Qualitative elemental analysis of selected potential anti-asthmatic medicinal plant taxa using EDXRF technique

S. Jyothsna a,*, G. Manjula a, Sateesh Suthari b,1, A.S. Nageswara Rao a

a Department of Physics, Kakatiya University, Warangal, Telangana 506 009, India
b Department of Plant Sciences, University of Hyderabad, Hyderabad 500 046, Telangana, India

1. Introduction

Asthma is an ubiquitous and important disease which is affected about 300 million people globally (Pires et al., 2011), nearly 7–10% of population suffers from it (Kasirajan et al., 2007). It is a chronic inflammatory lung disease which inflames and narrows the airways (Clarke et al., 2005) and causes recurring period of shortness of breath, cough, chest tightness, wheezing, mast cells, lymphocytes, changes in the level of eosinophils, cytokines, etc. (Teotia et al., 2014). Mast cells are immune cells, play an important role in some types of allergic reaction due to the antibody the allergic reaction which IgE have the mast cells and basophils. The increasing prevalence of allergic disease in westernized countries is a significant health problem (Athari, 2013).

Suitable curative therapies are not properly found to treat asthma (Bielory et al., 2004). Several studies have reported that the treatment of asthma is cost effective and modern drugs show side effects on human beings (Franco et al., 2007). To avoid the damage with allopathic medicines, present scenario search for natural drugs which are obtained from medicinal plants plays a vital role on management and control of asthma disease. Number of studies have been conducted for the activity of organic components of medicinal plants compared to inorganic compounds. The role of inorganic compounds in the treatment of various diseases has received less importance and attention. Different chemical compounds such as alkaloids, amines and glycosides are highly responsible for medicinal property (Reddy et al., 2013). In addition to these, macronutrients and trace (micro-) elements are rich source in medicinal plants and play a significant role to prevent various diseases. Trace elements are very important components in the plants for various physiological activities and cofactors in the production of enzymes (Sattar et al., 2012). The deficiency and excess amount of trace elements are also responsible for toxicity in plants (Chrzan, 2016). So, the present study is very important to measure the trace elemental concentration and to understand the effect of trace elements on human health (Reddy et al., 2013).

The main aim of the present study is to determine the concentration of trace elements in selected medicinal plants which are used for the treatment of asthma. Seven anti-asthmatic medicinal plants were selected for the present study, namely, Acacia catechu (bark), Argemone mexicana (whole plant), Aegle marmelos (fruit), Datura metel (leaf), Phyllanthus emblica (fruit), Sapindus margaritatus (fruit) and Senna occidentalis (leaf) (Suthari et al., 2016).
2. Materials and methods

2.1. Sample preparation

Fresh plant materials of seven medicinal plant taxa were collected from different locations of Telangana State, India. The list of medicinal plants with their botanical name, vernacular name, family name and plant parts used for the analysis was provided in Table 1 and figures in Picture 1.

The selected parts such as leaves, bark and fruits of medicinal plants were washed under tap water and rinsed thoroughly with double distilled water in order to remove dust, surface contamination, dried in an oven at about 60 °C overnight and subsequently powdered using Agate mortar and pestle. A quantity of pure 150 mg of each powder sample was weighted and compressed using a 150 ton hydraulic press machine and made into pellets of 13 mm dia and about 1 mm thickness. Triplicates of each sample were done. These pellets were used as targets for the EDXRF experiment. Certified values of biological reference material NIST 1515 apple leaves were used as a control for accuracy.

2.1.1. Experimental energy dispersive X-ray fluorescence (EDXRF) analysis

Most of the techniques namely, Atomic Absorption Spectrometry (AAS), Atomic Emission/Fluorescence spectrometry (AES/AFS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Neutron Activation Analysis (NAA), X-ray Fluorescence techniques (XRF, XPS, EDXRF, WDXRF), Proton Induced X-ray Emission (PIXE), Proton Induced Gamma-ray Emission (PIGE), etc. are generally used for trace elemental analysis present in minor quantity down to the level of ppm (parts per million) or ppb (parts per billion). Elemental analysis techniques were used in the detection of various fields of science, agriculture, engineering, mining, environmental analysis, clinical analysis, pharmaceuticals and forensic sciences. Of these, the present study reports Energy Dispersive X-ray Fluorescence (EDXRF) technique for the elemental analysis of medicinal plant samples. The EDXRF is a non-destructive tool for the quantitative and qualitative determination of major and trace elements in a wide range of sample types. EDXRF is small, simpler in design, low cost and less time to operate with high source of efficiency and process analyzers, and lack of moving parts. This technique is safe to prepare samples without wastage of chemicals by choosing energy of the exciting radiation, no need for re-calibration, permits multi-elemental analysis to be completed in less time and accuracy is high with precision compared to other techniques. Sensitivity from ppm to major elements can be optimized for a particular elemental region of interest and easy to used powerful windows inbuilt software of nEXT. X-rays are used to irradiate the sample with sufficient energy to knock out the inner shell electrons of the sample atoms. Electrons from outer shells then drop down into the vacant inner shell positions, and characteristic X-rays are emitted. This is called as x-ray fluorescence (xrf). In EDXRF, the energies of the x-rays emitted by the sample are measured using Si(Li) detector and are processed by a pulse height analyzer. The nEXT system software runs with windows operating system. It defines the composition of elements in the sample. The energy level of the peak gives the identification of element and number of x-rays counted in the peak gives the amount of element present in the sample. The experimental study part was carried out at Trace Elemental Laboratory, UGC-DAE CSR Kolkata Center, Kolkata, India (Figure 2).

Table 1. List of selected anti-asthmatic medicinal plants from Telangana, India.

| S. No. | Botanical name | Vernacular name | Family name | Part used |
|-------|----------------|----------------|-------------|-----------|
| 1     | Acacia catechu | Thella tumma    | Fabaceae    | Bark      |
| 2     | Argemone mexicana | Brahmadandi | Papaveraceae | Whole plant |
| 3     | Aegle marmelos | Mareda         | Rutaceae    | Fruit     |
| 4     | Datura metel   | Ummetta        | Solanaceae  | Leaf      |
| 5     | Phyllanthus emblica | Usiri | Phyllanthaceae | Fruit     |
| 6     | Sapindus emarginatus | Kunkudu | Sapindaceae | Fruit     |
| 7     | Senna occidentalis | Kasinta    | Fabaceae    | Leaf      |

The setup consists of Xenometric (previously Jordan valley) EX-3600, energy dispersive X-ray fluorescence (EDXRF) spectrometer, which consists of oil cooled Rh anode X-ray tube (maximum voltage 50 kV, current upto 1mA). The measurements were carried out in vacuum chamber using different filters (between the source and sample) for optimum (1–10 ppm) detection of elements. Si(Li) detector with a resolution and enhanced modular signal processor (emsp) of 143 eV at 5.9 k eV and 10 samples turret enables mounting and analyzing 10 samples at a time.

The targets were positioned at an angle of 45° to the beam direction. The X-ray beam was collimated to a diameter of 4 mm and was made to fall on the targets. The detector was kept at an angle of 45° to the target position and at an angle of 90° to the X-ray beam direction. The characteristic X-rays emitted from each sample were recorded with a high resolution Si(Li) detector which has sensitive area of 30 sq mm and provided with a thin beryllium window of 8 mm thickness. The spectra were collected for a sufficiently long time so that good statistical accuracies can be achieved. The quantitative analysis is carried out using nEXT software (Sudarshan et al., 2011).

3. Results and discussion

In the present study, 13 trace elements P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb and Sr were determined and measured their concentrations using EDXRF technique. The results of average concentrations and EDXRF spectrum of trace elements were shown in Table 2, and Figure 3, respectively. These results compared with certified values of NIST 1515 apple leaves for accuracy which gives good agreement within the standard values. Analysis of present investigation documents a wide range of variation in their elemental concentrations in different plant species. Toxic elements namely Cd, Pb, Hg and Sn were not detected in present selected medicinal plants. The concentration of K, Ca, S, P, Fe, Cu, Cl and Sr are high and remaining elements are present at minor level in all medicinal plants (Table 2). Trace elements are essential micronutrients that exist in very low concentrations in the body, forming less than 0.01% of the total body weight (Ohammad et al., 2016). They play an important role in inflammation, various physiological processes, and are crucial for proper functioning of the immune system may affect asthma status (Gray et al., 2010). Copper (Cu), Selenium (Se), Zinc (Zn) and Manganese (Mn) have been hypothesized to play important roles in the pathogenesis of asthma (Ermis et al., 2004). Changes in patterns of dietary consumption, associated with development of a more affluent lifestyle, may have contributed to the rise in asthma over the past few decades (Seaton et al., 1994; Burney, 1987). Although a good number of studies have been examined the possible role of dietary micronutrients and antioxidants in asthma, little data is present about the influence of either the severity of the disease or its treatment on intake and on the plasma levels of these micronutrients. These data suggest that study on the association between micronutrient and asthma is essential to support the information of trace elements.

Acquiring EDXRF spectra is the first and one of the important steps performed in both qualitative and quantitative analysis. Acquisition parameters which are used to capture the spectra determine the spectra profile and parameters and generally chosen to enhance the counts obtained for the elements. Since spectra acquisition is central to both qualitative and quantitative analysis. Energy range is the range of where the spectrum is acquired, it does not necessarily cover the entire range of the radiation emitted from the sample. Generally the range that includes the energies of all the analyzed peaks should be selected. There are 3 energy ranges exists in the system, i.e. 0-10 k eV is the parameter of e-1, 0-40 k eV is the parameter of e-2 and 0-60 k eV is the parameter of e-3. Low energy ranges offer somewhat improved resolution for low Z-elements.

In Figure 2, the EDXRF spectrum of Left panel consists the energy range of 0-10 k eV, in that energy range exits only certain elements. In Middle, the EDXRF spectrum consists of the energy range of 0-40 k eV in that energy range also exits some elements. In Right, the EDXRF spectrum consists of the energy range of 0-60KeV in that energy range they exits heavier Z-elements.
3.1. Elemental analysis

3.1.1. Phosphorus (P)

High concentration of P (2011.75 ppm) found in Senna occidentalis whereas low concentration is observed in Acacia catechu (143.53 ppm). Datura metel has permissible level of phosphorus (1464.34 ± 135 ppm). Concentration of P is varied in between of 2011.75 ppm to 143.53 ppm which are compared with standard reference material NIST 1515 apple leaves (Table 2) (Figure 4a). Excess and deficiency of trace elements may lead to abnormal development in the human body. Excess P can cause heart disease and deficiency of P may get bone related diseases.
Elemental concentrations (in ppm) in the selected medicinal plants with their standard deviation (SD) and compared with standard reference material of NIST 1515 apple leaves.

| Sample code | Sample name          | P | S   | Cl | K     | Ca | Mn | Fe  | Cu  | Zn  | Se  | Br  | Rb  | Sr  |
|-------------|----------------------|---|-----|----|-------|----|----|-----|-----|-----|-----|-----|-----|-----|
| TET         | Acacia catechu       |   | 1222.27 | 927.83 | 114 | 1078 | 607 | 1264 | 4.05 | 1490 | 67.33 | 3.8 | 7.44 | 0.29 |
| ARG         | Argemone mexicana    |   | 1222.27 | 927.83 | 114 | 1078 | 607 | 1264 | 4.05 | 1490 | 67.33 | 3.8 | 7.44 | 0.29 |
| MAR         | Phyllanthus emblica  |   | 1222.27 | 927.83 | 114 | 1078 | 607 | 1264 | 4.05 | 1490 | 67.33 | 3.8 | 7.44 | 0.29 |
| DTR         | Datura metel        |   | 1222.27 | 927.83 | 114 | 1078 | 607 | 1264 | 4.05 | 1490 | 67.33 | 3.8 | 7.44 | 0.29 |
| KN          | Sapindus emarginatus|   | 1222.27 | 927.83 | 114 | 1078 | 607 | 1264 | 4.05 | 1490 | 67.33 | 3.8 | 7.44 | 0.29 |
| OTL         | Senna occidentalis   |   | 1222.27 | 927.83 | 114 | 1078 | 607 | 1264 | 4.05 | 1490 | 67.33 | 3.8 | 7.44 | 0.29 |

3.1.2. Sulphur (S)

The concentration of S varies in between 8970.59 ± 6759 ppm to 360.06 ± 89 ppm. High concentration of S is found in *Senna occidentalis* (8970.59 ppm) and low concentration is noticed in *Phyllanthus emblica* (360.06 ppm). *Argemone mexicana* plant material has sufficient S concentration (1706.31 ± 86.99 ppm). Sulphur helps in protecting the cells in the body from environmental hazards such as heavy radiation and pollution (Kaur et al., 2012). Relatively high quantities of S are required, but there is no RDA (recommended dietary allowance) for sulphur it is a critical nutrient is obtained from used amino acids. Although there is no official RDA for S, it is a parous nutrient. The S deficiency can lead to a number of health problems and without sufficient S you may experience joint pain.

3.1.3. Chlorine (Cl)

The concentration of Cl is maximum in all plant samples and highest concentration of Cl was found in *Argemone mexicana* (34472.76 ppm). *Aegle marmelos* (579 ppm) and *Sapindus emarginatus* (579 ppm) have equal concentration as that of standards reference material (579 ppm). These are compared with standard reference material of NIST 1515 apple leaves (Figure 4c). DRI (Dietary Reference Intake) of Cl is 3.6 g/d. Chlorine helps man to digest his food properly and to absorb other important elements and that he need to survive (Kaur et al., 2012). Excessive chloride levels on the other side can results in water retention.

3.1.4. Potassium (K)

The range of K concentration changed between 19786 ± 624 ppm to 5546.59 ± 607 ppm. The concentration of K is high in *Datura metel* (19786 ppm) and low concentration is observed in *Acacia catechu* (5546.59 ppm). *Aegle marmelos* has sufficient K concentration (16211.39 ppm). Dietary reference intake (DRI) of K is 4.7 g/d (Figure 4d). Potassium is a major element in all plant samples and it is extremely important to cells and without it we could not survive. K participates actively in the maintenance of cardiarhythm (Srinivas et al., 2016) and deficiency of K may cause weakness as cellular processes are affected. Excess of K can cause Hyperkalemia (Bihl and Meyers, 2001).

3.1.5. Calcium (Ca)

The maximum concentration of Ca is noticed in *Acacia catechu* (12644.05 ppm) sample and minimum concentration in *Phyllanthus emblica* (1210.26 ppm) (Figure 4e). Calcium is an important mineral for normal functioning of human body and plays a key role on the electrophysiology of cardiac tissue (Rajurkar and Damame, 1997).

3.1.6. Manganese (Mn)

The Concentration of Mn is high in *Aegle marmelos* (135.74 ppm) and low concentration is found in *Sapindus emarginatus* (7.15 ppm). *Acacia catechu* and *Senna occidentalis* has sufficient concentration of Mn (Table 2). Mn is very essential trace element used for reproduction and normal ducting of the central nervous system (Kaur et al., 2012). It is mainly present in mitochondria. RDA of Mn is 1.2 mg/d for females and 2.3 mg/d for males (Dutta and Mukta, 2012). Deficiency of Mn can causes for humans myocardial infarction and other cardiovascular diseases. Toxic exposure to Mn containing dust produces hallucinations, psychosis and neurological symptoms resembling Parkinson's disease (Dutta and Mukta, 2012). Mn overload is generally toxic in brain it can cause a Parkinson type syndrome (Aschner, 2000).

3.1.7. Iron (Fe)

The concentration of Fe is Maximum in *Aegle marmelos* (691.74 ppm) and minimum concentration of Fe is found in *Phyllanthus emblica* (58.34 ppm). All medicinal plants had maximum concentration of Fe except *Phyllanthus emblica* plant sample. Iron is a necessary trace element found in nearly all living organisms (Figure 4f). Recommended Dietary
Allowances (RDA) of Fe is 8 mg/d for males and 18 mg/d for females (RDAS, 1989). Iron containing enzymes and proteins, often containing heme prosthetic groups participate in many biological oxidations and in transport. Proteins found in higher organisms include hemoglobin, cytochrome and catalase (FSA, 2013). Excess amount of Fe causes rapid increase pulse rate, coagulation of blood vessels, hypertension and drowsiness (Naziri et al., 2015). Generally iron deficiency is very dangerous associated with impairment in the immune system and increased by physiological requirements (Reddy et al., 2013).

### 3.1.8. Copper (Cu)

The permissible limit of copper for plants is 10 mg/kg recommended by World Health Organization (FSA, 2013). Cu is the third largest trace element found in human body (after Iron and Zinc). It is a major component of the oxygen carrying part of blood cells (Kaur et al., 2012) and main constituent of bone, connective tissues, brain, heart and other body organs (Morabad et al., 2013). The concentration of Cu is high in all the selected plant materials and Cu has very high in *Acacia catechu* plant sample (27.77 ppm) (Figure 4g). Cu deficiency occurs due to malnutrition, malabsorption disorders, liver damage, depigmentation of hair and skin.

### 3.1.9. Zinc (Zn)

The high concentration of Zn was found in *Senna occidentalis* (43.52 ppm) whereas low is found in *Phyllanthus emblica* (7.02 ppm), compared with standard values of NIST 1515 apple leaves (12.50 ppm) (Figure 4h). Zn is crucial to play vital processes, playing a unique role in growth, development, release and use of hormones in the body, wound healing and the reduction of skin irritation (Olivare and Uauy, 1996; Prasad, 1998). Zinc is defined as an essential trace element or micronutrient and it is indispensable to growth and development of microorganisms, plants and animals. Excess Zn may cause toxic for human health and deficiency of Zn associated with decreases in cell proliferation. Zn deficiency can cause growth retardation, skin rash like dermatitis, diarrhea, hypogonadism and acrocyanodermatitis and toxicity of Zn leads to Cu deficiency, swelling, gastritis fever, nausea and vomiting (Dutta and Mukta, 2012). Zn deficiency affects the regulation of T-cell lymphocytes, biochemical functions and for optimal activity of the immune system. Zn plays a key role in DNA and protein synthesis and involved with copper as cofactors in several important enzyme systems (Beisel, 1982; Fraker et al., 1986).

### 3.1.10. Selenium (Se)

The maximum concentration of Se is observed in *Aegle marmelos* (1.46 ppm) and all the plant samples had higher concentration than that of standard reference. Se exists at very trace level in all plant samples. Se might be able to prevent the development of diabetes and cancer. Se is known for its protective action against oxidative stress (Patching and Gardiner, 1999). Se deficiency contributes to heart diseases (Fraga, 2005).

### 3.1.11. Bromine (Br)

The maximum concentration of Br is found in *Argemone mexicana* (87.03 ppm) and minimum is observed in *Sapindus emarginatus* (0.40 ppm). Though Br is a non-essential element for living organisms (Berdanier, 1994), obviously harmful to human health in excess amount such as malfunction of nervous system and genetic material disturbance.

### 3.1.12. Rubedium (Rb)

The maximum concentration of Rb is found in *Datura metel* (20.23 ppm) and minimum in *Acacia catechu* (4.48 ppm). Rb is a non-essential element for human organism and causes eye and skin burns (Srinivas et al., 2016).

### 3.1.13. Strontium (Sr)

Sr concentration is very high in *Argemone mexicana* (244.19 ppm) and very low in *Sapindus emarginatus* (0.40 ppm). Though Br is a non-essential element for living organisms (Berdanier, 1994), obviously harmful to human health in excess amount such as malfunction of nervous system and genetic material disturbance.

### 4. Conclusion

The main aim of the present original research work is to analyze trace elements namely P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb and Sr using EDXRF spectrometer and to estimate their elemental...
concentrations in ppm level. There is a substantial difference in elemental concentration of medicinal plants in the present study. The variation in elemental concentrations is mainly attributed to the differences in chemical structure, mineral composition of the soil, the place where they grow, the climatic conditions, age, water in which the plants are cultivated and environment effect. The conventional treatment of asthma disease have many short coming side effects, cost effective and high rates of secondary failure on the other hand herbal extracts are expected to have similar efficacies without the side effects. The trace elemental concentrations of medicinal plants are almost near to the concentrations of standard reference material of NIST 1515 apple leaves. The present data will be helpful for the physicians to prescribe the type of extract, exact dosage, and combination of such medicinal plants. Further studies on the relation between asthma disease and trace elements are needed to understand the details of potential with traditional use. Finally, the present study concludes that their no excess quantities of toxic elements are investigated and major and minor trace elements in present medicinal plants are not harmful to human health. The present study will be very useful for phytochemists, pharmacology and ayurvedic clinicians who would like to pursue further study in the area of herbal and alternative medicines.

Figure 4. The graphs show the essential elemental concentrations in select anti-asthmatic plant samples with NIST 1515 (apple leaves) Standard value. a) Phosphorus (P); b) Sulphur (S); c) Chlorine (Cl); d) Potassium (K); e) Calcium (Ca); f) Iron (Fe); g) Copper (Cu); h) Zinc (Zn) concentrations.
Declarations

Author contribution statement

S. Jyothsna: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

G. Manjula: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Sateesh Suthari: Performed the experiments; Analyzed and interpreted the data.

A.S. Nageswara Rao: Conceived and designed the experiments; Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

Aschner, M., 2000. Manganes: brain transport and emerging research needs. Environ. Health Perspect. (Suppl.) 108, 429–432.

Atbari, S.S., 2013. Best treatment for allergic asthma with traditional herbal medicine: a brief report. Am-EurAsian J. Agric. Environ. Sci. 13, 291–292.

Beisel, W.R., 1982. Single nutrients and immunity. Am. J. Clin. Nutr. 35, 417–468.

Bielory, L., Russin, J., Zuckerman, G.B., 2004. Clinical ef... of a new hypothesis. Chest 97, 635–641.

Gray, R.D., Duncan, A., Noble, D., Imrie, M., O'Reilly, D.S., Innes, J.A., 2010. Sputum trace metals are biomarkers of inflammatory and suppurative lung disease. Chest 137, 174–180.

Greenberger, P.A., 2003. Therapy in management of rhinitis asthma complex. Allergy Asthma Proc. 24, 403–407.

Kaziraj, B., Maruthamutha, R., Gopalakrishnan, V., Arumugam, K., Asirvatham, H., Murali, V., Mohandas, R., Bhaskar, A., 2007. A data base for medicinal plants used in Treatment of Asthma. Bioinformation 2, 105–106.

Kaur, R., Kumar, A., Kaur, N., Mohanty, B.P., Onwal, M., Singh, K.P., Singh, G., 2012. Investigation of major and trace elements in some medicinal plants using PIXE. Int. J. PIXE 22, 113–119.

Morobad, R.B., Patil, S.J., Tapash, R.R., 2013. First series transition elemental analysis in some therapeutically important medicinal plants by AAS method. J. Mater. Environ. Sci. 4, 171–176.

Nazi, R., Khan, M., Masabi, M., Rehman, H.U., Rauf, N.U., Shahab, S., Ameer, N., Sajed, M., Ullah, M., Rafique, M., Shaheen, Z., 2015. Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water collected from Tanda Dam Kohat. J. Pharmaceut. Sci. Res. 7, 89–97.

Ohammad, M., Sherbeny, E.I., Behairy, O.G., Mohammad, O.I., Elsayed, A.M., 2016. Serum levels of lead and copper in a group of Egyptian children with bronchial asthma. Egypt J. Pediatr. Allergy Immunol. 14, 47–52.

Oliveira, M., Uasy, R., 1996. Copper as an essential nutrient. Am. J. Clin. Nutr. 63, 7915–7965.

Patching, S.G., Gardiner, P.H., 1999. Recent developments in selenium metabolism and chemical speciation: a review. J. Trace Elem. Med. Biol. 13, 193–214.

Pires, T., Santos, P., de, M., Machado, A.S., Noblat, L.D.A.B.C., Cruz, A.A., 2011. Use of medicinal herbs by patients with severe asthma managed at a Referral Center. Braz. J. Pharmaceut. Sci. 47, 643–649.

Prasad, A.S., 1998. Essential and Toxic Elements in Human Health and Disease: an Update. Wiley-online Library, New York.

Raju, G.J.N., Saritha, P., Murthy, G.A.V.R., Reddy, S.B., Charles, M.J., Lakshminarayana, S., Reddy, T.S., Reddy, S.B., Vijayan, V., 2003. Estimation of trace elements in some anti-diabetic medicinal plants using PIXE technique. Appl. Radiat. Isot. 64, 893–900.

Rajurkar, N.S., Damame, M.M., 1997. Elemental analysis of some herbal plants used in the treatment of cardiovascular diseases by NAA and AAS. J. Radioanal. Nucl. Chem. 219 (1), 77–80.

RDAS, 1989. Recommended Dietary Allowances Subcommittee on the Tenth Edition of the RDAS, Commission on Life Sciences, Food and Nutrition Board. National Academy Press, Washington DC.

Reddy, B.S., Dessalegn, F., Sheikh, A.S., 2013. Trace element analysis of some indigenous medicinal plants of Ethiopia by PIXE. Eng. Sci. Tech. An Int. J. 3, 633–637.

Rout, G.R., Debata, B.K., Das, P., 1991. Somatic embryogenesis in callus cultures of Rosa hybrida L. cv. Landora. Plant Cell. Tissue Organ Cult 27, 65–69.

Sattar, S.A., Reddy, B.S., Rao, V.K., Pradeep, A.S., Raju, G.J.N., Ramanarayana, K., Rao, P.V.M., Reddy, S.B., 2012. Estimation of trace element in some anti-epileptic medicinal plants by PIXE. J. Radioanal. Nucl. Chem. 294, 337–341.

Senthil, A., Godden, D.J., Brown, K., 1994. Increase in asthma as a more toxic environment or a more susceptible population. Thorax 49, 171–174.

Srinivas, C., Raju, V.P., Babu, N.G., Ram, S.S., Sudarshan, D., N.L., 2016. Estimation of elemental concentrations of Indian medicinal plants using energy dispersive X-ray fluorescence (EDXRF) technique. Int. J. Multidiscip. Res. Dev. 3, 299–304.

Sudarshan, M., Ram, S.S., Majumdar, S., Maity, J.P., Ray, J.G., Chakraborthy, A., 2011. Energy-dispersive X-ray fluorescence-A tool for interdisciplinary research PRAMANA. J. Phys. (Paris) 76, 241–247.

Suthari, S., Kandagada, R., Ragan, A., Raju, V.S., 2016. Plant wealth of a sacred grove: Mullar gutta, Telangana State, India. Int. J. Gen. Med. 9, 369–381.

Teotia, U.S.V., Mishra, A.K., Kumar, R., Deepa Kumar, V., 2014. Anti asthmatic potential of some medicinal plants. Asian Pac. J. Health Sci. 1, 155–161.