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Evaluation of total mineral, calcium, selenium, iron content of ten medicinal plants extracts of Manipur having anti-inflammatory properties

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Abstract

Evaluation of total mineral, calcium, selenium, iron content of ten medicinal plants extracts of Manipur having anti-inflammatory properties” was undertaken to analyse the mineral content of ten medicinal plants of Manipur (*Cissus adnata*, *Debregeasia longifolia*, *Clerodendrum serratum*, *Polygonum barbatum*, *Colocasia gigantean*, *Allium hookerii*, *Houttuynia cordata*, *Oenanthe javanica*, *Allium odorum*, *Solanum xanthocarpum*). The total mineral content was ranged from 5.093±0.015% to 17.486±0.005% on dry weight. The calcium and selenium content ranged from 16.66±0.005 mg/100g to 117.77±1.92 mg/100g, 99.0.46µg/100g to 188.65±0.01 µg/100g respectively. The iron content of ten medicinal plants was ranged from 8.87±0.03 mg/100g to 112.37±0.04 mg/100g respectively. From the present study it can be concluded that the ten medicinal plant which are frequently used by the traditional healers/*Maibas/Maibis* and local people of Manipur for the treatment of different inflammatory diseases are rich in minerals like calcium, iron, selenium. Nevertheless, further in-depth investigation for identification of specific trace minerals is needed.

Keywords: Minerals, calcium, iron, selenium, medicinal plants, inflammatory diseases

1. Introduction

Natural Minerals are inorganic compounds and their structure is usually nothing more than a molecule, or molecules, of an element. The functions of minerals do not include participation in the yielding of energy. But they do play vital roles in several physiological functions, including critical involvement in nervous system functioning, in cellular reactions, in water balance in the body, and in structural systems, such as the skeletal system. Problem of mineral malnutrition are prominent in developing countries like India which causes degenerative diseases like osteoporosis, heart disease and cancer which can be prevented with proper mineral supplementation (Everitt *et al.*, 2006) [7]. Minerals are required for normal cellular function, enzyme activation, bone formation, haemoglobin composition, gene expression and amino acid, lipid and carbohydrate metabolism. Minerals in the diet are required for proper growth and good health (Bamji *et al.*, 2003) [4]. Minerals like calcium plays an important role in the prevention of degenerative and inflammatory diseases like heart diseases, skin infections, arthritis, gout, respiratory tract infections etc. and also enhanced the activity of superoxide dismutase, peroxidase, glutathione peroxidase, glutathione reductase enzymes which suppress the inflammation and also reduced the activity of superoxide anions which is the most common free radicals produced in the body (Patak, 2013) [11]. Minerals like selenium are unique mineral because it exerts its biological effects through direct incorporation into proteins (selenoproteins) as the amino acid selenocysteine and selenium-dependent enzyme reduces lipid peroxidation by catalyzing the reduction of peroxides, including hydrogen peroxide. Seleno-proteins help to prevent cellular damage from free radicals (Lee *et al.*, 2003) [8]. Selenium is also involved in several biochemical pathways associated with rheumatic diseases. Iron is also an essential micro-nutrient for almost all organisms. Its essentiality is largely based on its ability to exist in two redox states (Fe^{2+}/Fe^{3+}), which makes it ideal to act as a catalytic molecule in numerous biochemical reactions. The transport of oxygen in the blood and its storage in tissues, the transfer of electrons in the electron transport chain to supply cells with energy, and DNA synthesis, all require iron. Furthermore, iron is incorporated into and is essential for the function of ROS-producing enzymes such as nicotinamide adenine dinucleotide phosphate hydride (NADPH) oxidases and can catalyze the

production of reactive Oxygen species (ROS), which in turn can lead to peroxidation and radical chain reactions with molecular damage (Aust and Eveleigh, 1999) [3]. The ten medicinal plants namely, *Cissus adnata* (Kongouyen), *Debregeasia longifolia* (U-Khajing), *Clerodendrum serratum* (Moirang khanam), *Polygonum barbatum* (Yelang), *Colocasia gigantea* (Yendem), *Allium hookerii* (Napakpi), *Houttuynia cordata* (Tuningkhok), *Oenanthe javanica* (Komprek), *Allium odorum* (Nakuppi), *Solanum xanthocarpum* (Leipung-kangkha) (shown in plate 1 and 2) were selected to carry out the present investigation.

2. Methodology

2.1 Collection of Plant Material

The required fresh plants/plant parts were collected on the advice of the traditional healers, from various places of Thoubal district (24°37'N and 93°30'E), Manipur, India and also from the local market of Manipur. The samples were collected during the month of December and January and also June and July, in the year 2013-2014.

2.2 Chemical and reagents

Hydrochloric acid (HCl), Calcium carbonate (CaCO₃), Potassium iodide (KI), Azure B, Nitric acid (HNO₃), Sodium hydrogen selenite (NaHSeO₃), Sulphuric acid (H₂SO₄), Potassium thiocyanate (KSCN), Potassium per sulphate (K₂S₂O₈) ferrous ammonium sulphate [(NH₄)₂Fe(SO₄)₂.6H₂O].

2.3 Preparation of sample

After collection the tender leaves or required plant parts were cleaned by removing the infested and diseased portion. The leaves were thoroughly washed under running water and finally in distilled water and shade dried till the leaves became very crisp. The dried plant material were then ground properly into fine powder in an electrical grinder and stored in an airtight container with identification labels. The ground plant species were stored in a refrigerator at 4°C. These powdered materials were used for further different chemical analysis.



Cissus adnata (Kongouyen)



Debregeasia longifolia (U-Khajing)



Clerodendrum serratum (Moirang khanam)



Polygonum barbatum (Yelang)



Colocasia gigantea (Yendem)

Plate 1: Selected medicinal plant samples



Allium hookerii (Napakpi)



Houttuynia cordata (Tuningkhok)



Oenanthe javanica (Komprek)

*Allium odorum* (Nakuppi)*Solanum xanthocarpum* (Leipung-kangkha)**Plate 2:** Selected medicinal plant samples

2.4 Estimation of total mineral content

Total mineral content was determined as per the A.O.A.C method (2000) [2]. Procedure. 5 gm of the samples in duplicate were weighed into the previously heated and weighed crucibles. The crucibles were placed over a hot plate on low flame of a burner and the samples were charred completely. The crucibles containing the charred samples were transferred to a muffle furnace maintained at 600 degree Celsius. The heating was continued for 3-5 hrs till the ash become white in color or grayish white. The crucible was then transferred to desiccators, cooled and weighed. The ash % was then calculated using the following formula
($w_3 - w_1$)

$$\text{Total mineral (\% w/w)} = \frac{(w_3 - w_1)}{(w_2 - w_1)} \times 100$$

Where, W_1 = weight of crucible (g)

W_2 = weight of crucible +sample (g)

W_3 = weight of crucible + ash (g)

2.5 Estimation of mineral content

2.5.1 Preparation of ash solution

The ash is moistened with a small amount of distilled water and 5 ml of distilled HCl is added to it. The mixture is evaporated to dryness on a boiling water bath. Another 5 ml of HCl is added again and the solution evaporated to dryness as before. 4ml of HCl and few ml of water are then added and the solution warmed over a boiling water bath and filtered into a 100 ml volumetric flask using whatman number 40 filter paper. After cooling the volume is made up to 100 ml and suitable aliquots are used for mineral estimation.

2.5.2 Estimation of calcium

Calcium was determined by using flame photometer according to the method A.O.A.C (1984) [1]. A stock solution of Ca having concentration 200 ppm was prepared by dissolving 500 mg CaCO_3 in 1000ml distilled water. A few drops of (1:1) HCL were to be added to dissolve the CaCO_3 . From the stock solution another 3 solutions having concentration 50 ppm, 100 ppm and 150 ppm were prepared by appropriate dilution. The solutions were now placed under the Nebuliser and reading was taken. The concentration of calcium present in the sample was calculated from the standard curve and expressed as mg Ca/100g sample.

2.5.3 Determination of Selenium

Selenium was assayed by the method proposed by Mathew and Narayana (2006) [9]. An aliquot of the solution containing 2-10 $\mu\text{g/ml}$ of selenium was transferred into a series of 10mL calibrated flasks. A volume of 1mL of 2% potassium iodide solution followed by 1 ml of 2 M hydrochloric acid was added to it. The mixture was gently shaken until the appearance of yellow colour, indicating the liberation of iodide. Azure B (0.1%, 0.5mL) was added to the solution and the reaction mixture was shaken for 2 minutes. The contents were diluted to 10ml in a standard flask with distilled water. The absorbance of the resulting solution was measured at 644nm against a reagent blank. A blank was prepared by replacing the selenium solution with distilled water. The absorbance corresponding to the bleached colour, which in turn corresponds to the selenium concentration, was obtained by subtracting the absorbance of the blank solution from that of the test solution. The amount of the selenium present in the volume taken was computed from the calibration graph. A sample of plant material 5 g was digested with 10ml of nitric acid for 20 min. After cooling, the digested sample was treated by the above procedure and analyzed for selenium content according to general procedure.

2.5.4 Estimation of iron

Iron content was determined according to the method described by Ranganna (1997) [12]. by using spectrophotometer. To an aliquot (6.5ml or less) of the ash solution, added water to the final volume of 6.5 ml. then added one ml of 30% sulphuric acid, one ml of 7% potassium per sulphate solution, one ml of potassium per sulphate solution, one ml of potassium thiocyanate solution respectively. Mixed the content of the tube well and measured the intensity of the red colour at 540nm. Standard curve was prepared by taking different concentration of iron ranging from 10 μg to 50 μg . The concentration of iron present in the sample was calculated from the standard curve and expressed as mg Fe/100 g sample.

2.6 Statistical analysis

The results of all experiments performed were expressed as Mean \pm SD of three determinations.

3. Results and Discussions

3.1 Total mineral content

The total mineral content of the ten medicinal plants of Manipur are presented in the Fig 1.

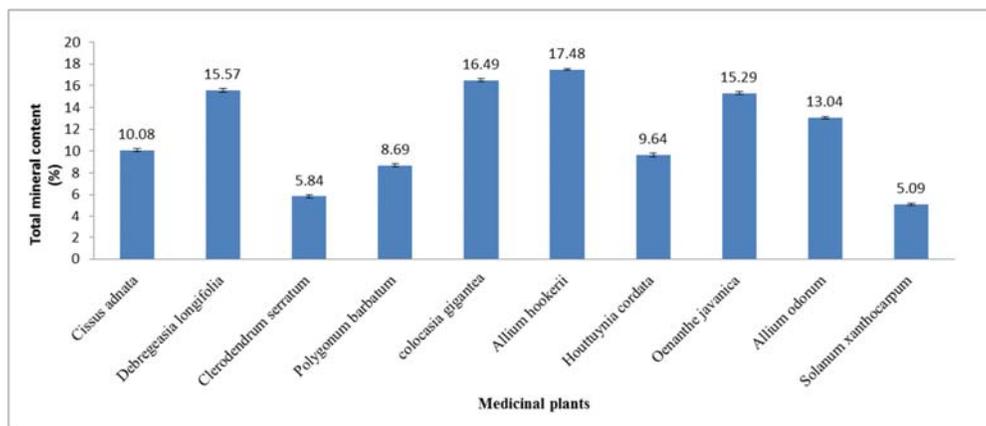


Fig 1: Total mineral content in ten medicinal plant extracts

From the Fig. 1, it was observed that the ash content of ten medicinal plants showed significant variation and it was ranged from $5.093 \pm 0.015\%$ to $17.486 \pm 0.005\%$ respectively. The highest mineral content was found in leaves of *Allium hookerii* ($17.486 \pm 0.005\%$), followed by *Colocasia gigantean* ($16.493 \pm 0.015\%$), *Debregeasia longifolia* ($15.57 \pm 0.026\%$), *Oenanthe javanica* ($15.29 \pm 0.020\%$), *Allium odorum* ($13.036 \pm 0.015\%$), *Cissus adnata* ($10.086 \pm 0.025\%$), *Houttuynia cordata* ($9.64 \pm 0.0360\%$), *Polygonum barbatum* ($8.696 \pm 0.020\%$), *Clerodendrum serratum* ($5.84 \pm 0.017\%$), and *Solanum xanthocarpum* ($5.093 \pm 0.015\%$). Problem of mineral malnutrition are prominent in developing countries like India which causes degenerative diseases like

osteoporosis, heart disease and cancer which can be prevented with proper mineral supplementation (Everitt *et al.*, 2006) [7]. Minerals are required for normal cellular function, enzyme activation, bone formation, haemoglobin composition, gene expression and amino acid, lipid and carbohydrate metabolism (Bamji *et al.*, 2003) [4]. Minerals in the diet are required for proper growth and good health (Bamji *et al.*, 2003) [4]. High mineral contents in ten selected medicinal plants may helps in protection and prevention of many health's issues related with mineral malnutrition.

3.2 Calcium content

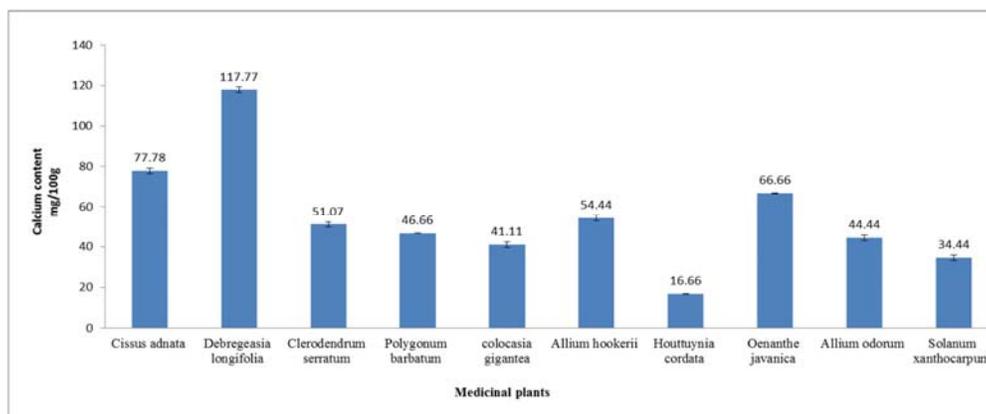


Fig 2: Calcium content in ten medicinal plants extracts

It was observed that the calcium content of ten medicinal plants shows significant variation and it was ranged from 16.66 ± 0.005 mg/100g to 117.77 ± 1.92 mg/100g respectively. The highest calcium content was found in *Debregeasia longifolia* (117.77 ± 1.92 mg/100g), followed by *Cissus adnata* (77.78 ± 1.92), *Oenanthe javanica* (66.66 ± 0.01), *Allium hookerii* (54.44 ± 1.92), *Clerodendrum serratum* (51.07 ± 1.8), *Polygonum barbatum* (46.66 ± 0.005), *Allium odorum* (44.44 ± 1.92), *Colocasia gigantean* (41.11 ± 1.92), *Solanum xanthocarpum* (34.44 ± 1.92), and *Houttuynia cordata* (16.66 ± 0.005). The antioxidant activity of calcium is that they enhanced the activity of superoxide dismutase, peroxidase, glutathione peroxidase, glutathione reductase enzymes which suppress the inflammation. Another mechanism of antioxidant activity of calcium is that calcium also reduced the activity of superoxide anions which is the most common free radicals produced in the body (Young and Woodside, 2001) [13]. Calcium also plays an important part in

nerve-impulse transmission and in the mechanism of neuromuscular system. Many researchers have identified important role for calcium in cell signalling in immune responses to chronic infection resulting from mycobacterium tuberculosis causing tuberculosis. Several epidemiological studies have also revealed that calcium play an important role in prevention of degenerative diseases like Alzheimer's disease. Calcium is the most common mineral in the human body which helps in strengthening bones and teeth, regulating muscle functioning, such as contraction and relaxation, regulation of heart function, blood clotting, transmission of nervous system messages and enzyme function. It plays a key role in skeletal mineralization, as well as a wide range of biologic functions. The calcium content in ten selected medicinal plants may help in prevention of degenerative and inflammatory diseases like heart diseases, skin infections, arthritis, gout, respiratory tract infections etc.

3.3 Selenium content

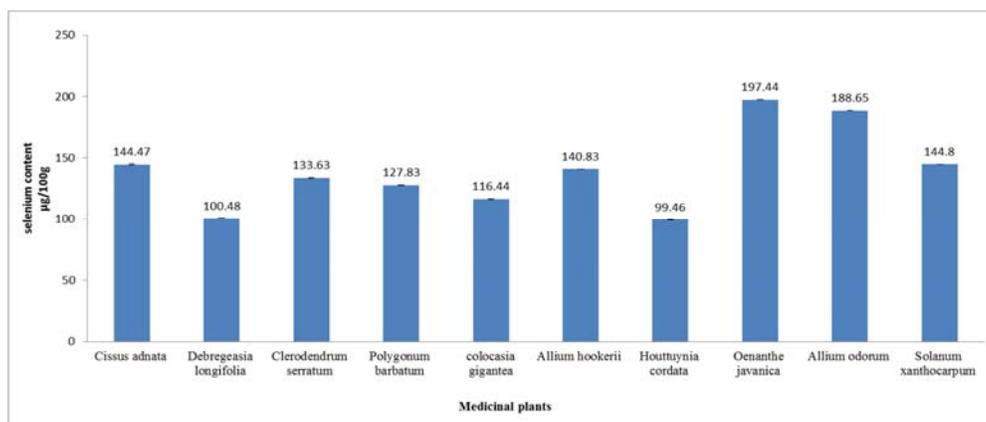


Fig 3: Selenium content in ten medicinal plants extracts

The selenium content of ten medicinal plants showed significant variation and it ranged from 99.046µg/100g to 188.65±0.01 µg/100g respectively. The highest selenium content was found in *Oenanthe javanica* (197.44±0.03) followed by *Allium odorum* (188.65±0.01), *Solanum xanthocarpum* (144.80±0.01), *Cissus adnata* (144.47±0.30), *Allium hookerii* (140.83±0.01), *Clerodendrum serratum* (133.63±0.03), *Polygonum barbatum* (127.83±0.03), *Colocasia gigantea* (116.44±0.02), *Debregeasia longifolia* (100.48±0.10), *Houltuynia cordata* (99.046±0.03). Recent studies have shown the importance of selenium in the prevention of different inflammatory diseases like cancers, carcinogenesis, cardiovascular diseases, diabetes mellitus and enhancing immune function, regulation of cell proliferation etc. (Papp *et al.*, 2014) [10]. Selenium is unique antioxidants as it exerts its biological effects through direct incorporation into proteins (selenoproteins) as the amino acid selenocysteine and selenium-dependent enzyme reduces lipid

peroxidation by catalyzing the reduction of peroxides, including hydrogen peroxide (Young and Woodside, 2001) [13]. The anti-oxidant properties of seleno-proteins help to prevent cellular damage from free radicals (Ching *et al.*, 2002) [6]. Because of its antioxidant role, selenium has been studied for its potential to protect the body from many degenerative diseases, including Parkinson's and cancer (Young and Woodside, 2001) [13]. Selenium is involved in several biochemical pathways associated with rheumatic diseases. Emerging evidence from many studies in humans and animals strongly suggested that the beneficial effects of selenium supplementation in prevention and treatment of diseases occur via the mitigation of inflammatory signalling pathways (Bouba *et al.*, 2012) [5]. So, the selenium present in ten selected medicinal plants has the potential to protect the body from many health diseases.

3.4 Iron content

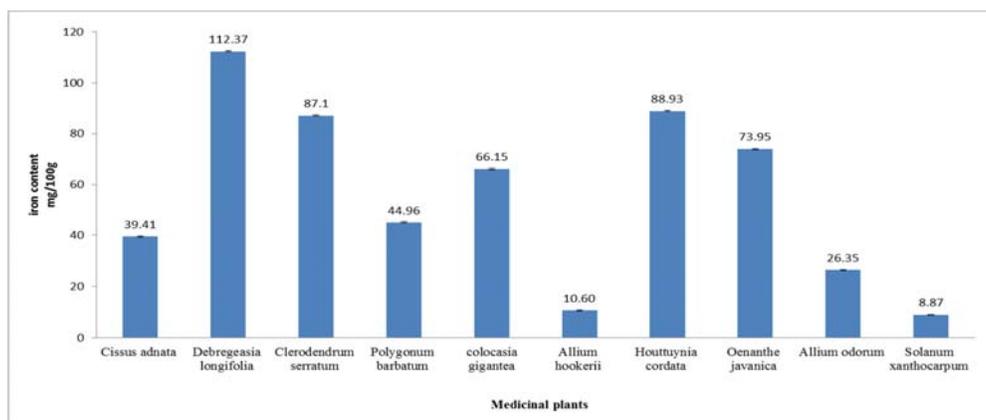


Fig 4: Iron content in ten medicinal plants extracts

The iron content of ten medicinal plants was ranged from 8.87±0.03 mg/100g to 112.37±0.04 mg/100g respectively. The highest iron content was found in *Debregeasia longifolia* (112.37±0.04 mg/100g), whereas it was lower in *Solanum xanthocarpum* (8.87±0.03 mg/100g). Iron is also an essential micro-nutrient for almost all organisms. Its essentiality is largely based on its ability to exist in two redox states (Fe^{2+} / Fe^{3+}), which makes it ideal to act as a catalytic molecule in numerous biochemical reactions. The transport of oxygen in the blood and its storage in tissues, the transfer of electrons in the electron transport chain to supply

cells with energy, and DNA synthesis, all require iron. Furthermore, iron is incorporated into and is essential for the function of ROS-producing enzymes such as nicotinamide adenine dinucleotide phosphate hydride (NADPH) oxidases and can catalyze the production of reactive oxygen species (ROS), which in turn can lead to peroxidation and radical chain reactions with molecular damage (Aust and Eveleigh, 1999) [3]. The ten selected medicinal plants is also rich in iron content which helps in prevention and protection from various health related issues.

4. Conclusion

This present study showed that the studied ten medicinal plants contained high minerals like calcium, selenium, iron, which demonstrated that the ten medicinal plants might have good antioxidant activity and could be a good source of natural antioxidants. Consumption of these medicinal plants will result in improved health thereby reducing many diseases like cardiovascular diseases, diabetes, anaemia, rheumatic arthritis, skin diseases, cancers etc. Creating mass awareness regarding the need for conservation of such plants will promote ethno-botanical knowledge within the region. These efforts may help in upliftment of the rural economy as well as long term security of the traditional healthcare system.

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