



Original Research Article

Evaluation of important mineral nutrients and vitamins in poly herbal medicines used for the treatment of tuberculosis in the Eastern Cape Province, South Africa

Elizabeth Bosede Famewo¹, Anna Maria Clarke¹ and Anthony Jide Afolayan^{1*}**Abstract**

Polyherbal medicines are widely used for the treatment of various diseases in the developing countries. In order to validate their ability in boosting the immune system of tuberculosis patients, the mineral nutrients and vitamins present were determined. Their nutritive properties were analyzed using an inductively coupled plasma optical emission spectrometer, while the vitamins were determined using standardized methods. The poly herbal preparations were found to be rich in mineral nutrients and vitamins. Calcium was the highest mineral nutrient detected, while the lowest nutrient was phosphorus. Quantitatively, calcium and magnesium contents in the remedies ranged from 973.30 to 6503.30 mg/100g and 80.00 to 406.00 mg/100g respectively. The amount of phosphorus and potassium was between 20.00 and 263.30 mg/100g; 160.00 and 2050.00 mg/100g respectively. Micro nutrients such as iron, manganese, zinc, aluminum and copper were also detected. Iron was the highest nutrient in the majority of the poly herbal preparations while the lowest value was recorded for copper. However, vitamin C was absent in the herbal preparations while vitamin A and E were detected. These findings indicate that these poly herbal formulations contain the essential mineral nutrients and vitamins that could probably be boosting the immune system of tuberculosis patients.

Keywords: Mineral nutrients; polyherbal medicines; tuberculosis; vitamins

Introduction

Tuberculosis (TB) is one of the deadliest infectious diseases and has remained a major public health threat in most parts of the world [1]. Currently, it is estimated that over 2 billion people are infected with TB, with 10.4 million new cases per year; leading to the death of 1.8 million, including deaths of human immunodeficiency virus-TB co-infected individuals [2] [3]. In South Africa, about 80% of the population is infected with latent TB among the age group 30-39 years [4]. The disease is related to poverty, under-nutrition and poor immune function [5]. TB and malnutrition constitute a substantial problem in the developing countries [6]. Patients with active tuberculosis usually have low

nutritional status, thus, suffer macro nutrient and micro nutrient malabsorption, reduction in appetite and altered metabolism of the body [6]. Vitamins and minerals are essential nutrients needed for normal growth and maintenance of body functions. The minerals (macro and micro nutrients) work together for tissue regeneration, cellular integrity and play important roles in the treatment of tuberculosis [5] [6]. However, vitamins are biomolecules that maintain the physiology of the body and boost the immune system. They are responsible for a spectrum of vital functions in the body due to their anti-oxidant, pro-oxidant, anti-inflammatory effects and metabolic functions. While vitamin A, C and E largely contribute to the anti-oxidant system of the human body [7] [8], vitamin C and E are effective in improving the immune responses to tuberculosis when given as an adjuvant to multi drug tuberculosis therapy [9]. The use of herbal medicines for the treatment of various illnesses in the developed and developing countries has increased tremendously

*Correspondence: aafolayan@ufh.ac.za

¹Faculty of Science and Agriculture, University of Fort Hare, Alice, South Africa

Full list of author information is available at the end of the article.

over the past decade. These remedies contain multiple active constituents which act synergistic ally [10]. They are believed to contain pharmacological properties capable of boosting the immunity of TB-infected individuals and aid quick recovery. The nutritive potential of a few poly herbal preparations have been investigated and found to be rich in mineral nutrients [11] [12]. However, the mineral constituents and vitamins of poly herbal medicines used for the treatment of tuberculosis in Eastern Cape Province have not been investigated. Thus, this research work was designed to evaluate some important mineral nutrients and vitamins in poly herbal medicines used for the treatment of tuberculosis in this region.

Material and Methods

Collection of poly herbal medicines

Polyherbal medicines evaluated in this study were purchased from herbal healers in five communities namely; Alice, Fort Beaufort, Hogsback, King Williams Town and East London, all within the Amathole District Municipality, Eastern Cape Province, South Africa (Figure 1). The remedies were already prepared and well packaged into clean containers by the herbal healers before being transported to the Medicinal Plants and Economic Development Research Centre, University of Fort Hare, Alice, for analysis. All the herbal preparations were air dried, ground to homogeneous powder and code-named according to their respective place of collection: viz; King Williams Town site A (KWTa), King Williams Town site B (KWTb), King Williams Town site C (KWTc), Hogsback first site (HBfs), Hogsback second site (HBss), Hogsback third site (HBts), East London (EL), Alice (AL) and Fort Beaufort (FB) [13].

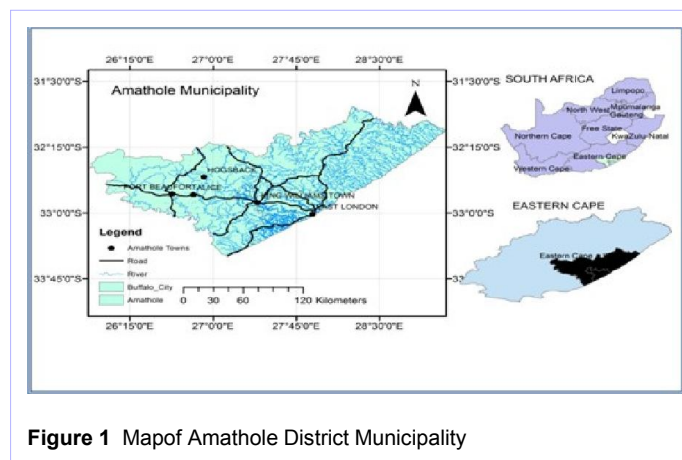


Figure 1 Map of Amathole District Municipality

Determination of macro and micro nutrients

Digestion and analysis

The method described by Bvenura and Afolayan [14] used for the digestion of the dried poly herbs. Selenium powder, sulfuric acid and salicylic acid were the reagents used for digestion. The finely ground material was divided into samples of 0.3 g, which were placed in dry and clean digestion tubes. A volume of 2.5 ml of the digestion mixture was added to each tube and allowed to react at room temperature for 120 min. The tubes were heated in a block digester at 110°C for 60 min. After removal from the digester, the tubes were allowed to cool and three successive portions of 1.0 ml hydrogen peroxide were added, allowing at least 10 sec between additions because of the volatility of the reaction. The tubes were returned to the block digester at a temperature of 330°C and were removed from the block digester when the digest was colorless. The tubes were allowed to cool to room temperature before their contents were transferred to 50 ml volumetric flasks and deionized water was added to attain volumes of 50 ml. Standards were prepared for all the elements. The samples were then analyzed for the following nutrients: calcium, magnesium, sodium, potassium, nitrogen, phosphorus, iron, zinc, aluminum, manganese and copper using an inductively coupled plasma optical emission spectrometer (ICP OES; Varian 710-ES series, SMM Instruments, Cape Town, South Africa). All analyses were performed in triplicates. The macro and micro nutrient contents were expressed as mg/100 g.

Vitamin analysis

Vitamin A content

The vitamin A (Retinal) content of each herbal remedy was determined using the method of Onyesife et al. [15]. A quantity, g of ground sample was macerated with 20 ml of petroleum ether. This was decanted into a test tube and evaporated to dryness. About 0.2 ml of chloroform-acetic anhydride (1:1, v/v) was added to the residue. Two milliliters of TCA-chloroform (1:1 v/v) was added to the resulting solution and absorbance was measured at 620 nm using UV-3000PC spectrophotometer. Retinal standard was prepared in like manner and the absorbance was taken at 620 nm. The concentrations of standard working solutions were 10 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml respectively. The concentration of vitamin A in the sample was extrapolated from the standard curve using the equation: $Y = 0.001x + 0.0008$.

Vitamin C content

The vitamin C (Ascorbic acid) contents of the poly herbal remedies were determined by the spectrophotometric method of Njoku et al. [16]. Briefly, 1.0 g of each remedy was macerated

with 20 ml of 0.4% oxalic acid. This was filtered using Whatman No. 1 filter paper. 9 ml of indophenol reagent was added to 1.0 ml of the filtrate. Standard solution of vitamin C was prepared similarly and the absorbances of the standard solutions and the remedies were read at 540 nm using UV-3000PC spectrophotometer. The concentrations of the standard working solutions were 0.1 mg/ml, 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml and 1.0 mg/ml respectively. The concentration of vitamin C was extrapolated from the standard curve of vitamin C using the following equation:

$$Y = 0.67x + 0.0824$$

Vitamin E content

The vitamin E (α -tocopherol) contents of the remedies were determined using the method of Njoku et al. [16]. A quantity, 1.0 g of each remedy was macerated with 20 mL of ethanol and then filtered using Whatman No. 1 filter paper. One milliliter of 0.2% ferric chloride in ethanol and 1.0 mL of 0.5% α - α -dipyridine solution were added to 1.0 mL of the filtrate. The solution was diluted to 5.0 mL with distilled water before the absorbance was read at 520 nm using UV-3000PC spectrophotometer. The concentrations of standard working solutions ranged between 10 μ g/mL and 100 μ g/mL. The concentration of vitamin E in the samples was extrapolated from the standard curve using the equation: $Y = 0.0086x - 0.0216$. The vitamin contents were expressed as mg/100 g.

Statistical analysis

Each analysis was carried out in triplicates and the results were expressed as the mean \pm SD. Minitab program version 17 for windows was used for the Analysis of Variance (Min tab Inc., Pennsylvania, USA). Statistical significance was evaluated at $P \leq 0.05$.

Results

Macro nutrient compositions

The macro nutrients detected in the poly herbal remedies include calcium, magnesium, sodium, potassium, nitrogen and phosphorus (Table 1). The results clearly indicated that these remedies are rich sources of macro nutrients. The level of potassium content was markedly higher in EL remedy, followed by calcium and nitrogen while the least content was phosphorus. Calcium was the highest content found in KWTa and HBfs remedies. This was followed by nitrogen, potassium, sodium, magnesium and phosphorus in descending order respectively. Nitrogen was markedly higher in FB remedy while the nutrient found in the

lowest level was phosphorus. KWTb and KWTc remedies contained high calcium content, followed by nitrogen and potassium with sodium being the lowest nutrient observed in the remedies. Calcium, potassium and nitrogen were the nutrients having the highest values in AL, HBss, and HBts remedies respectively. However, across the herbal formulations, calcium was the nutrient with the highest values detected, except in EL and FB remedies where potassium and nitrogen were the highest respectively. However, the lowest macro nutrients in majority of the remedies were phosphorus and magnesium in HBts remedy (Table 1).

King Williams Town site A (KWTa), King Williams Town site B (KWTb), King Williams Town site C (KWTc), Hogsback first site (HBfs), Hogsback second site (HBss), Hogsback third site (HBts), East London (EL), Alice (AL) and Fort Beaufort (FB). Each value represents the mean \pm standard deviation for triplicate determinations. Different superscripts within the same column are significantly different ($P \leq 0.05$).

Micro nutrient compositions

The micro nutrients that were determined include iron, aluminium, zinc, manganese and copper (Table 2). Iron was the highest mineral nutrient detected in KWTa, AL, HBfs, HBss and HBts remedies. This was followed by aluminium, manganese, zinc and copper in descending order respectively. Also, KWTc remedy had iron as the mineral with the highest micro nutrient while manganese was the highest in FB remedy. The element with the highest concentration in EL remedy was aluminium, with copper being the mineral content with the lowest value in the remedy. In KWTb remedy, aluminium was the highest mineral content, followed by iron, manganese, zinc while the lowest was copper. In general, these poly herbal remedies are good sources of iron. However, the lowest micro nutrient content in all the remedies was copper (Table 2).

King Williams Town site A (KWTa), King Williams Town site B (KWTb), King Williams Town site C (KWTc), Hogsback first site (HBfs), Hogsback second site (HBss), Hogsback third site (HBts), East London (EL), Alice (AL) and Fort Beaufort (FB). Data expressed as mean \pm SD of three replicates. Different superscripts within the same column are significantly different ($P \leq 0.05$).

Vitamin compositions

Table 3 shows the vitamin contents in each of the poly herbal preparation. The vitamin A and E contents observed in the remedies varies from 95.10 to 458.20 mg/100 g and 9.00 to 9.70 mg/100 g respectively indicating that these preparations are rich sources of retinol and α -tocopherol. However, vitamin C content was not detected in all the herbal remedies.

Table 1 Macro nutrient compositions (mg/100 g) of nine polyherbal remedies used for the treatment of tuberculosis in the Eastern Cape Province, South Africa.

Sample	N	Ca	Mg	Na	K	P
EL	803.30 ± 0.08ac	1956.70 ± 0.02ae	406.70 ± 0.02a	381.66 ± 8.20a	2050 ± 0.20a	263.33 ± 5.77a
KWTa	626.70 ± 0.16bd	2113.30 ± 0.89a	160.00 ± 0.04b	184.25 ± 64.58b	483.33 ± 0.11b	76.67 ± 25.17b
AL	713.30 ± 0.03bc	973.30 ± 0.02b	216.67 ± 0.00c	362.57 ± 5.99a	806.67 ± 0.01c	146.67 ± 5.77c
HBfs	583.30 ± 0.05bd	1376.70 ± 0.09bc	80.00 ± 0.01d	300.59 ± 9.07c	373.33 ± 0.03b	73.33 ± 15.28b
HBss	590.00 ± 0.03bd	1363.30 ± 0.11bc	156.67 ± 0.01b	113.25 ± 10.81d	760.00 ± 0.05c	123.33 ± 5.77c
HBts	903.30 ± 0.07ae	2180.00 ± 0.03a	110.00 ± 0.00e	201.57 ± 10.92b	970.00 ± 0.04d	143.33 ± 5.77c
FB	1866.70 ± 0.08f	1120.00 ± 0.02bc	260.00 ± 0.00f	276.27 ± 5.59c	1660.00 ± 0.04e	200.00 ± 10.00d
KWTb	560.00 ± 0.03d	1567.00 ± 0.21ce	140.00 ± 0.01b	20.52 ± 1.34e	160.00 ± 0.03f	46.67 ± 5.77f
KWTc	1023.30 ± 0.08e	6503.30 ± 0.07d	76.67 ± 0.00d	6.73 ± 0.02e	200.00 ± 0.01f	20.00 ± 0.00e

Table 2 Micronutrient compositions (mg/100g) of nine polyherbal remedies used for the treatment of tuberculosis in the Eastern Cape Province, South Africa.

Sample	Zn	Cu	Mn	Fe	Al
EL	8.13 ± 0.65a	1.31 ± 0.11a	6.70 ± 1.60a	112.70 ± 48.54a	158.70 ± 69.57a
KWTa	1.70 ± 0.56b	0.61 ± 0.44b	39.13 ± 14.75b	54.6 ± 38.54b	39.87 ± 23.09b
AL	3.03 ± 0.12c	0.56 ± 0.04b	7.43 ± 0.15a	35.57 ± 1.04bc	30.50 ± 0.85 b
HBfs	0.90 ± 0.05d	0.19 ± 0.01c	8.57 ± 0.37a	24.63 ± 15.91bc	11.8 ± 0.70 b
HBss	3.03 ± 0.35c	0.51 ± 0.09bd	3.40 ± 0.20a	18.93 ± 5.21bc	13.07 ± 1.40 b
HBts	2.17 ± 0.12b	0.56 ± 0.18b	5.37 ± 0.12a	20.17 ± 4.38bc	17.97 ± 1.85 b
FB	2.17 ± 1.49b	0.67 ± 0.19b	18.67 ± 0.29c	14.70 ± 1.18c	15.07 ± 1.04 b
KWTb	0.70 ± 0.02de	0.25 ± 0.04cd	23.77 ± 2.79c	32.67 ± 4.15bc	45.57 ± 5.84 b
KWTc	0.30 ± 0.17e	0.25 ± 0.03cd	58.03 ± 2.49d	76.50 ± 30.20bc	44.30 ± 0.06 b

Table 3 Vitamin contents (mg/100g) detected in nine polyherbal remedies used for the treatment of tuberculosis in the Eastern Cape Province, South Africa.

Sample	Vitamin A	Vitamin E
EL	459.20 ± 0.02a	9.40 ± 0.01ae
KWTa	262.20 ± 0.02b	9.50 ± 0.02ab
AL	124.80 ± 0.02c	9.0 ± 0.02cd
HBfs	197.00 ± 0.00d	9.50 ± 0.01bd
HBss	200.80 ± 0.00d	9.30 ± 0.03e
HBts	333.10 ± 0.02e	9.40 ± 0.01e
FB	365.40 ± 0.00e	9.00 ± 0.03f
KWTb	201.80 ± 0.00d	9.70 ± 0.05c
KWTc	96.10 ± 0.01c	9.30 ± 0.04e

Data expressed as mean ± SD of three replicates. Different superscripts within the same column are significantly difference ($P \leq 0.05$).

Discussion

Polyherbal remedies are extensively used for the treatment and management of various diseases in the developing countries. According to Bhope et al. [17] these remedies are mixtures of two or more medicinal herbs, which contain multiple active constituents and act synergistically against infections. These results have clearly indicated that the nine polyherbal remedies are rich sources of macro and micro nutrients. Calcium which was the highest mineral detected in the herbal preparations is an important mineral required in the body for the normal development and maintenance of the skeleton. It helps in the proper function-

ing of neuromuscular, blood clotting, nerve transmission, oocyte activation and cardiac function [18]. The mineral is stored in the teeth and bones where it provides structure and strength to the body [18] [19]. Inadequate intake of this mineral could lead to rickets in children or osteoporosis in aging adults, particularly among women [20]. The potassium content present in the herbal remedies was between 160.00 and 2,050.00 mg/100g. The level of this mineral was markedly higher in EL remedy when compared with other polyherbal formulations. The nutrient is the major cation of intracellular fluid in the body, playing an important role in the synthesis of proteins and normal cell functioning such as digestion and neurotransmission [19]. It helps to maintain the pH inside every cell and acts as an electrolyte, a molecule that transmits electrical activity between cells. It also helps in maintaining proper nerve function and muscle contraction [21]. Thus, the presence of this mineral in the remedies could aid the normal cell functioning of the consumers considering their immunocompromised status. In addition, the results revealed that FB and KWTc remedies are rich in nitrogen. This element is required for the growth and repair of worn-out tissues [19]. Also, the polyherbal formulations are good sources of magnesium, the nutrient that acts as a co-factor to more than 300 enzyme systems which regulate diverse biochemical reactions in the body [22] [23] [24]. It is involved in many physiologic pathways such as energy production, oxidative phosphorylation and glycolysis. Magnesium is required for the synthesis of nucleic acid and antioxidant glutathione, as well as contribut-

ing to the structural development of the bone. This element also plays a role in the active transport of calcium and potassium ions across cell membranes [24]. Sodium, which is a cation required to maintain extracellular fluid volume and serum osmolality in the body was also detected in the remedies. The nutrient plays an essential role in keeping the fluids and electrolytes in the body balance, transmit nerve impulses, helps in maintaining the membrane potential of cells and active transport of molecules across cell membranes [25]. The sodium contents in these herbal formulations were between 6.70 and 381.00 mg/100 g. The low sodium content observed in these therapies is of great advantage because, excessive intake of sodium in human results in arterial hypertension. This mineral is required in small amount; too much intake of it has been associated with increased risk of high blood pressure leading to heart disease, stroke and kidney disease [19]. The lowest macro nutrient in the majority of the remedies was phosphorus. The main importance of this mineral is in the formation of bones and teeth. Phosphorus, which is present in smaller amounts in cells and tissues helps to filter out waste in the kidneys. It plays an essential role in the storage and utilization of energy in the body. The element is required for the growth, maintenance and repair of all tissues and cells [22]. Also, it helps in the production of genetic building blocks, DNA and RNA in the body. However, since calcium and phosphorus are associated with each other for growth and maintenance of bones, teeth and muscles; the presence of calcium in the poly herbal formulations can augment for the low phosphorus observed. Also, these poly herbal remedies are good sources of iron, which is an essential component of a number of proteins including hemoglobin [26], myoglobin [27], cytochromes and enzymes involved in redox reactions [19]. It also plays an important role in metabolism, development, normal cellular functioning and synthesis of some hormones and connective tissues [28]. The deficiency of this nutrient could increase the susceptibility of an individual to infection such as tuberculosis [29]. Thus, the presence of iron in the poly herbal remedies is of great importance. Copper was the lowest mineral detected in the herbal preparations. The nutrient is one of the co factors for antioxidant enzymes called super oxide dismutase in the body. It is required to manufacture collagen, a major structural protein in the body. It helps with incorporation of iron into red blood cells, prevents anaemia and involved in the generation of energy from carbohydrates. It is also important for nerve function, building strong tissue, bone growth, maintaining blood volume and helps the body in the utilization of sugar [30]. The presences of this mineral in conjunction with other minerals detected could improve the immunity of the consumers. Also, manganese was equally observed in the polyherbal remedies. This nutrient plays an important role in the formation of bone, metabolism of fat and

carbohydrate, cholesterol and amino acids [19, 30]. It helps the body to form connective tissue, blood-clotting factors and sex hormones. Manganese, a component of the antioxidant enzyme which helps to fight free radicals also plays a role in calcium absorption and blood sugar regulation [21]. Zinc is another important micro nutrient playing a significant role in the immune system of an individual and helps to protect the cells against various free radicals. While some of these herbal formulations had low zinc level, three of the remedies namely; EL, AL and HBss were rich in zinc. The importance of this element cannot be ignored as it is involved in various functions such as cellular metabolism, catalytic activity of enzymes, protein synthesis, wound healing, DNA synthesis and cell division. The deficiency of Zn can lead to impairment of immunity and thus increases susceptibility to infections such as TB [31] [32]. Vitamins are important in the body. They help to maintain the physiology of the body and boost the immune system. In this study, vitamin A had the highest concentrations followed by vitamin E. However, vitamin C was not detected in any of the remedies. While Vitamin Supports the vision and bone growth in the body, vitamin E strengthens the immune system and repairs the DNA [33], Vitamin E is an anti-oxidant capable of playing the role of vitamin C in cavity healing in active cavitory tuberculosis [1]. The supplementation of vitamin E and selenium reduces oxidative stress and enhances total antioxidant status in patients with pulmonary TB treated with standard chemotherapy [34]. This vitamin also helps in preventing the per oxidation of membrane phospholipids and cell membrane oxidation through its antioxidant properties [16]. These vitamins and mineral constituents play important roles in the treatment of tuberculosis and also serve as co factors for many physiologic and metabolic functions (Safarian et al., 1990). According to SANDH [35], patients of TB requires multivitamin and mineral supplement which provides about 50 to 150% of the recommended daily allowance. In conclusion, the findings of this study indicated that this poly herbal medicines are rich sources of essential nutrients and vitamins. Considering the amount of mineral constituents and vitamins in the poly herbal preparation, it could be deduced that these remedies are probably be boosting the immune system of TB patients in the study area against the disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' contributions

This project was designed by AJ Afolayan and AM Clarke. EB Famewo performed the experiments and involved in the writing

of the manuscript. AJ Afolayan and AM Clarke gave conceptual ideas and were fully involved in the reading of the manuscript. All authors read, agreed and approved the manuscript.

Acknowledgments

The authors acknowledge National Research Foundation for funding this project.

References

- [1] Chakraborty S, Syal K, Bhattacharyya R, Banerjee D. Vitamin deficiency and tuberculosis: need for urgent clinical trial for management of tuberculosis. *J Nutr Health Food Sci*;2014(2):1–6.
- [2] World Health Organization, “Global tuberculosis report,”. Accessed. 2016 7;27. Available from: <http://apps.who.int/iris/bitstream/10665/250441/1/9789241565394-eng.pdf>.
- [3] Baldwin PR, Reeves AZ, Powell KR, Napier RJ, Swimm AI, Sun A, Giesler K, Bommarius B, Shinnick TM, Snyder JP, Liotta DC. Monocarbonyl analogs of curcumin inhibit growth of antibiotic sensitive and resistant strains of *Mycobacterium tuberculosis*. *Euro J med Chem*;2015:92–693.
- [4] TBFACTS.ORG |Information about Tuberculosis. <http://www.tbfacts.org/tb-statistics> South-Africa/2015. Accessed. 2015 5;23.
- [5] World Health Organization, “Guideline: Nutritional care and support for patients with tuberculosis.”. World Health Organization. 2013;10665(94836/1).
- [6] Gupta KB, Gupta R, Atreja A, Verma M, Vishvkarma S. Tuberculosis and nutrition. *Lung India*;2009(26):9.
- [7] Ciccone MM, Cortese F, Gesualdo M, Carbonara S, Zito A, Ricci G, De Pascalis F, Scicchitano P, Riccioni G. Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. *Mediators of inflammation*; 2013.
- [8] Wahlqvist ML. Antioxidant relevance to human health. *Asia Pac J Clin Nutr*;2013(22):171–176.
- [9] Safarian MD, Karagezian KG, Karapetian ET, Avanesian NA. The efficacy of antioxidant therapy in patients with tuberculosis of the lungs and the correction of lipid peroxidation processes. *Problemy*; 1989.
- [10] Peltzer K. Utilization and practice of traditional/complementary/alternative medicine (TM/CAM) in South Africa. *Afr J Trad Complement Alter Med*;2009(6):175.
- [11] Soni HK, Ribadiya NC, Bhatt SB, Sheth NR. Evaluation of herbal formulation (capsule) containing Ashwagandha as a single herb with their nutritional value determination. *Inter J Appl Biol Pharm Technol*;2010(1):960–967.
- [12] Dickson RA, Amponsah IK, Annan K, Fleischer TC. Nutritive potential of a polyherbal preparation from some selected Ghanaian Herbs; 2014.
- [13] Famewo EB, Clarke AM, Afolayan AJ. Identification of bacterial contaminants in polyherbal medicines used for the treatment of tuberculosis in Amatole District of the Eastern Cape Province, South Africa, using rapid 16S rRNA technique. *J Health Popul Nutr*;2016(35):27.
- [14] Bvenura C, Afolayan AJ. Heavy metal contamination of vegetables cultivated in home gardens in the Eastern Cape. *South African Journal of Science*;2012(108):10–1.
- [15] Onyesife CO, Ogugua VN, Anaduaka EG. Investigation of some important phytochemicals, vitamins and mineral constituents of ethanol leaves extract of *Piper nigrum*. vol. 5;.
- [16] Njoku NE, Ubbaonu CN, Alagbaoso SO, Eluchie CN, Umelo MC. Amino acid profile and oxidizable vitamin content of *Synsepalum dulcificum* berry (miracle fruit) pulp. *Food Sci Nutr*;2015(3):252–256.
- [17] Bhope SG, Nagore DH, Kuber VV, Gupta PK, Patil MJ. Design and development of a stable polyherbal formulation based on the results of compatibility studies. *Pharmacog Res*;2011(3):122.
- [18] Pravina P, Sayaji D, Avinash M. Calcium and its role in human body. *Inter J Res Pharm Biomed Sci*;2013(4):659–68.
- [19] Reference Values for Australia and New Zealand including Recommended Dietary Intakes. Department of Health and Ageing, Australian Government, Australia;2005:35.
- [20] IOM Institute of Medicine. Food and Nutrition Board. Committee to Review Intakes for vitamin D and Calcium. Dietary Reference Intakes for Calcium and Vitamin. D. Washington, D.C: National Academy Press;2010.
- [21] Young DB. Role of potassium in preventive cardiovascular medicine. Springer Science & Business Media; 2012.
- [22] IOM Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes for Calcium Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington, DC: National Academy Press; 1997.
- [23] Magnesium RRR. In: Coates PM, Betz JM, Blackman MR, Cragg GM, Levine M, Moss J, White JD. *Encyclopedia of Dietary Supplements*. New York, NY: Informa Healthcare; 2010.
- [24] Magnesium RRR. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR. *Modern Nutrition in Health and Diseases*. Baltimore, Mass: Lippincott Williams; 2012.
- [25] Capra S. Nutrient reference values for Australia and New Zealand: Including recommended dietary intakes. *Common wealth of Australia*. 2006;.

- [26] Iron WRM. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR. *Modern Nutrition in Health and Diseases*. Baltimore, MD: Lippincott Williams; 2014.
- [27] Iron APJ. In: Jr Erdman JW, MacDonald IA, Zeisel SH, "Present knowledge in nutrition,". Washington, DC: Wiley-Blackwell; 2012.
- [28] Murray-Kolbe LE, Iron BJ. In: Coates PM, Betz JM, Blackman MR et al. *Encyclopedia of Dietary Supplements*. New York: Informa Healthcare; 2010.
- [29] Karyadi E, Schultink W, Nelwan RH, Gross R, Amin Z, Dolmans WM, van der Meer JW, Hautvast JG, West CE. Poor micronutrient status of active pulmonary tuberculosis patients in Indonesia. *The J Nutr*;2000(130):2953–2958.
- [30] IOM Institute of Medicine. Food and Nutrition Board. *Dietary reference intakes for vitamin A, vitamin K, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc*. Washington, D. C: National Academy Press; 2001.
- [31] Ramakrishnan K, Shenbagarathai R. Kavitha K, Uma A, Balasubramaniam R, Thirumalaikolundusubramanian P. Serum zinc and albumin levels in pulmonary tuberculosis patients with and without HIV. *Japanese J Infect Dis*;2008(61):202.
- [32] Muthuraj M. Kamatchiyammal S, Usharani B, Manupriya S, Ayyappan AN, Divyalakshmi K. Serum zinc, calcium and albumin levels in pulmonary tuberculosis patients co-Infected with HIV. *Global J Biotech Biochem*;2010(5):27–35.
- [33] Martini LA, Catania AS. Ferreira SR. Role of vitamins and minerals in prevention and management of type 2 diabetes mellitus. *Nutr Rev*;2010(68):341–54.
- [34] Seyedrezazadeh E, Ostadrahimi A, Mahboob S, As-sadi Y, Ghaemmagami J, Pourmogaddam M. Effect of vitamin E and selenium supplementation on oxidative stress status in pulmonary tuberculosis patients. *Respirology*;2008(13):294–298.
- [35] South African National Guidelines on Nutrition for People Living with HIV, Aids, TB and other Chronic Debilitating Conditions; 2001.