chlorine has

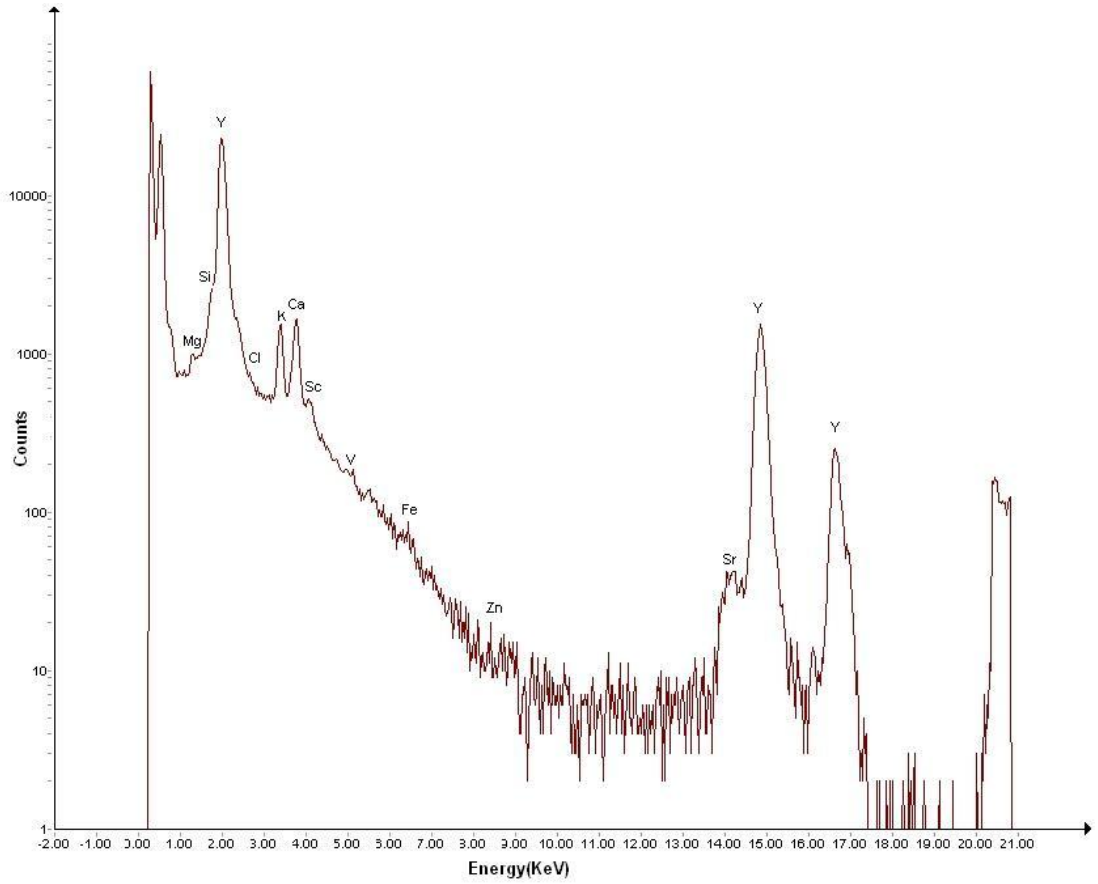


Figure 1. Typical PIXE spectrum of a medicinal herb sample.

Table 3. Elemental concentrations (µg/g±sd) of medicinal herbs using PIXE.

| Element | Fennel | Black cumin seeds | Pepper mint | Omum | Liquorice | Violet flower | Tragacanth | Field marigold | Pot marigold | Indian lilac | Rose |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mg | 1569±79 | 2914±143 | 2017±103 | 3823±199 | 2052±103 | 4364±236 | 1524±76 | 5142±273 | 2217±106 | 8526±426 | 1455±74 |
| Al | - | - | 168±20 | 1120±137 | 151±18 | 1498±178 | - | 1558±189 | 1125±135 | 2677±324 | 1060±129 |
| Si | 5461±328 | 4911±304 | 24130±1472 | 10810±638 | 4160±258 | 32161±1930 | 2454±153 | 12633±771 | 11357±681 | 12728±751 | 11081±676 |
| S | 744±89 | - | 2227±274 | 1173±143 | 173±21 | - | - | 2923±357 | 1201±144 | 3308±407 | - |
| Cl | 1908±95 | 284±15 | 783±41 | 4485±224 | - | 917±45 | - | 10142±517 | 2521±131 | 5106±255 | - |
| K | 10445±522 | 6633±338 | 10542±559 | 12227±611 | 3912±192 | 22470±1123 | 3117±162 | 42776±2267 | 22736±1136 | 18318±879 | 7491±375 |
| Ca | 9534±381 | 7932±341 | 6736±283 | 16353±670 | 4193±168 | 12210±476 | 9507±380 | 9827±383 | 4659±191 | 21806±872 | 2986±119 |
| Sc | 476±71 | 567±87 | 499±75 | 478±73 | 387±57 | 384±57 | 336±50 | 518±78 | 528±81 | 529±80 | - |
| Ti | 22±6 | - | - | 53±13 | - | 63±16 | - | 12±3 | - | - | - |
| V | - | 69±10 | 129±19 | 158±24 | 89±13 | 206±31 | - | - | - | 194±29 | - |
| Cr | - | - | - | - | - | - | - | 264±48 | 54±10 | - | - |
| Mn | 68±41 | - | 58±35 | 88±52 | 50±29 | 113±70 | 50±30 | | - | 179±113 | 133±77 |
| Fe | 137±14 | 96±8 | 200±21 | 555±57 | 225±22 | 509±50 | 19±2 | 565±57 | 405±41 | 561±56 | 311±33 |
| Ni | - | - | 12±9 | - | 12±9 | 23±17 | - | 31±23 | 39±29 | - | - |
| Cu | - | - | - | 17±12 | - | - | - | 26±19 | 39±27 | - | 45±31 |
| Zn | - | 41±23 | 11±6 | 34±18 | 12±7 | 76±44 | 23±11 | 54±30 | - | 60±33 | - |
| Ge | - | - | - | - | - | 68±23 | - | - | - | - | - |
| Br | - | - | - | - | 37±24 | - | 45±29 | - | - | - | - |
| Sr | 950±114 | 826±99 | 981±118 | 1060±127 | 682±82 | 1650±198 | 1039±125 | - | - | - | - |

a maximum value of 10142 µg/g for field marigold and lowest value 284 µg/g for black cumins and is not detected in sweet wood, tragacanth and rose. Chlorine together with K and Na carries the electrical charge for the functioning of nerve cells. Potassium combined with Na helps to maintain body water balance which in turn maintains blood pressure [9]. Potassium is one of the major elements present in all the medicinal plants with the highest concentration of 42776 µg/g in field marigold. Calcium is also present in all medicinal herbs as major trace element. The concentration of Ca ranges from 2986 µg/g to 21806 µg/g in Neem. Ca plays an important role for the development of bones and teeth in human body. In addition it controls muscle growth and the electrical pulses to the brain. Deficiency of Ca causes osteoporosis [10]. Very small amounts of Ti have been detected in fennel, omum, violet flowers and calendula. The role of Ti in human metabolism is not yet known. However, Ti due to its inert nature is compatible with human body and is used in several types of transplants in human body. Manganese supports the immune system, regulates blood sugar level and plays an important role in the clot formation by vitamin K [8]. It is present in almost all the medicinal herbs with the highest concentration of 179 µg/g in Neem, Manganese is an antioxidant trace element and helps the breakdown of amino acid and production of energy in the body.

Iron is an important constituent of haemoglobin in blood that helps transport energy to various parts of the body [11]. The concentration of Fe varies from 19 µg/g to 565 µg/g and is present in all the medicinal herbs under study. Zinc plays a vital role in healing wounds and ulcers [12]. It is present as minor trace element ranging from 11-76 µg/g. Violet flower has the highest concentration of Zinc. Ni has been detected only in five medicinal herbs with highest concentration in pot marigold. It is a toxic element and can cause ailments connected with heart, kidneys and liver [8]. Copper has been detected only in three medicinal herbs, namely omum seeds, field marigold and pot marigold. Copper is an important trace element for the growth and development of bones, connective tissues, brain and heart. Copper also helps immune system to fight infections [13].

Strontium has been detected in seven medicinal herbs investigated in the present study. Concentration of Sr varies from 682-1650 µg/g. Cr plays a vital role in strengthening of insulin as a constituent of glucose tolerant factor [14]. Pot marigold and field marigold contain 264 and 54 µg/g of Cr respectively.

## 4. Conclusions

In the present study eleven medicinal herbs commonly used by Hakims in Pakistan have been analyzed for their major and minor trace element content by proton induced X-ray emission. These medicinal herbs have proved very effective for the cure of a large number of diseases and has provided relief to a large segment of population in the subcontinent. The role of trace elements for the control of various metabolic functions and their therapeutic effectiveness is now well established, however. The precise knowledge of the concentration of trace elements could be used for prescribing the appropriate dosage of these herbal medicines thus leading to improved treatment. Lot more needs to be investigated to understand the role and functioning of trace elements in the human body.

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