COMPARATIVE ANALYSIS OF MINERAL CONTENT OF AQUEOUS AND ETHANOL EXTRACT OF LEAVES OF PHYLLANTUS NIRURI

Supported by tetfund

Obiwulu E.N. O, Ezeokafor D.C., Okoh F and Adu M.E.
Department of Chemical Sciences (Biochemistry), Faculty of Science, University of Delta, Agbor.
Corresponding author: esther.obiwulu@unidel.edu.ng

Received: May 15, 2025, Accepted: July 28, 2025

Abstract

Phyllanthus niruri, a member of the Phyllantaceae family, is a small sized herb with a wide range of medicinal properties. It is utilized globally for managing and treating various conditions. In Nigeria, it is incorporated into the ayurvedic system of medicine for addressing diabetes, urinary tract issues, asthma, cough, and fever. The objective of this research is to evaluate the mineral composition of Phyllanthus niruri plant extracts in both aqueous and ethanol forms. The extracts were obtained through the use of ethanol and water, and their mineral content was analyzed using established procedures. The analysis of Phyllanthus niruri leaf extracts in aqueous and ethanol form revealed the presence of Zn, Fe, Na, Mn, K, Mg, Ca, and Cu in a natural blend. Nevertheless, the aqueous extract exhibited a greater concentration compared to the ethanol extract. Potassium levels were notably higher in the aqueous extracts at 542.371 ppm, in contrast to the ethanol extract at 6.036 ppm. On the other hand, Iron, Calcium, and Sodium levels were lower in the aqueous extract but higher in the ethanol extract. This suggests that the aqueous solution had a higher efficiency in extracting these minerals. The findings indicate the potential of P. niruri leaf extract and provide evidence to support its usefulness in the fields of medicine and nutrition.

Keywords: Ayurvedic, Aqueous extract, Ethanol extract, Herb, Mineral contents, Phyllanthus niruri,

Introduction

Herbal medicine, also referred to as Herbalism or Botanical medicine, is a field of study within medicine that focuses on the examination and utilization of plants or plant components for therapeutic purposes. Though the field dates to prehistoric times, it remains important and relevant in today's medical research, and about a quarter of modern medicines are sourced from plants (Aisha et al, 2022). According to World Health Organization (2014) report, herbal or traditional medicine is fundamental to meeting the primary health care needs of the population in many developing countries. A sizeable number of people in even in developed countries now rely on use of alternative medicine to manage diseases and ailments. This can be attributed to several factors which includes; high cost of conventional drugs, problems associated with side effects of some drugs and inability of existing conventional drugs succumbing to resistance (Srivastava et. al.,1996).

Medicinal plants are widely distributed throughout the world and have been used by almost all cultures (Barnes et. al., 2007).While there have been numerous studies identifying plant species that show promise in combating microorganisms, a significant number of plants have yet to be thoroughly investigated in this regard. Phyllanthus niruri Linn is one of the herbs widely used in Ayurvedic procedures. The plant group known as Phyllanthus, belonging to the Phyllanthaceae family, comprises 1,301 species and is widely distributed across tropical and subtropical regions of Asia, Africa, America, and Australia (GBIF 2021). P. niruri Linn is commonly known as 'Gale of the wind' (stone breaker) and 'Seed under a leaf' according to its morphological and pharmacological actions (Lee et al., 2016). It is called 'Obukeyeke' in the Urhobo speaking parts of Southern Nigeria.

Phyllanthus niruri contains diverse bioactive constituents such as alkaloids, anthocyanins, chlorogenic acids, coumarins, flavonoids, lignans, phenolic acids, saponins and glycosidic substitutes, tannins and terpenoids (Bagalkotkar et al., 2006). Phyllanthus niruri L has demonstrated its potential as an immunomodulator in countering Covid-19 (Marhaeny et al. 2021).P. niruri was also found to have anti- oxidant and heptoprotective properties and antiinflammatory potential (Kiemer et al, 2003). Jantan and colleagues (2019) reported that P.niruri Linn possess antiviral activity against hepatitis B virus, hepatoprotective activities, litholisis action of kidney and gall bladder stones, inhibitory action on HIV, anti-inflammatory, anti-diabetic, lipid lowering, analgesic and immunomodulatory activities.

Minerals are inorganic elements or substances that do not come from animals or plants (i.e., natural compounds that typically do not contain carbon, hydrogen, oxygen, or nitrogen). Inorganic elements constitute the major part of dry ash that remains after ignition of organic matter, and consequently dry-ashing techniques are still the main stay way to determine total minerals in foodstuffs (Nielsen, 2010). Minerals are classified based on their essentiality, nutritional value or toxicity. Minerals are reported in mg/kg, trace elements in µg/kg andtoxic elements in maximum limits (MLs) (O'Dell and Sunde, 1997). Minerals are nutritional elements use as cellular and structural building materials taking part in osmotic/oncotic and acid/ base regulation as exemplified by, calcium (Ca), potassium (K), sodium (Na), phosphorus (P), magnesium (Mg), and chlorine (Cl) (Adebisi et al, 2021). Having explored the importance of herbal medicine in the health care system and the benefits of minerals to the body, and the paucity of data on this subject, this research intends to evaluate the mineral composition of aqueous and ethanol extracts derived from P. Niruri leaves in Agbor.

Materials and Method

Plant Collection, Authentication

The plant Phyllantus niruri was gathered from its native environment near the University of Delta in Agbor, Delta State, Nigeria. The plant was then verified and confirmed by an Angiosperm Taxonomist at the Botany Department of Delta State University in Abraka, Delta state, Nigeria. A voucher number of DELSUH: 181 was assigned to the plant specimen, which is displayed in Figure 1.

Fig. 1. Phyllantus Niruri Plant

Sample Preparation

Fresh leaves from P. niruri plants were harvested and weighed (6.25g) before being washed in cold tap water to eliminate any sand or debris. The leaves were then crushed using a mortar and pestle for homogenization. Following this, the homogenized mixture was extracted using various aqueous and ethanol solvents (100ml each). The resulting extract was subsequently preserved in a sealed plastic container.

Procedure

1 gram of the specimen was measured and placed into a 50ml beaker. Following this, 2.5ml of hydrochloric acid and 7.5ml of nitric acid aqua regia solution (in a 1:3 ratio) were added to the beaker. The mixture in the beaker was then placed on a hotplate set at a temperature between 100-170 degrees Celsius until it was nearly dried or precipitation was visible. The beaker was then taken off the heat and allowed to cool, after which a few drops of distilled water were added. The solution was combined and poured into a funnel set with filter paper. The resulting filtrate was quantified and diluted to a volume of 50 milliliters. Subsequently, the test sample was moved to a sample bottle for analysis using MP-AES. (Uddin and colleagues, 2016, as cited in Balaram, 2020).

Results and Discussion

The results of the study are shown in the graphs below. There was a significant variation in mineral concentrations between the two extraction methods. The aqueous extract exhibited higher levels of potassium (543.37 ppm), iron (91.812ppm), calcium (196.48ppm), and sodium (109.00ppm). The minerals present in the aqueous extract, followed this sequence: Potassium > calcium > sodium > Iron > magnesium > manganese > zinc > copper. In the ethanol extract, the minerals followed same pattern as above but at a lesser concentration.

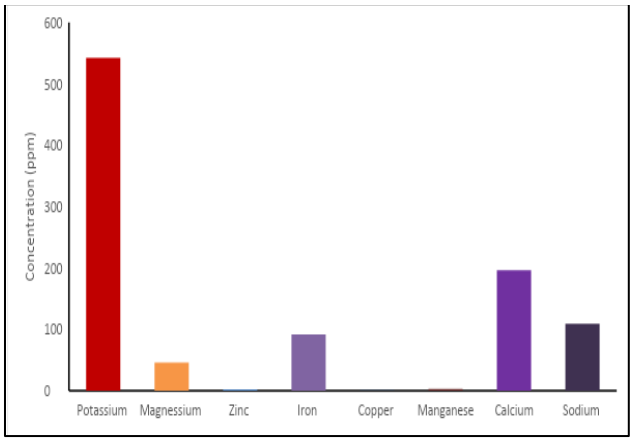

Fig 2: Showing concentration Aqueous extract of minerals in ppm

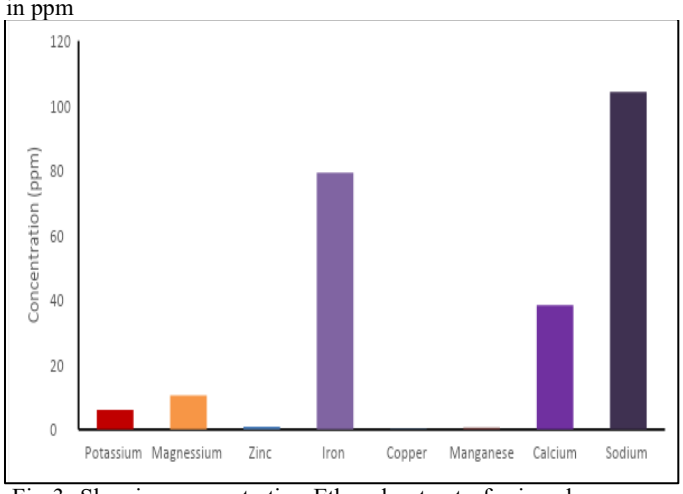

Fig 3: Showing concentration Ethanol extract of minerals in ppm Below are the calibration curve as obtained from the MP-AES

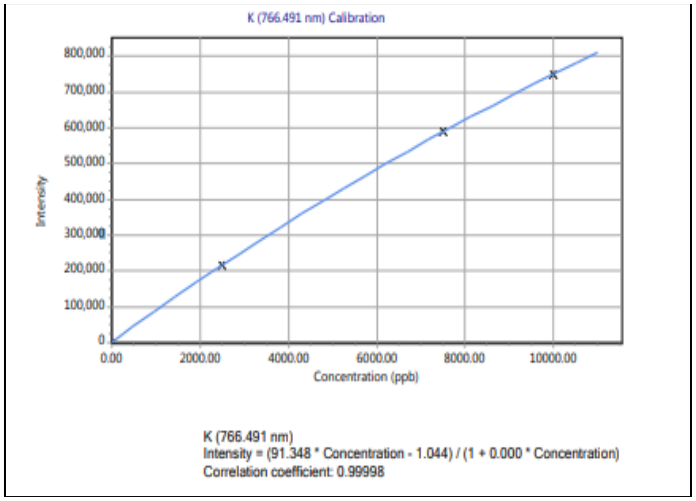

Figure 4; Calibration curve for Potassium obtained from the MP-AES

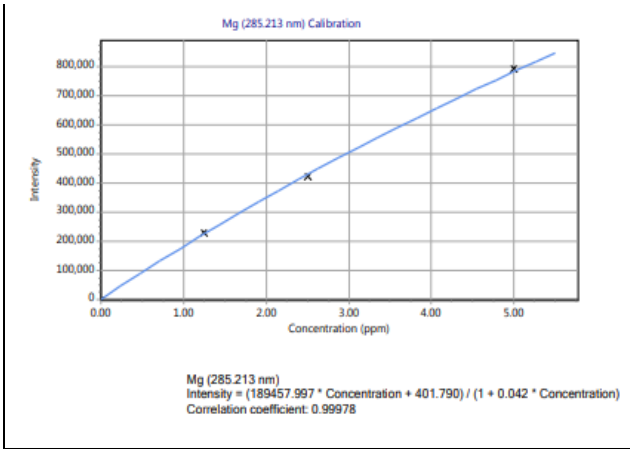

Figure 5: Calibration curve for Magnesium obtained from the MP-AES

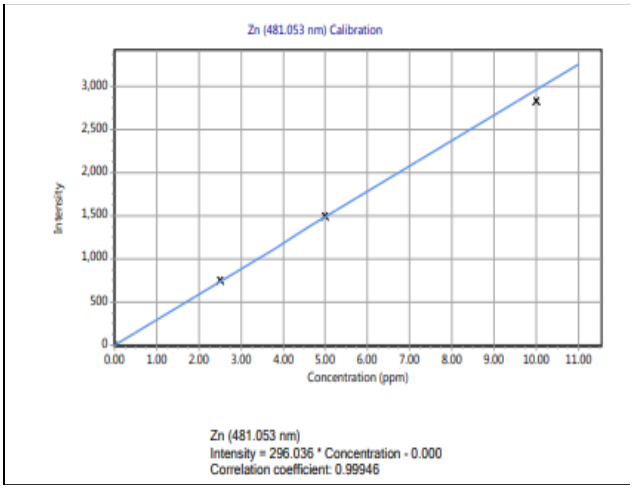

Figure 6: Calibration curve for Zinc obtained from the MP-AES

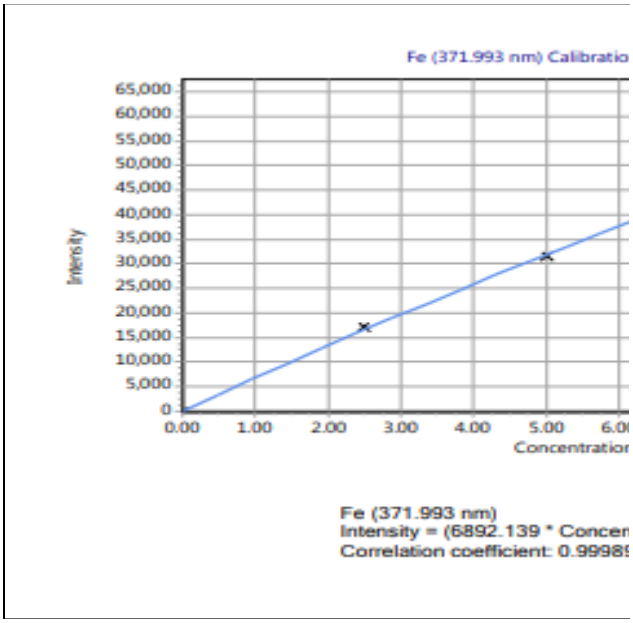

Figure 7: Calibration curve for Iron (Fe) obtained from the MP-AES

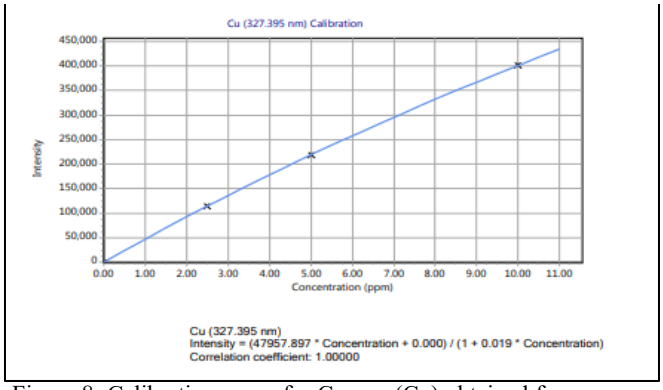

Figure 8: Calibration curve for Copper (Cu) obtained from the MP-AES

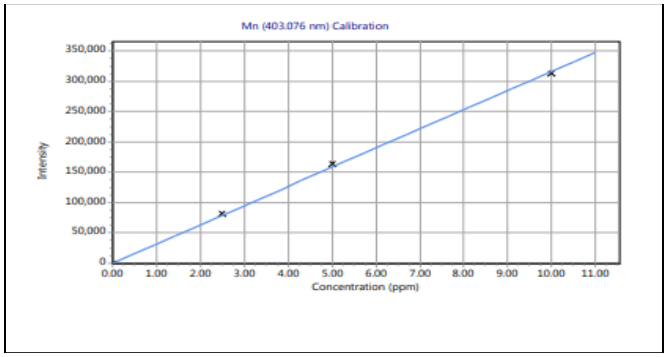

Figure 9: Calibration curve for Manganese (Mn) obtained from the MP-AES

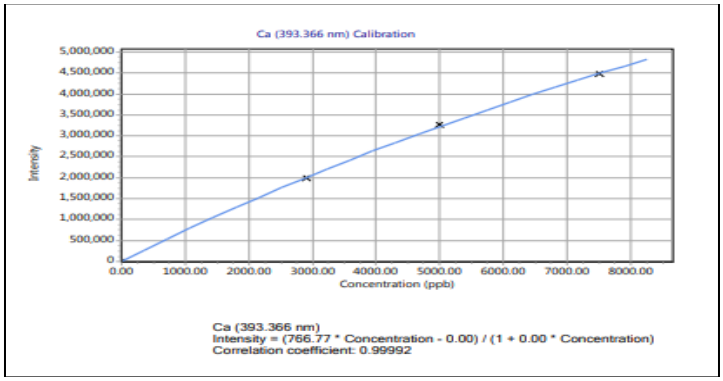

Figure 10: Calibration curve for Calcium (Ca) obtained from the MP-AES

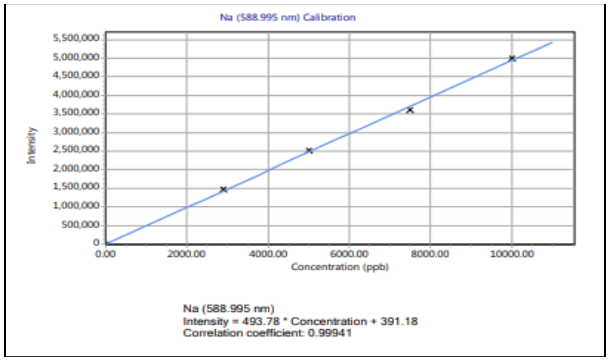

Figure 11: Calibration curve for Sodium (Na) obtained from the MP-AES

Discussion

The results of the study show significant variations in mineral concentrations between the two extraction methods. The aqueous extract exhibited higher levels of potassium (543.37 ppm), iron (91.812ppm), calcium (196.48ppm), and sodium (109.00ppm) compared to the ethanol extract, indicating better extraction efficiency of these minerals in the aqueous solution. This is in accordance with earlier report by Buba et al (2023) and observed that minerals have different solubility pattern who accentuate the varying mineral solubility in different extraction methodologies. The aqueous extract exhibited higher levels for the mineral extracts compared to the ethanol extract, indicating a potential difference in the mineral solubility and extraction efficiency of the two solvents. These findings suggest that the choice of extraction solvent can influence the mineral composition of the plant extract, which may impact its potential therapeutic properties and bioavailability (Ahamefula et al, 2024). The differences in mineral content between the aqueous and ethanol extracts may be attributed to the varying solubility of minerals in different solvents. Aqueous extracts tend to contain higher levels of minerals, as water is a more polar solvent, allowing for better solubility of ionic compounds. Ethanol, being less polar, may extract more non-polar compounds or minerals bound to organic molecules. This indicates that these minerals are also more soluble in water (Owabhel and Eboh, 2022).

The high concentration of potassium, magnesium and calcium is in tandem with earlier study by Olufayo et al (2021), which studied the nutritive value of Phyllantus Niruri Linn. But the high concentration of Iron (Fe) in this study, is in contrast with the same study by Olufayo et al (2021). In general, these results bolster the mineral abundance of Phyllanthus niruri, emphasizing its plausible nutritional and therapeutic significance.

The traditional use of the plant to prevent oedema, kidney problems and oliguria as observed by Oyewo et al, (2012) may be due to the high concentrations of potassium, magnesium and sodium in the leaf which may serve the purpose of the maintenance of osmotic pressure, water balance and pH in the body. Presence of minerals such as zinc, iron and magnesium in the leaf extract may possibly contribute to immunomodulatory action, since these substances have been implicated in immune modulation (Ravalglia et al., 2000). These mineral elements also functions as cofactors for enzymes and ultimately modulates the immune system (De la Fuente et al., 2005), thus they enhance the activities of antioxidant enzymes (Prasaid, 2000). This property could be attributed to its neuroprotective function.

Conclusion

In conclusion, this research has demonstrated the significant presence of minerals in both the aqueous and ethanol extracts of P. nuriri leaves. Nonetheless, the aqueous extract of P. leaves contains a higher amount of minerals compared to the ethanolic extract. These results are significant for the application of plant extracts in diverse fields like nutrition, medicine, and industrial processes. Knowledge of the mineral content and extraction effectiveness can enhance the extraction procedure and maximize the plant's full potential.

Acknowledgement

We are grateful to Mr. Abdulhamid Zubairu Abdullahi from the MultiUser Science Research Laboratory at Ahmadu Bello University for his invaluable contribution that has brought this project to fruition.

Conflict Of Interest

The authors declare that there is no conflict of interest with regards to this publication.

References

Adebisi JA, Okunloye NA, Togun VA & Okwusidi JI (2021). Phytochemical screening, proximate and mineral composition analyses of phyllantus niruri Leaves. *International Journal of Public Health, Pharmacy and Pharmacology.* 6(2): 1-10.

Ahamefula A, Ahuchaogu A, Nwaokoro C, Nwaogwugwu CJ, Bello MO, & Umoh SD (2024). Phytochemical investigation of methanolic leaf extract of phyllanthus niruri using fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. *Scientific African,* doi: 10.4314/sa.v23i1.7

Aisha U & Hauwa'u, BA (2022). Evaluation of the phytochemical, proximate and mineral contents of phyllanthus niruri (L). found in Sokoto State. International Journal of Science for Global Sustainability. 8: 2 July: https://fugus-ijsgs.com.ng

AOAC. (2005). Methods of Analysis of Association of Official Analytical Chemistry 16th edn. Washington D.C, USA, pp. 600-792.

Bagalkotkar G, Sagineedu SR, Saad MS, & Stanslas J (2006). Phytochemicals from phyllanthus niruri Linn. and their pharmacological properties: a review. *J Pharm Pharmacol* 58: 1559–1570.

Balaram, V (2020). Microwave Plasma Atomic Emission Spectrometry (MP-AES) and its Applications – A Critical Review. *Microchemical Journal,* 159:105483

Barnes J, Anderson LA & Phillipson JD (2007). Herbal Medicines, 3rd edn. London: Pharmaceutical Press. pp.349-350.

Buba F, Olaide, HA, Abdulrahman AA, Milala MA (2023). Evaluation of aqueous leaf extract of phyllantus niruri in vitro. *Iraqi Journal of Science, doi:* 10.24996/ijs.2023.64.1.12.

De la Fuente M, Hernanz A & Vallejo MC (2005). The immune system in the oxidative stress conditions of aging and hypertension: Favourable Effects of Antioxidants and Physical Exercise. *Antioxidant Redox Signal,* 7: 1356-1366.

GBIF Backbone Taxonomy (2021). Phyllanthus L. in GBIF Secretariat. Checklist dataset. https://doi.org/10.15468/39omei [accessed via GBIF.org on 2022-05-08]

Jantan I, Haque A, Ilangkovan M, & Arshad L (2019). An insight into the modulatory effects and mechanisms of actions of phyllanthus species and their bioactive metabolites on the immune system. *Front. Pharmacol.* 10:878.

Kiemer AK, Hartung T, Huber & Vollmar AM (2003). Phyllanthus amarus has anti-inflammatory potential by inhibition of iNOS, ox- 2 and cytokine via the NF-KB pathway. *J. Hepatol*. 38: 289-297.

Lee NYS, Khooa WKS, Adnana MA, Mahalingam TP, Fernandez AR & Jeevaratnam K (2016). The pharmacological potential of Phyllanthus Niruri. *J. Pharm. Pharmacol.* 68:953-969.

Marhaeny HD, Widyawaruyanti A, Widiandani T, Hafid AF & Wahyuni TS (2021). Phyllanthin and Hypophyllanthin, the isolated compounds of phyllanthus niruri inhibit protein receptor of corona virus (COVID-19) through in silico approach. *J Basic ClinPhysiol Pharmacol*. 32: 809–815.

Nielsen S. (2010). In food analysis, S. Nielsen edn Springer, New York, NY. doi:10.1007/978-1-4419-1478-1
O'Dell BL,& Sunde RA (1997). Handbook of Nutritionally Essential Mineral Elements, Marcel Dekker Inc., New York, NY.

Olufayo OO, Tayo GO, Olumide MD & Akintunde AO (2021). Assessment of the nutritive value of phyllanthus niruri linn. (stonebreaker) leaves. *Nigerian J. Anim. Sci.,* 23 (3): 108-115.

Owabhel FR & Eboh AS (2022). Antioxidant, chelating and HPLC quantification of phenols in extract of phyllanthus niruri. World Journal of Advanced Pharmaceutical and Life Sciences, doi: 10.53346/wjapls.2022.3.1.0029

Oyewo EB, Akanji MA & Adekunle AS (2012). Immunomodulation capabilities of aqueous leaf extract of phyllanthus amarus in male Wistar Rats. *Report and Opinion,* 4:1.

Prasaid AS (2000). Effects of zinc deficiency on Th1 and Th2 cytokinesshift. *Journal of Infectious Diseases,* 182: 62-68

Ravalglia, G., Forti, P. and Maioli, F. (2000). Effects of micronutrient status on natural killer cell immune function in healthy free-living Subjects age>90years. *American Journal of Clinical Oncology* 3(2): 871.

Srivastava, J.J., Lambert, J. & Vietmeyer, N. (1996). Medicinal Plants: An Expanding Role in the Development of Medicine: World Bank technical paper. No. 320. The Phytoalexin 6-Metaxy Mulleins from Culture Carrot Cells. *Journal of Phytochemistry,* 22 (3), 666-672.

Uddin, A. B. H., Khalid, R.S., Alaama, M.A., Abdualkaer, A.M., Kasmuri, A.R. and Abbas, S. (2016). Comparative Study of Three Digestion Methods for Elemental Analysis in Traditional Medicine Products Using Atomic Absorption Spectrometry. *J Anal Sci Technol* 7, 6. https://doi.org/10.1186/s40543-016-0085-6

World Health Organization (2014). WHO Traditional Medicine Strategy. DOI: http://apps.who.int/iris/bitstream/10665/92455/1/9789241506090_eng.pdf?. Retrieved on 12/06/2017