OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2019.

Research Article
Nutrient Content, Mineral Content and Antioxidant Activity of *Muntingia calabura* Linn

[1]Lukman Muslimin, Rini, [1]Isna Hasyim, [1]Nurul Fatimah Yusuf, [1]Fhahri Mubarak and [2]Risfah Yulianty

[1]Sekolah Tinggi Ilmu Farmasi Makassar, Perintis Kemerdekaan Street KM 13.7, Makassar 90241, Indonesia
[2]Faculty of Pharmacy, Hasanuddin University, Perintis Kemerdekaan Km.10, Makassar 90245, Indonesia

Abstract

Background and objective: The protective action of fruits and vegetables are associated with the presence of anti-oxidants, notably anti-oxidant vitamins, such as ascorbic acid, α-tocopherol and β-carotene. The aim of this research was to measure the nutritional content, mineral composition and antioxidant activities of *Muntingia calabura* Linn. fruit, which is grown in Makassar, Indonesia. **Materials and Methods:** Proximate parameters (moisture, ash, protein, carbohydrate and ascorbic acid) were evaluated using the Association of Official Analytical Chemists (AOAC) method, oxalate and phytic acid were evaluated by redox titration method and saponin and alkaloid content were evaluated using the gravimetric method. Mineral analysis was conducted using the inductively coupled plasma-optical emission spectrometry (ICP-OES) technique and antioxidant activity was evaluated by the fluorescence recovery after photobleaching (FRAP) method. **Results:** The results showed that the fruit of *M. calabura* had a high content of moisture and ash. The fruit had iron (Fe) as the higher component in trace elements and potassium (K) as the higher component in major elements. The anti-nutrients oxalate, phytate, alkaloids and saponins were detected while alkaloids are the highest content. In addition, from the study, the fruit showed antioxidant activity. **Conclusion:** *Muntingia calabura* is easily accessible and contributes remarkably to the amount of nutrient intake in the human diet. Further study is needed to analyze the type of alkaloids contained in the fruit.

Key words: Anti-nutrient regeneration, antioxidant, mineral composition, *Muntingia calabura*, nutritional content, proximate parameters

Received: Accepted: Published:

Citation: Lukman Muslimin, Rini, Isna Hasyim, Nurul Fatimah Yusuf, Fhahri Mubarak and Risfah Yulianty, 2019. Nutrient content, mineral content and antioxidant activity of *Muntingia calabura* Linn. Pak. J. Nutr., CC: CC-CC.

Corresponding Author: M. Lukman, Sekolah Tinggi Ilmu Farmasi Makassar, Perintis Kemerdekaan Street KM 13.7, Makassar 90241, Indonesia
Tel: +62 8971561010

Copyright: © 2019 Lukman Muslimin *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fruits and vegetables are functional foods that can provide health benefits in addition to meeting physiological needs. Routine or periodic consumption of fruits and vegetables provides significant benefits to human health[1].

Protein, carbohydrates, vitamins, sterols and lipids are primary metabolites in plants, which are essential for the growth and development of plants. These are the basis of nutrition worldwide[2,3].

Anti-nutritional factors are substances that reduce the bioavailability of some nutrients, diminution of the digestive process, or inhibit food diffusion[4]. Trace elements that have been involved in combating several human ailments and disease are found chiefly in medicinal plants[5,6]. The functional activities of specific organs may be affected by the continuous dietary consumption of specific elements; which can lead to their bioaccumulation beyond normal or safe levels[7].

The presence of anti-oxidants, notably anti-oxidant vitamins, such as beta-carotene, vitamin C and vitamin E are associated with the defense mechanism of fruits and vegetables[1]. The primary purpose of this research was to determine the nutritive potential and antioxidant activity of the *M. calabura* fruit growing in Makassar, South Sulawesi which is lacking in the literature.

MATERIALS AND METHODS

Muntingia calabura fruits were collected from Makassar, South Sulawesi. Fruits were harvested during the rainy season in December 2017. The sample was rinsed under the tap water to eliminate dirt and wash again with aquadest. Fresh *M. calabura* fruits were processed to separate the juice and bulb.

Determination of moisture content: Moisture was determined using an oven-drying method at 105°C for 24 h until a constant weight was achieved.

Determination of total ash content: The total ash contents were determined by incinerating in a furnace at 550°C for 3 h.

Determination of N-protein content: The N-protein contents were obtained from deducting the crude protein contents with non-protein nitrogen (NPN) contents. Crude protein content was evaluated as described by Maubois and Lorient [8]. with modification. NPN was determined by precipitation protein contents using copper sulfate. Continue with Kjeldahl assay without the destruction process.

Determination of total carbohydrate content: The carbohydrate contents were determined by Luff Schoorl method. A 3 g sample was weighed. Then 200 mL of 3% HCl was added and the mixture was simmered for 3 h and chilled for a few minutes or longer before being neutralized with 30% of sodium hydroxide (qualitative test with phenolphthalein indicator). Three percent acetic acid was added to the solution. The solution was added to a volumetric flask using a pipette and filled to the mark with distilled water. Ten milliliters of the obtained filtrate was added to 25 mL of Luff-Schoorl solution and 15 mL of purified water in an Erlenmeyer flask. Heated in a water bath followed by boiling on reverse cooler for 10 min. When the solution was cooled, 15 mL of 20% potassium iodide and 25 mL of 25% H_2SO_4 were added. The obtained mixture was titrated with $Na_2S_2O_3$ 0.1 N with a few drops of 0.5% starch solution added.

Determination of ascorbic acid content: The iodometric titration determined the ascorbic acid content. Thirty grams of sample was weighed and put into a flask, aquadest was added to 100 mL, then centrifuged to separate the filtrate. Twenty milliliters of filtrate was placed into the flask and 1% starch solution and 20 mL of aquadest was added. Then, the solution was titrated with an iodine standard of 0.01 N.

Determination of oxalate content: Oxalate content was measured as described by Unuofin *et al.*[3] with modification. The sample was weighed (1 g) in a flask; 75 mL of 3 mol L^{-1} of H_2SO_4 was added and homogenized with a magnetic stirrer for an hour before filtering. Twenty-five milliliters of collected filtrate was heated to 80-90°C. This filtrate was kept above 70°C at all times. The hot aliquot was titrated continuously with 0.05 mol L^{-1} of $KMnO_4$ until the end point revealed by a light pink color which persisted for 15 s was reached. One milliliter of 0.012 mol L^{-1} of $KMnO_4$ as equivalent to 0.528 mg of oxalate.

Determination of phytate content: Phytic acid was determined as described by Magomya *et al.*[9]. The sample (2 g) was weighed into a flask and 100 mL of 2% HCl was added and allowed to stand at room temperature for 3 h before filtering. Twenty-five milliliters of the filtrate was placed in a separate conical flask and the end of titration point was observed by adding 5 mL of 0.3% ammonium thiocyanate solution. To achieve the desired activity 53.5 mL of distilled water was added. Then the sample was titrated with standard iron (III) chloride solution (0.00195 g of iron per mL) until a brownish yellow color persisted for 5 min. Phytic acid was calculated as:

$$\text{Phytic acid (\%)} = \text{Titer value} \times 0.001\ 95 \times 1.19 \times 100$$

Determination of saponins: Saponin content was estimated as described by Unuofin *et al.*[3] with modification. Five grams of the fruit juice was added to 75 mL of 20% ethanol, placed on a shaker incubator for 30 min and then heated in a water bath at 55°C for 4 h. The mixture was filtered and the residue added to 150 mL of 20% aqueous ethanol. The filtrates were mixed and transferred to 40 mL in a water bath at 90°C. The concentrate was transferred into a separating flask, 20 mL of chloroform was added and the mixture was shaken. The chloroform layer which was the upper layer was removed and the bottom layer (aqueous) was retained in a beaker. The aqueous layer was re-introduced into a separating funnel and 60 mL of n-butanol was added and shaken vigorously. The butanol phase was retained and rinsed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was evaporated in a water bath, then dried until a constant weight was achieved at 40°C in an oven. The saponin content was counted using the equation:

$$\text{Saponin content (\%)} = \frac{\text{Weight of residue}}{\text{Weight of original sample}} \times 100$$

Determination of alkaloids: The described method was adopted by Omoruyig *et al.*[10]. Approximately 5 g of fruit juice was mixed with 200 mL of 10% acetic acid in ethanol, placed in untransparent plastic and left standing at room temperature for 4 h. The mixture was filtered and the filtrate was concentrated in a water bath up to a quarter of the first volume. Concentrated ammonium hydroxide was in drops until precipitation (cloudy fume) was completed. The solution was allowed to settle, washed twice with ammonium hydroxide and filtered with paper. The residue collected was dried and weighed and the alkaloid content was calculated using the formula:

$$\text{Alkaloid (\%)} = \frac{\text{Weight of precipitate}}{\text{Weight of original sample}} \times 100$$

Elemental analysis: The method was described by a previous researcher using inductively coupled plasma-optical emission spectrometer[11].

Determination of antioxidant activity: The method was described by Karim *et al.*[12]. with slight modifications. Ferric (III) chloride solution (3 mM in 5 mM citric acid) and TPTZ solution (2,4,6-tripyridyl-s-triazine; 1 mM in 0.05 M hydrochloric acid) were prepared as fluorescence recovery after photobleaching (FRAP) solutions. One thousand microliters of the tested sample was added to 3000 µL of TPTZ solution and 1000 µL of $FeCl_3$ solution, incubated for 10 min and its wavelength was measured at 500-700 nm. The absorbance data were calculated against serial dilution of ascorbic acid and recorded as similar ascorbic acid capacity using the general linear regression model.

RESULTS AND DISCUSSION

Proximate composition: The result of the proximate content of *M. calabura* fruit is presented in Table 1. The moisture content was high (77.26%), with a high ash value of 1.66%. The percentage of crude protein was 1.68% and N-non-protein content was 0.05%. By deducting the result of crude protein and N-non-protein content the N-protein content obtained was 1.63%. The carbohydrate content was 1.75% and the ascorbic acid content was 0.11%.

Moisture content in food is a source of nutrients that can help to ensure adequate intake of fluids in the body. According to the European Food Safety Authority (EFSA)[13], the number of daily needs in adult men averaged 2.5 L and adult females averaged 2.0 L. The total ash content can indicate the amount of mineral content contained in the food[14]. Dietary protein is the primary source of amino acids, particularly the essential amino acids, which cannot be synthesized from endogenous precursors and are required for growth, development and maintenance of human health[15]. The primary function of carbohydrates in the body is as a primary source of energy for cells, particularly the brain. The excess carbohydrate that is not metabolized is stored in glycogen form[16,17]. Ascorbic acid plays a vital role in physiological functions of living organisms, like in the collagen synthesis process in connective tissues[18,19]. A condition in which the body lacks vitamin C can lead to the scurvy state. The features of scurvy include the absence of wound healing and the failure of fractures to repair. This occurs because of the impaired of collagen formation[19].

Antioxidants have an essential role in cellular function. They have been implicated in processes associated with aging, such as vascular, inflammatory damage and cancer. One of the

Table 1: Proximate composition of *Muntingia calabura*

Parameters	Percentage
Water content	77.26
Ash content	1.66
Carbohydrate	1.75
Protein	1.63
Vitamin C	0.11

antioxidant vitamins is ascorbic acid. Its antioxidant role is useful because it contributes to the maintenance of the cardiovascular system and prevents the formation of atherogenesis through regulation of collagen synthesis and the production of prostacyclin (eicosanoid family of lipid molecules that inhibits activation of platelet) and nitric oxide (vasodilator)[20]. The antioxidant capacity, in this case, is measured by the ability of an antioxidant to reduce the TPTZ-Fe^{3+} complex to Fe^{2+}. Compounds that have reductive ability may be able to act as antioxidants because they can stabilize radicals by donating electrons or hydrogen atoms and become more stable[21]. The result showed that *M. calabura* fruit has antioxidant activity with a reduction value of 3.2688 mg AAE g^{-1}.

Mineral content: The result of the mineral analysis of *M. calabura* fruit is presented in Table 2. Potassium (K) content (1,966.8 mg/220 mL) was highest compared to other minerals analyzed while selenium was the lowest mineral present in *M. calabura* juice.

Minerals are needed in human nutrition for mental and physical well-being, or as essential constituents of bones, teeth, tissues, muscles, blood and nerve cells[22].

Muntingia calabura can contribute approximately 9% of the amount of calcium (Ca) needed. Calcium plays a role in the growth and maintenance of bones, teeth and muscles and can be used as a dietary supplement[23]. Chlorine (Cl) plays a role in fluid and electrolyte balance, is a major negative ion of extracellular fluid (NaCl) and results in the production of acid in the stomach (HCl)[24]. K was the highest mineral content in samples. K is useful for reducing hypertension, improving heart rhythm and function in various physiological functions of the body[25]. It can also regulate heart rate, neurotransmission and air balance in the body[26].

Table 2: Macro and micro minerals of *Muntingia calabura*

Minerals	Contents	RDA per day
Al	3.476 mg	*
B	5.214 mg	20 mg
Cu	0.904 mg	0.9 mg
Fe	5.39 mg	8 mg
Mn	2.706 mg	2.3 mg
Mo	5.258 µg	45 µg
Se	0.22 µg	55 µg
Si	2.42 mg	*
Ca	88.22 mg	1,000
Cl	72.842 mg	2,300
K	1,966.8 mg	4,700
Mg	66.11 mg	320
N	1,188 mg	*
Na	24.948 mg	1,500
P	110.726 mg	700
S	44.88 mg	*

Mineral content in *Muntingia calabura* equivalent to 1 cup (220 mL) of *Muntingia calabura* juice, *There is no recommended value by RDA

Magnesium (Mg) improves beta cell function in diabetes and hypertension[27,28]. It is also involved in enzymatic reactions such as oxidative metabolism of nutrients, cell synthesis, nerve impulses transmission, regulation of body temperature, detoxification, energy production and bone and tooth formation[29,30]. The body needs nitrogen to increase protein synthesis, producing compounds and amino acids that play a role in growth, hormones, brain function and body systems. There is no Research Data Alliance (RDA) data showing nitrogen levels and there is no maximum limit for nitrogen consumption per day. Sodium can improve the osmotic pressure in the body, muscle impairment and cell permeability as well as having an essential role in enhancing membrane potential, nerve impulse transmission and the absorption of monosaccharides, amino acids, pyrimidines and bile salts[24,31].

Phosphorus (P) is an essential mineral that is affordable for the absorption of calcium needed for growth and repair of bones, teeth and muscles[32]. Sulfur in plants is available in inorganic sulfates derived from air and food. Sulfur compounds that have a biological significance are cyanates (SCN) in saliva and other liquids, ergothioneine in red blood cells, glutathione, present in all cells and chondroitin sulfate, which functions as a structural function of cartilage, bone, tendon, blood wall, etc. Sulfur food sources are adequate protein foods and these meet the daily needs of sulfur[33].

Aluminum (Al) is one of the nonessential minerals but consumption of excessive amounts of Al can modify enzyme activity in the body and second messenger pathways as well as interfere with mitochondrial function and cause oxidative stress[34].

Boron (B) has a role in steroid hormone metabolism, such as estrogen and testosterone, healthy bone development and maintenance of cell membranes. However, B can be toxic when consumed over the recommended dose, as it may lead to growth and reproductive abnormalities[35].

Copper (Cu) plays a role in erythropoiesis, regulation and erythrocyte function in the survival of red blood cells. However, the concentration does not go beyond the maximum tolerable limit (10 mg per day). High consumption of Cu can cause diarrhea, gastric ulcers, the presence of blood in urine, liver damage, hypotension and vomiting[36].

Iron (Fe) is needed to produce red blood cells that are necessary to transport oxygen throughout the body tissues. Fe deficiency is a nutritional problem that occurs in most people. It can be caused by chronic bleeding, infection, lack of iron bioavailability, folic acid deficiency, vitamin A or vitamin B12,

pregnancy and menstruation in women during reproductive life[37]. Manganese (Mg) has an important function in bone growth and development and also functions for the formation of prothrombin with vitamin K. Mg serves as a catalyst and cofactor in some enzymatic processes, is a co-factor in the synthesis of fatty acids and cholesterol and is required in the synthesis of mucopolysaccharides and glycoprotein[38]. Molybdenum (Mo) is a vital micronutrient both in animal and plant nutrients. Mo affects the metabolism of copper in humans because high molybdenum intake can trigger copper deficiency[24].

Silicon (Si) is one of the micro minerals that acts as a co-factor of enzymes in the body. Based on the RDA table, the maximum limit of Si consumption has not been determined, as no data has stated the amount of Si that can cause adverse effects. As with Al, daily consumption of Si should be limited to prevent excess Al in the body[39]. Zinc (Zn) is one of the most critical micro minerals in cell growth, brain development, behavioral response, bone formation and wound healing. Zn plays an essential key role in the metabolism of carbohydrates and proteins, storage of vitamin A and nucleic acid synthesis in cells. Zn deficiency can cause diseases such as Crohn's disease, hypothyroidism and some viral infections[40,41]. Selenium (Se) is a micromineral that is necessary for the essential biological functions of the body. Differently from the other (semi) metals, it is incorporated into proteins by a co-translational mechanism as part of the amino acid selenocysteine (SeCys), the 21st amino acid used for protein synthesis in humans[42].

Anti-nutrient compounds: The anti-nutritional composition of *M. calabura* is shown in Table 3. The saponin content was 1.640±0.313%, alkaloid was 255±0208 mg/100 mL and phytate was 0.062±0.007%, while oxalate content was 7.18±0.329%.

The phytate acid content of *M. calabura* was low. The content of dietary phytic acid decreased in monogastric animals by 1-6% in the long term[43]. Phytate has been known to form complexes with proteins. This complex formation changes the structure of proteins that can lead to decreased protein solubility, enzymatic activity and proteolytic digestibility. Many researchers have focused on the presence of phytate in food due to its adverse effect on mineral absorption[44]. Saponins were found to decrease nutrient bioavailability and decrease enzyme activity and they can affect protein digestibility via inhibiting various digestive enzymes such as trypsin and chymotrypsin[45]. The saponin content of *M. calabura* was within safe levels since amounts below 10% are harmless to the body.

Table 3: Anti-nutrition content in *Muntingia calabura* fruit

Anti-nutrition	Contents	Comparison
Phytic acid	0.062±0.007 (%)	1%
Oxalate	7.18±0.329 mg/1 g	50-60 mg day^{-1}
Saponin	1.640±0.313 (%)	10%
Alkaloid	255±208 mg/100 mL	140 mg day^{-1}*

*Exceeds the intake limit per day

The oxalate content of this fruit is quite low. Nowadays, patients are suggested to limit their intake of foods with a total intake of oxalate not exceeding 50-60 mg per day[46]. Consumption of oxalate can cause irritation of the gut lining and can prove fatal in large doses. The insoluble calcium oxalate has the tendency to precipitate thus forming sharp-edged calcium oxalate crystals. These crystals play a role in the formation of kidney stones in the urinary tract. Alkaloids are believed to be anti-nutrients due to their action on the nervous system, disrupting or inappropriately augmenting electrochemical transmission[47]. Lethal doses for humans range from 3-6 mg kg^{-1} body weight, although susceptibility varies considerably among individuals. A dose of more than 2 mg/kg is usually considered toxic[48]. They have been found to cause poison symptoms including vomiting, diarrhea, abdominal pain, apathy, weakness and unconsciousness. The alkaloid content recorded in this study was quite high.

CONCLUSION

The finding from this study indicates that *M. calabura* fruit are nutrient-dense foods that can be good sources of carbohydrates, moisture and many minerals including Fe and K. It also showed that the juice of *M. calabura* fruit possesses high levels of vitamin C as an antioxidant. However, the validation of the specific alkaloid is needed to ensure the safety of this fruit.

SIGNIFICANCE STATEMENT

This study found complete data on the nutrient content, mineral estimate and antioxidant capacity of *M. calabura*. There are few studies on the nutritional composition of this fruit in Makassar, Indonesia. Therefore, this study is important to record data and to expand our knowledge regarding the nutritional composition of food grown in Makassar. Further laboratory analysis to explore the kind of safe or unsafe alkaloid should be conducted.

ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support from the Almarisah Madani foundation.

REFERENCES

1. Vijayanand, S. and A.S. Thomas, 2016. Screening of *Michelia champacca* and *Muntingia calabura* extracts for potential bioactives. Int. J. Pharma Sci. Res., 7: 266-273.
2. Verma, K.S. and V.S. Kaushal, 2014. Nutritive assessment of different plant parts of *Carica papaya* Linn of Jabalpur region. J. Nat. Prod. Plant Resour., 4: 52-56.
3. Unuofin, J.O., G.A. Otunola and A.J. Afolayan, 2017. Nutritional evaluation of *Kedrostis africana* (L.) Cogn: An edible wild plant of South Africa. Asian Pac. J. Trop. Biomed., 7: 443-449.
4. Soetan, K.O. and O.E. Oyewole, 2009. The need for adequate processing to reduce the antinutritional factors in plants used as human foods and animal feeds: A review. Afr. J. Food Sci., 3: 223-232.
5. Shirin, K., S. Imad, S. Shafiq and K. Fatima, 2010. Determination of major and trace elements in the indigenous medicinal plant *Withania somnifera* and their possible correlation with therapeutic activity. J. Saudi Chem. Soc., 14: 97-100.
6. Achudume, A.C. and D. Owoeye, 2010. Quantitative assessment of heavy metals in some tea marketed in Nigeria-bioaccumulation of heavy metals in tea. Health, 2: 1097-1100.
7. Jaishankar, M., T. Tseten, N. Anbalagan, B.B. Mathew and K.N. Beeregowda, 2014. Toxicity, mechanism and health effects of some heavy metals. Interdisciplin. Toxicol., 7: 60-72.
8. Maubois, J.L. and D. Lorient, 2016. Dairy proteins and soy proteins in infant foods nitrogen-to-protein conversion factors. Dairy Sci. Technol., 96: 15-25.
9. Magomya, A.M., D. Kubmarawa, J.A. Ndahi and G.G. Yebpella, 2014. Determination of plant proteins via the Kjeldahl method and amino acid analysis: A comparative study. Int. J. Sci. Technol. Res., 3: 68-72.
10. Omoruyig, B.E., G. Bradley and A.J. Afolayan, 2012. Antioxidant and phytochemical properties of *Carpobrotus edulis* (L.) bolus leaf used for the management of common infections in HIV/AIDS patients in Eastern Cape Province. BMC. Complement Altern. Med., Vol. 12. 10.1186/1472-6882-12-215
11. Bvenura, C. and A.J. Afolayan, 2012. Heavy metal contamination of vegetables cultivated in home gardens in the Eastern Cape. S. Afr. J. Sci., 108: 1-6.
12. Karim, A.A., A. Azlan, A. Ismail, P. Hashim, S.S.A. Gani, B.H. Zainudin and N.A. Abdullah, 2014. Phenolic composition, antioxidant, anti-wrinkles and tyrosinase inhibitory activities of cocoa pod extract. BMC Complement. Altern. Med., Vol. 14. 10.1186/1472-6882-14-381
13. EFSA Panel on Dietetic Products, Nutrition and Allergies, 2010. Scientific opinion on dietary reference values for water. EFSA J., Vol. 8, No. 3. 10.2903/j.efsa.2010.1459
14. Bradley, R.L., 2010. Moisture and Total Solids Analysis. In: Food Analysis, Nielsen, S.S. (Ed.). Springer, Boston, MA., USA., ISBN: 978-1-4419-1478-1, pp: 85-104.
15. Pasiakos, S., S. Agarwal, H. Lieberman and V. Fulgoni, 2015. Sources and amounts of animal, dairy and plant protein intake of US adults in 2007-2010. Nutrients, 7: 7058-7069.
16. Institute of Medicine, 2005. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids. The National Academies Press, Washington, DC., Pages: 1358.
17. Trumbo, P., S. Schlicker, A.A. Yates and M. Poos, 2002. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J. Am. Dietetic Assoc., 102: 1621-1630.
18. Aguirre, R. and J.M. May, 2008. Inflammation in the vascular bed: Importance of vitamin C. Pharmacol. Therapeut., 119: 96-103.
19. Chambial, S., S. Dwivedi, K.K. Shukla, P.J. John and P. Sharma, 2013. Vitamin C in disease prevention and cure: An overview. Indian J. Clin. Biochem., 28: 314-328.
20. Barrita, J.L.S. and M.S.S. Sanchez, 2013. Antioxidant Role of Ascorbic Acid and his Protective Effects on Chronic Diseases. In: Oxidative Stress and Chronic Degenerative Diseases: A Role for Antioxidants, Morales-Gonzalez, J.A. (Ed.). Chapter 18, InTech Publ., Rijeka, Croatia, ISBN: 978-953-51-1123-8, pp: 449-484.
21. Kim, J.S., 2005. Radical scavenging capacity and antioxidant activity of the E vitamer fraction in rice bran. J. Food Sci., 70: C208-C213.
22. Bhattacharya, P.T., S.R. Misra and M. Hussain, 2016. Nutritional aspects of essential trace elements in oral health and disease: An extensive review. Scientifica, Vol. 2016. 10.1155/2016/5464373
23. Peacock, M., 2010. Calcium metabolism in health and disease. Clin. J. Am. Soc. Nephrol., 5: S23-S30.
24. Soetan, K.O., C.O. Olaiya and O.E. Oyewole, 2010. The importance of mineral elements for humans, domestic animals and plants: A review. Afr. J. Food Sci., 4: 200-222.
25. Houston, M.C., 2011. The importance of potassium in managing hypertension. Curr. Hyperten. Rep., 13: 309-317.
26. Grandi, E., M.C. Sanguinetti, D.C. Bartos, D.M. Bers and Y. Chen-Izu *et al*., 2017. Potassium channels in the heart: Structure, function and regulation. J. Physiol., 595: 2209-2228.
27. Barbagallo, M. and L.J. Dominguez, 2015. Magnesium and type 2 diabetes. World J. Diabetes, 6: 1152-1157.
28. Grober, U., J. Schmidt and K. Kisters, 2015. Magnesium in prevention and therapy. Nutrients, 7: 8199-8226.
29. Castiglioni, S., A. Cazzaniga, W. Albisetti and J. Maier, 2013. Magnesium and osteoporosis: Current state of knowledge and future research directions. Nutrients, 5: 3022-3033.

30. Stendig-Lindberg, G., D. Moran and Y. Shapiro, 1998. How significant is magnesium in thermoregulation? J. Basic Clin. Physiol. Pharmacol., 9: 73-85.
31. Lumbers, E.R., R.S. Moore and A.D. Stevens, 1995. Effects of changes in colloid osmotic pressure on excretion of sodium by the ovine fetal kidney. Reprod. Fertil. Dev., 7: 1321-1327.
32. Penido, M.G.M.G. and U.S. Alon, 2012. Phosphate homeostasis and its role in bone health. Pediatr. Nephrol., 27: 2039-2048.
33. Nimni, M.E., B. Han and F. Cordoba, 2007. Are we getting enough sulfur in our diet? Nutr. Metab., Vol. 4 10.1186/1743-7075-4-24
34. Dolara, P., 2014. Occurrence, exposure, effects, recommended intake and possible dietary use of selected trace compounds (aluminium, bismuth, cobalt, gold, lithium, nickel, silver). Int. J. Food Sci. Nutr., 65: 911-924.
35. Uluisik, I., H.C. Karakaya and A. Koc, 2018. The importance of boron in biological systems. J. Trace Elem. Med. Biol., 45: 156-162.
36. Johnson, W.T., 2005. Copper and Brain Function. In: Nutritional Neuroscience, Lieberman, H.R., R.B. Kanarek and C. Prasad (Eds.). Chapter 17, CRC Press, Boca Raton, FL., USA., ISBN-13: 9780415315999, pp: 289-306.
37. Ooi, D.J., S. Iqbal and M. Ismail, 2012. Proximate composition, nutritional attributes and mineral composition of *Peperomia pellucida* L. (Ketumpangan Air) grown in Malaysia. Molecules, 17: 11139-11145.
38. Samsel, A. and S. Seneff, 2015. Glyphosate, pathways to modern diseases III: Manganese, neurological diseases and associated pathologies. Surg. Neurol. Int., Vol. 6. 10.4103/2152-7806.153876
39. Henstock, J.R., L.T. Canham and S.I. Anderson, 2015. Silicon: The evolution of its use in biomaterials. Acta Biomater., 11: 17-26.
40. Roohani, N., R. Hurrell, R. Kelishadi and R. Schulin, 2013. Zinc and its importance for human health: An integrative review. J. Res. Med. Sci., 18: 144-157.
41. Gimenez, M.S., L.B. Oliveros and N.N. Gomez, 2011. Nutritional deficiencies and phospholipid metabolism. Int. J. Mol. Sci., 12: 2408-2433.
42. Roman, M., P. Jitaru and C. Barbante, 2014. Selenium biochemistry and its role for human health. Metallomics, 6: 25-54.
43. Gupta, R.K., S.S. Gangoliya and N.K. Singh, 2015. Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. J. Food Sci. Technol., 52: 676-684.
44. Greiner, R. and U. Konietzny, 2006. Phytase for food application. Food Technol. Biotechnol., 44: 125-140.
45. Milgate, J. and D.C.K. Roberts, 1995. The nutritional and biological significance of saponins. Nutr. Res., 15: 1223-1249.
46. Massey, L.K., R.G. Palmer and H.T. Horner, 2001. Oxalate content of soybean seeds (*Glycine max*: Leguminosae), soyfoods and other edible legumes. J. Agric. Food Chem., 49: 4262-4266.
47. Friedman, M., P.R. Henika and B.E. Mackey, 2003. Effect of feeding solanidine, solasodine and tomatidine to non-pregnant and pregnant mice. Food Chem. Toxicol., 41: 61-71.
48. Korpan, Y.I., E.A. Nazarenko, I.V. Skryshevskaya, C. Martelet, N. Jaffrezic-Renault and A.V. El'skaya, 2004. Potato glycoalkaloids: True safety or false sense of security? Trends Biotechnol., 22: 147-151.