Chapter-V

Discussion, Conclusion and Scope of Future Study

The results obtained in Chapter IV are discussed in the light of literatures reported. The elemental content of the plants were correlated to some extent with their medicinal properties. Attempt is also made for the definition of the recommended doses of some of these plants for their use in the medicinal purposes. No toxic heavy metals such as As, Pd and Hg were detected in the studied plants. The study shows that many of these plants contain elements of vital importance in man's metabolism and that are needed for growth and developments, prevention and healing of diseases.

The results obtained in the present investigation and the correlations found between the medicinal use of the plants and their elemental contents are encouraging and offer the scope of further study.
5.1. PIXE Analysis

The results of the PIXE analysis of the plant samples are shown in Tables 4.1 and 4.2. The concentrations are expressed in parts per million (ppm) of the dried samples. The results document a wide range of variation in the elemental constitution of different plant taken as study samples. The range of the elemental concentrations in the samples is found to vary from 53091.14 ppm to 1.81 ppm. All the reported elemental concentration values of the studied plants are in agreement with the common values reported elsewhere [1-13] for medicinal plants. Some of the plants are found to be rich in one or more individual elements, which pertain their therapeutic value for treatment of different disease delicately, either singly or in combination.

Among the detected elements K and Ca are found to be most abundant elements followed by other trace elements. From the observed data, some of the traditional use of plants can be well correlated with their elemental composition.

5.1.1. Analysis at CCCM

In all the samples analysed at CCCM, the elements K, Ca, Mn, Fe Cu and Zn are present in varying concentrations (ppm level and above). Sr and Rb are present in some of the samples. Concentrations of K and Ca are observed to be in the range of 48380 ppm to 687 ppm. The relatively high concentration of K in Sample no. 3 (Curcuma domestica valeton) and sample no. 12 (Melothria purpusilla) is significant because K is the principal positively charged ion inside body cells. It
plays a major role in maintaining fluid and electrolyte balance and cell integrity. It is also critical to maintaining the heartbeat [14]. Potassium also helps in protein and carbohydrate metabolism of the body and its deficiency causes diabetic acidosis [15]. *Andrographis paniculata* contains the highest amount of Ca. Ca is known to enhance the qualities of bones and teeth and also of neuromuscular systemic and cardiac functions. Iron also occurred in all the specimens and is an important element in the human body and plays a role in oxygen and electron transport. *Lemania australis* contains highest amount of iron among the studied samples. This high amount may be correlated to the traditional use of this plant by nursing and pregnant mothers for better blood circulation. *Polygonum posumba* Bach which is use for controlling high blood pressure contains high amount of Mn (722.9 ppm). Manganese is an antioxidant nutrient and is important in the breakdown of fats and cholesterol and also helps in the nourishment of the nerves and the brain [16]. The Zn content of *Cissus javana* DC is significantly higher in comparison with all other plant samples studied. It offers the scope to further explore the therapeutic use of this plant in case of Zn deficiency related disorders.

Though many of the studied plants have varied medicinal properties, some of them have common applications as well. For example, sample nos. 2, 4, 5, 6, 8, 11, 15, 18 and 22 are use for cold and associated cough remedy by the local people apart from their other applications. A cold is a respiratory infection caused by one of hundreds of possible viruses. However, because these viruses are so widespread, it is perhaps more accurate to say that colds are caused by a decrease in immunity that allows one of these viruses to take hold. Hence, the only way to
remain away from cold is to have a strong immune system. The plants which are used by the local people for cold and cough remedy are found to contain trace elements which have a direct or indirect role in elevating the human immune system. All these plants are found to contain relatively high amount of Fe, Cu and Zn. Zinc and copper are necessary for the normal function of the immune system [17].

Zn is dietary trace mineral that, in addition to its many essential functions in growth and developments, is essential for the function of cells of the immune system and Zn has been shown to be effective in the treatment of the common cold [18, 19]. Zn is also required for the activity of more than 100 enzymes associated with carbohydrate and energy metabolism, protein degradation and synthesis, nucleic acid synthesis, heme biosynthesis, and CO$_2$ transport [18]. Zn deficiency impedes host defense systems [20], leading to increased susceptibility to a variety of pathogens and a deficiency of Zn is known to occur in many diseased states that involve the immune system [21]. These include alcoholism, renal disease, burns and gastrointestinal tract disorders [22] as well as HIV and diarrhoea [20]. It has been reported that oral zinc gluconate over the period of illness (13.3mg every 2h while awake) significantly reduced the duration of symptoms of the common cold [19, 23, 24]. The content of Zn in these plants range from 26.4 ppm to 155.7 ppm.

Again, it is well documented that iron regulates the function of T lymphocytes, and in most studies (in vivo and in vitro), a deficiency results in impaired cell-mediated immunity [25-29]. Iron deficiency may also delay the
development of cell-mediated immunity [25]. Copper is essential for a variety of biochemical processes and is needed for certain critical enzymes to function in the body. Copper is also involved in the functioning of the nervous system, in maintaining the balance of other useful metals in the body such as Zn and molybdenum, and other body functions.

Among the plants, *Andrographis paniculata* has been used historically in epidemics, including the Indian flu epidemic in 1919, during which this plant was credited with stopping the spread of the disease [30]. Although we don’t know how *Andrographis* might work for colds, some evidence suggests that it might stimulate immunity [31]. Interestingly, the ingredient of this plant used for standardization purposes, andrographolids, does not appear to affect the immune system as much as the whole plant extract [32]. According to a few well-designed studies, *Andrographis paniculata* can reduce the symptoms of colds. It may offer the additional useful benefit of helping to prevent colds [33-35, 36]. Hence the elemental content of the plants might have played a significant role in their medicinal property.

Again most of the rhizomes of the medicinal Zingiberales included in the present study, namely, *Curcuma domestica, Zingiber officinale, Zingiber cassumunar* (Roxb), *Hedychium marginatum* C.B Clarke, *Kaempferia galanga* Linn and *Curcuma caesia* are also found to contain relatively rich amount of the essential elements which are considered to possess good anti-oxidant property.
Medicinal Zingiberales are hot herbs today and a number of studies have shown that the rhizomes of these species possess a variety of potential antioxidant molecules [37-40]. These aromatic plants are not only used as food, spices and condiments, but they are also used in the traditional system of medicines for curing various human diseases [41, 42]. The antioxidant and anticarcinogenic properties of curcumin, the chrome orange yellow coloring compound present in turmeric rhizome have been reported [43, 44]. Rhizome extracts of Zingiber cassumunar, Kaempferia galanga, Curcuma caesia and Zingiber officinale have been shown to possess antioxidant [45] properties. Supplementation of natural antioxidants through a balanced diet could be more effective and also more economical than the supplementation of an individual antioxidant, such as vitamin C or vitamin E, in protecting the body against oxidative damage under various conditions. Another advantage of using antioxidant agents of plant origin is that it can control the oxidative damage without any side effects. It is also reported that various elements present in plants have either direct or indirect role in the control and maintenance of the antioxidant defense system of the body. The trace elements copper, manganese, selenium and zinc act as co-factors of antioxidant enzymes to protect the body from oxygen free radicals that are produced during oxidative stress. It is necessary to maintain a balance between the harmful pro-oxidant components produced and the antioxidant compounds that counter these effects [46-48]. A delicate balance also exists for the redox trace elements such as copper, which can initiate free radical reactions but is also a co-factor of copper/zinc- superoxide dismutase, a free radical scavenging enzyme. Manganese
is an antioxidant nutrient and is important in the breakdown of amino acids and the production of energy. It is an essential requirement for the metabolism of vitamin B1, C and E and for activation of various enzymes, which are important for proper digestion, and utilization of foods. The rhizomes show the presence of high amounts of Mn, an antioxidant rich element, ranging between 203ppm and 632ppm except in *Curcuma domestica* where the content is 24ppm. But this value is also well within the common values reported elsewhere [1-12] for medicinal plants which range between 18ppm and 700ppm. The high content of Mn, Fe, and Zn in these rhizomes may again support their effective use in the traditional system of medicine by the local people of Manipur. The low incidence of various tumors among the populations in northeast India, particularly in Manipur [49], may be linked with the high level consumption of these plant-based foods rich in high antioxidant elements in routine dietary intake. Another point to be noted here is that selenium, the element of much importance because of its antioxidant properties, has not been detected in any of the plants. It is possible that Se is present at a very low amount in these rhizomes and that with a higher exposure time and by using a thicker filter, it could be detected. As characteristic X-ray intensities emanating out from elements at very low concentrations are often too weak to be separated from the background, there is a possibility that Se is also present in these rhizomes.

5.1.2. Analysis at IOP

All the six medicinal plants analysed at IOP contains K and Ca as the major element followed by Fe, Mn, Sr, Zn, Cu and Ni. The concentration of Ni in the
studied plants ranges from 2.8 ppm to 46 ppm. Nickel is an essential micronutrient and is required for normal growth and reproduction in animals, and presumably in human beings as well. It appears to have a role in the modulation of the immune system and in development of brain [16]. Ca content in sample no. 23, 24 and 25 are quite high compared to those analysed at CCCM.

*Ficus glomerata*, which is used for the treatment of diabetes mellitus, is found to contain the highest amount of Ca (29467.86 ppm). Sufficient levels of calcium are required for release of insulin [50]. The elements K, Ca, Cr, Mn, Cu and Zn are responsible for the secretion of insulin from beta cells of the islets of langerhans and are involved in potentiating insulin action [51-57]. Calcium is reported to play an important role in glucose tolerance factor (GTF), which decreases the blood glucose level by utilizing insulin [55]. The potassium content in this plant is 3680.6 ppm. Normal concentrations of potassium are required for optimal secretion of insulin [56]. Helderman et.al [58] have reported that hypokalemia reduces the capacity of the pancreas to secrete insulin and therefore is recognized as a reversible cause of glucose intolerance. Some studies suggest that serum potassium below optimum levels can also cause insulin resistance [58, 59]. Since this plant contains reasonable amounts of K and Ca, the usage of this plant as dietary supplements might prove beneficial to those people with diabetes whose potassium and calcium levels are subnormal. It is well known that trace elements play a pivotal role in the various biochemical and physiological processes in humans. As diabetes is a disease of metabolic abnormality, elements as such or as a component of enzymes may play a significant role in the
development and control of diabetes mellitus. Appropriate levels of Mn, Zn and Cu are also needed for normal synthesis and secretion of insulin [50, 57, 60-62].

Fig. 5.1. Concentrations of Trace elements in ppm of the six samples analysed at IOP

All the six samples analysed at IOP contains good amount of Sr with concentrations ranging from 27.4 ppm to 621 ppm (Fig. 5.1). Rb is also found in sample no. 25 and sample no.26 while Br is detected in sample no. 24, though these three elements have no such functions reported till now and need to be subject of detailed investigation. *Panax gingseng* which is remarkably known for helping the body to adapt to situations of stress, fatigue, cold, hunger, temperature extremes and mental/emotional stress contains relatively good amount of Mn, Fe,
Zn and Cu. The roles of these trace elements in various human metabolisms are discussed previously.

5.2. PIGE Analysis

Table 4.4 shows the result of the PIGE analysis. Light elements Na, Al, Mg and P were detected in all the samples studied by PIGE technique in varied concentrations. The concentrations of the elements range from 36 ppm to 71500 ppm. In all the studied samples Mg and P are present at higher concentrations. Al content is significant in sample no. 1, 8 and 16 where it is as high as 1191 ppm. Na content in sample no. 4 is also high.

Phosphorus is the second most abundant mineral in the body. About 85 percent of it is found combined with calcium in the crystals of the bones and teeth. Phosphorus salts are critical buffers, helping to maintain the acid-base balance of cellular fluids. Each cell also depends on phosphorus as part of its DNA and RNA, the cells' genetic material. Thus phosphorus is essential for growth and renewal of tissues. In metabolism of energy nutrients, phosphorus compounds carry, store and release energy and they assist many enzymes and vitamins in extracting the energy from nutrients.

Like phosphorus, magnesium is critical to many cell functions. It assists in the operation of more than 300 enzymes, is needed for the release and use of energy from the energy-yielding nutrients, and directly affects the metabolism of potassium, calcium and vitamin D. Magnesium acts in the cells of all the soft
tissues, where it is part of the protein-making machinery and is necessary for the release of energy. Magnesium works with calcium in contracting and relaxing muscles: calcium promotes contraction, and magnesium helps the muscles relax afterward, so both are needed for proper functioning. In teeth, magnesium promotes resistance to tooth decay by holding calcium in tooth enamel. Magnesium deficiency may also be related to cardiovascular disease, heart attack and high blood pressure [63]. Sodium is the chief ion used to maintain the volume of fluid outside cells. Sodium also helps maintain acid-base balance and is essential to muscle contraction and nerve transmission. Most of the studied medicinal plants contain good amount of P, Mg and Na and hence could be use for supplementation of these elements. Fig 5.2 and Fig 5.3 shows the major and trace elemental concentrations of the eighteen samples analysed by both PIXE and PIGE techniques.

![Graph of elemental concentrations](image)

**Fig. 5.2.** Concentration of major elements of the eighteen samples analysed by both PIXE and PIGE
It is interesting to note that the comparison of the elemental content of medicinal plants with that of non-medicinal plants shows that medicinal plants are richer in elemental content than non-medicinal plants [64]. When deficiency of certain trace elements is associated with a particular disease, then restoring the normal levels of these elements through diet and supplementation would ameliorate that disease's condition. Supplementation of these medicinal plants cannot be considered as the sole source of trace elements, but can be considered...
as a potential source for providing a reasonable amount of the elements other than diet.

We carefully searched for certain toxic elements such as Pb, As, Hg, etc. which are of prime interest in toxicological studies [65] but none was found in the plants. The maximum permissible levels in raw plant materials for these elements as cited by the World Health Organization (WHO) [66] are 10 ppm, 1 ppm and 0.5 ppm respectively and for the proton energy of 2.4 MeV used in our study the minimum detectable concentration of these toxic elements range from 0.5 ppm to 1 ppm [67]. Hence, it can be safely concluded the studied plants do not contain these toxic elements. Since mineral content of plant varies according to the composition of the soil on which the plant is grown, [68] the absences of these heavy metals in all the samples suggested that most of the herbs were collected from their unpolluted natural habitats and therefore reflect their natural levels of heavy metals. In Manipur, there is no industry and the places of collection were far from farmlands where fertilizers are used.

The results obtained in the present study would also be very useful for the definition of the recommended doses of these plants for their use in the medicinal purposes. All trace minerals are toxic in excess. The hazards of overdose are among the chief risks faced by people who take multiple nutrient supplements. Table 5.1 shows the elemental content of all the twenty-eight samples along with their Recommended Daily Dietary Allowance (RDA) in mg. It is seen from the table that at the dose of 10g of dried matter of the medicinal plants the concentrations of all the elements except Mn in Polygonum posumba Bach are
within the RDA. At this dose, consumption of the medicinal plants under study would be safe except for *Polygonum posumba* Bach which has a Mn concentration above normal. Manganese in higher concentration can be toxic for all forms of life. The disease caused by higher concentration of manganese in human body is called manganism. Manganism is a collection of symptoms that result from excessive exposure to manganese. It occurs because high concentration of manganese injures a part of the brain that helps to control body movements [69]. It has also been called “Parkinson’s syndrome” because its symptoms closely resemble those of Parkinson’s disease [70-72]. Levy and Nasseta [73] reported that many of the cases diagnosed as Parkinson’s disease in the United States and elsewhere are related to manganese exposure. Also high levels of essential elements such as Fe, Mn, Zn and Ca have been demonstrated to influence the retention of toxic elements in animals or human beings [65, 74] and hence care should be taken while using these medicinal plants as non-prescription drugs.

Several trace elements are essential micronutrients and are required for various body functions and well being of the human body system. The deficiency or excess of a particular trace element can influence changes in the functioning, forms, activities of some organs or concentrations of such element in the body tissue and fluids can rise above the permissible limit. In addition to identifying the active secondary metabolites of these medicinal plants, the knowledge of their elemental composition is very important in determining their toxicity/safety for use.
Table 5.1. Elemental Concentrations (in ppm) of the twenty eight (28) medicinal plants under study.

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| Na      |            | 413 | 301 | 338 | 6030 | 313 | 530 | 159 | 610 | 131.7 | 319 | 292 | 500 | **Recommended Daily Dietary Allowance (in mg) [Source: Recommended Dietary Allowances, © 1989 by the National Academy of Sciences, National Academy Press, Washington D.C.]
|         | Sample No: | 1 (Centella asiatica Linn); 2 (Zanthoxylum acanhipodium DC); 3 (Curcuma domestica Valeton); 4 (Ocimum sanctum Linn.); 5 (Hedychium marginatum C.B.Clarke); 6 (Kaempferia galanga Linn); 7 (Acacia farnesiana Willd); 8 (Zingiber cassumunmar (Roxb.)); 9 (Lemanea australis Atkins); 10 (Leucas aspera Spreng); 11 (Andrographis paniculata Wall. Ex Nees); 12 (Melothria purpurilla (Blume) Cogn); 13 (Aegle marmelos Correa ex Roxb); 14 (Celtis tinctoria); 15 (Meriandra bengalensis Benth)
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**Sample No: 16 (Polygonum posumba Bach); 17 (Cissus javana DC); 18 (Zingiber officinale Rosc); 19 (Xylous longifolia); 20 (Bryophyllum pinnatum (Lam.) Kru); 21 (Elaeocarpus floribundus Blume); 22 (Curcuma coesia); 23 (Ficus glomerata); 24 (Ficus palmate Forsk); 25 (Oroxylum indicum Vent.); 26 (Terminalia arjuna (Roxb)); 27 (Panax ginseng); 28 (Plumeria acuminata Ait)

**Recommended Daily Dietary Allowance (in mg) [Source: Recommended Dietary Allowances, © 1989 by the National Academy of Sciences, National Academy Press, Washington D.C.]

NA: Data not available; ND: Not Detected
Several trace elements are essential micronutrients and are required for various body functions and well being of the human body system. The deficiency or excess of a particular trace element can influence changes in the functioning, forms, activities of some organs or concentrations of such element in the body tissue and fluids can rise above the permissible limit. In addition to identifying the active secondary metabolites of these medicinal plants, the knowledge of their elemental composition is very important in determining their toxicity/safety for use.

5.3. Conclusion

PIXE and PIGE techniques are well suited for multielemental determinations in plant samples. The elements K, Ca, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr, P, Mg, Na and Al were identified and their concentrations were estimated. The results show that the studied medicinal plants contain elements of vital importance in man's metabolism and that are needed for growth and developments, prevention and healing of diseases. The concentrations of the constituent elements in some of the medicinal plants have been found to have good correlations with their medicinal usage. As discussed in previous sections, some traditional remedies seem to have their roots in the elemental contents of these plants. Some important points found are:

1. Relatively higher content of Mn in *Polygonum posumba* Bach which is used for controlling high blood pressure. Manganese, which is an antioxidant nutrient and important in the breakdown of fats and
cholesterol, might be responsible for efficiency of this plant in the
treatment of high blood pressure.

2. *Lemania australis* traditionally used by pregnant and nursing mother is
found to contain very high amount of iron.

3. The phytomedicines used traditionally for cold and cough (*Zanthoxylem
acanthopodium* DC, *Oscimum sanctum* Linn, *Hedychium marginatum*
C.B.Clarke, *Kaempferia galanga* Linn, *Zingiber cassumunar* (Roxb.),
*Andrographis paniculata* Wall. Ex Nees, *Meriandra bengalensis* Benth,
*Zingiber officinale* Rosc, *Curcuma caesia*) has been found to contain good
amount of Fe, Cu and Zn which are necessary for elevating the human
immune system and hence has a direct role in preventing the cold.

4. Most of the studied rhizomes of the Zingiberales are found to contain
relatively higher amount of the essential trace elements.

Thus it is seen that, the medicinal properties of these folk medicines though might
originally became known to the human kind through trial and error method or by
simple observation or by some other means and the knowledge transferred
through generations, are indeed having the active principles for effective
remedies. The results also emphasize the need to have good understating of trace
elements and their roles in health and diseases. A fair amount of information on
these elements and their roles in various physiological processes, their ways of
functioning and necessity would be of paramount importance to understand the
progression of various diseases and their remedies. The data obtained in this work
could serve as an important resource for further studies on these medicinal plants.
As trace elements in biological and medicinal sciences is a growing and evolving field, and novel information are being added up regularly, the importance of mineral content of medicinally important herbs will become more pronounced with the increased knowledge of the role of elements in health and diseases. The correlations between the therapeutic usage of plants and their elemental profile that have been found in the present study point to this direction. No toxic elements are found in the plants and hence these plants could be considered safe to consume.

Our results also show that the analyzed medicinal plants can be considered as potential sources for providing a reasonable amount of the required elements other than diet. Moreover, these results can be used to set new standards for prescribing the dosage of the herbal drugs prepared from these plant materials. At the same time adequate and necessary precautions should be taken while supplementing the trace elements through these medicinal plants in order to avoid other complications of metal toxicity.

5.4. Scope of Future Study

The results obtained in the present investigation and the correlations found between the medicinal use of herbs and their mineral contents are encouraging and offer the scope of further study. In the present study, correlations have been made by closely analyzing the results. In any case, where a group of plant parts that are reported to be used in the treatment of any specific disease contain
relatively higher levels of a specific element in comparison with other samples, correlations have been made between this specific ailment with the element concerned. These propositions offer further studies on these plants, elements concerned and the corresponding diseases. Suitable animal experiments could be designed to further investigate the efficacy of these plants in treating the diseases and also the remedial roles of these elements.

The variation in elemental composition of medicinal plants with region warrants further research on medicinal plants in each geographical region in order to integrate their medicinal values in the modern system of medicine. This would provide the benefit of effective and cheaper drugs from the right plant resource available on earth. Focus on understanding the exact relation between the organic constituents and the trace elements will be an interesting area for further work. Also micro-PIXE investigation of the samples would prove useful in order to understand the spatial distribution of the trace elements.
References


