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Evaluation of the Influence of Geographical Location on Phytochemical Composition of *Moringa oleifera* Seeds

[1]C.K. Ukwueze, [1]O.I. Okogwu, [2]E.C. Ebem, [1]G.N. Nwonumara and [1]J.N. Nwodo

[1]Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria
[2]Department of Biotechnology, Ebonyi State University, Abakaliki, Nigeria

Abstract: Plants contain phytochemicals that are non-nutritive but have anti-oxidant activity, which protects the body cells from oxidative damage. An example is the seeds of *Moringa oleifera* which contain phytochemicals of great medicinal importance. Seed samples of *Moringa oleifera* were collected from three senatorial zones of Ebonyi State (Ebonyi North, Ebonyi Central and Ebonyi South Senatorial Zones) and from each senatorial zone, three local governments were chosen to serve as replicates. The seeds were sun-dried and pulverized to obtain 5.0 g powder which was defatted using 100 mL of petroleum ether for 24 hours. The extracts were quantitatively and qualitatively analyzed for flavonoid, alkaloid, saponin, tannin and glycoside. The soil analysis was also carried out on soil samples collected from each study site. Significant level was tested at $p < 0.05$ in which no significant difference was observed on seed's phytochemical parameters analysed across the zones but on soil quality tested, there were significant difference on % clay, % silt and % coarse sand. The result showed that the phytochemical constitution of *Moringa oleifera* seeds from the senatorial zones varied in presence and concentration across the senatorial zones. Ebonyi South Senatorial Zone had best soil features and seeds from the Zone contained the phytochemicals studied at averagely high concentrations, which suggests that enhanced soil quality enhances availability of phytochemicals and their concentrations.

Key words: Phytochemicals · *Moringa oleifera* · Environment · Soil

INTRODUCTION

Phytochemicals are literally chemicals produced by plants [1]. They could further be described as biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans other than those attributed to macronutrients and micronutrients [2]. Most of these phytochemicals are known to confer specific characteristics and properties to plants by protecting them from disease, damage, pollution, stress, drought, ultra-violet exposure, pathogenic attack and they also contribute to the plant's color, aroma and flavour. These chemicals have different relevance ranging from medicinal to industrial applications. Phytochemicals accumulate in different parts of the plants, such as roots, stems, leaves, flowers, fruits and seeds [3] and their levels vary from plant to plant depending upon the plant's variety, processing method and growing conditions [4]. *Moringa oleifera* contain many phytochemicals which include phenolics, saponins, steroids, alkaloids, flavonols, pro-anthocyanidins, terpenoids, tannin and cardiac glycosides among others. The plant is considered one of the world's most useful trees, as almost every part of the *Moringa* tree can be used for food and has some other beneficial purposes [5]. Studies have been done on *Moringa* seed which includes pharmacological properties of *Moringa oleifera* seed [6], oxidative rancification resistance of *Moringa oleifera* seed oil [7], physicochemical properties of *Moringa oleifera* seeds and their edible oil cultivated at different regions in Egypt [8], but there is none on the phytochemical constitution of the seeds from different geographical regions in Ebonyi State. Since phenotype results from the expression of an organism's genes, as well as the influence of environmental factors and the interactions between gene and environment [9], not all organisms with the same genotype gives similar phenotypic expression when exposed to dissimilar environments.

Corresponding Author: C.K. Ukwueze, Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria.

According to [10], agro-climatic conditions and environment can influence the chemical composition and therapeutic properties of medicinal plant species. The need to confirm the bio-constituents of plants across varied agro-ecologies is necessary in the selection and formulation of plant-based food supplement and development of food-based medicinal compounds. Thus, there is need to study the phytochemical content of *M. oleifera* seeds in different zones of Ebonyi State as there could be evidence differential gene expression. This research is aimed at identifying the presence and ranges of phytochemicals found in *M. oleifera* in different senatorial zones in Ebonyi State.

MATERIALS AND METHODS

Sample Collection: *Moringa oleifra* seeds were collected from three senatorial zones of Ebonyi State using three local government areas (L.G.A.) within each senatorial zone as replicates. The locations were Ebonyi North Senatorial Zone (Ebonyi, Abakaliki and Izzi Local Government Areas), Ebonyi Central Senatorial Zone (Ikwo, Ishielu and Ezza North Local Government Areas) and Ebonyi South Senatorial Zone (Afikpo North, Ohaozara and Ivo Local Government Areas). Soil samples were also collected from each location and labeled appropriately. The collection was done between April and July, 2015. Phytochemical analysis of the seeds and soil analysis were conducted at the Department of Pharmaceutical and Medicinal Chemistry Laboratory and the Department of Soil Science respectively, in University of Nigeria Nsukka, respectively.

Preparation of Plant Extracts: The air dried *Moringa oleifera* seeds were pulverized and the powdered materials (5.0 g) were each de-fatted with 100 mL of petroleum ether for 24 hours. The mixtures were filtered and then marc dried for 2 hours. The dried marc were macerated with 100 mL of methanol and extracted at room temperature for 24 hours with agitation. The filtrates were concentrated *in vacuo* at reduced pressure and temperature (40°C) to obtain the extracts. The percentage yields of the extracts were determined. The dried extracts were used for the qualitative phytochemical analysis of tannins, flavonoids, saponins, alkaloids and cardiac glycosides according to standard methods [11, 12]. For quantitative analysis of the seed samples for the phytochemicals, Folin-Ciocalteau method was used for the determination of the total tannins content of the seed extracts using gallic acid as an internal standard with slight modification as previously reported [13]. Aluminium-chloride colourimetric assay was used to determine the total flavonoids content in the seed extract as previously reported [14]. The total saponins content of the extracts were determined based on vanillin - sulphuric acid calorimetric reaction using diosgenin as standard as previously reported [15, 16] with slight modification. The total glycosides content of the extracts were determined based on picric acid in alkaline medium calorimetric reaction using digitalis as standard [17].

For the soil samples analysis, determination of particle size distribution was done by the Bonyoncos hydrometer method. For exchangeable bases (Ca^{+}, Mg, Na^{+} and k^{+}), ammonium acetate method was used [18]. Carbon content was determined by wet oxidation method. Total nitrogen was determined by modified Kjeldahl method. Potassium was determined colorimetrically using flame- photometer 100 N NH_4OAC Leachate whereas phosphorus was determined using Bray Li method. The soil pH was determined by dissolving 10.0 g of the soil samples in 10.0 mL distilled water, filtered and the filtrates used for the pH determination. All analysis was done in triplicate.

Data Analysis: Analysis of variance (ANOVA) was used to determine statistical difference between phytochemical contents of seeds from different geographical regions. The significance was assessed at $P < 0.05$ and Least Significance Difference used to separate the means.

RESULT

Flavonoid was not detected in seeds from Ebonyi North Senatorial Zone, Ishielu Local Government Area in Ebonyi Central Senatorial Zone, Afikpo North and Ivo Local Government Areas in Ebonyi South Senatorial Zone (Table 1). Alkaloid was present in seeds from all the senatorial zones with highest occurrence observed in Ivo LGA of Ebonyi Central Senatorial Zone. Saponin's highest occurrence was observed in seeds from Ebonyi North Senatorial Zone. Glycoside was not detected in seeds from Ebonyi Central Senatorial Zone but highly present in Afikpo North LGA of Ebonyi South Senatorial Zone. Tannin was highly present in seeds from all the senatorial zones. Across the senatorial zones, highest occurrence was observed in tannin while glycoside had the least number of occurrences.

No flavonoid contained in seeds from Ebonyi North Senatorial Zone while Ebonyi Central Senatorial Zone has highest flavonoid content (Table 2). The seeds with

Table 1: Qualitative phyto-constituents of *Moringa oleifera* seeds from Ebonyi State Senatorial Zones

Location	LGA	Flavonoid	Alkaloid	Saponin	Glycoside	Tannins
Ebonyi North Senatorial Zone	EBONYI	-	+	-	-	++
	ABAKALIKI	-	-	+	+	-
	IZZI	-	-	+	-	-
Frequency of occurrence		-	1	2	1	1
Ebonyi Central Senatorial Zone	IKWO	+	-	-	-	++
	ISHIELU	-	+	-	-	-
	EZZA-NORTH	+	+	-	-	++
Frequency of Occurrence		2	2	-	-	2
Ebonyi South Senatorial Zone	AFIKPO NORTH	-	-	+	++	-
	OHAOZARA	+	-	-	-	+
	IVO	_	++	-	-	++
Frequency of Occurrence		1	1	1	1	2
Total frequency		3	4	3	2	5

Key: - = absent; + = present; ++ = highly present

Table 2: Quantitative phyto-constituents of *Moringa oleifera* seeds from Ebonyi State Senatorial Zones

Location	Flavonoid (mg QE/g)	Alkaloid (mg A/g)	Saponin (mg DE/g)	Glycoside (mg DE/g)	Tannins (mg GAE/g)
Ebonyi North Senatorial Zone	0.00±0.00	0.06±0.10	0.33±0.29	0.24±0.42	0.14±0.24
Ebonyi Central Senatorial Zone	0.25±0.28	0.08±0.07	0.00±0.00	0.00±0.00	0.42±0.37
Ebonyi South Senatorial Zone	0.12±0.21	0.05±0.09	0.12±0.21	0.33±0.58	0.41±0.37

Result shows mean±SD. Significance determined at $P < 0.05$.

Table 3: Edaphic features of soil samples across senatorial zones of Ebonyi State

Locations	% Clay**	% Silt**	% Fine Sand	% Coarse Sand*	Soil pH	% C	% OM	% N	K (MG/100G)	P(PPM)
Ebonyi North Senatorial Zone	12.00±2^a	21.67±4.16^b	44.33±9.50^a	22.00±13.00^a	6.49±0.10^a	2.45±0.37^a	4.23±0.65^a	0.24±0.04^a	0.14±0.02^a	23.01±8.15^a
Ebonyi Central Senatorial Zone	10.00±0.00^a	12.33±2.31^a	46.67±4.04^a	31.00±2.65^a	6.76±0.24^a	4.44±0.58^a	4.21±1.00^a	0.20±0.06^a	0.15±0.02^a	25.18±18.01^a
Ebonyi South Senatorial Zone	6.00±0.00^b	8.33±3.06^a	31.33±11.24^a	54.33±13.80^b	6.69±0.05^a	1.88±1.38^a	3.24±2.39^a	0.15±0.05^a	0.13±0.05^a	32.33±5.47^a

Key: C = Carbon; OM = Organic matter; N = Nitrogen; K = potassium; P = Phosphorus.

Result shows mean±SD. Significance determined at *$P < 0.05$; **$P < 0.01$

* = Significant; ** = Highly significant

Means with the same superscript on same column are not significantly different

highest and least alkaloid content are found in Ebonyi Central and Ebonyi South Senatorial Zones respectively. Highest glycoside content was seen in seeds from Ebonyi South Senatorial Zone. Ebonyi Central and Ebonyi North Senatorial Zones seeds possessed highest and least tannin respectively. In all the parameters analysed across the entire research locations, no significant difference was observed at $P < 0.05$. High standard deviation was consistently observed across the whole locations under tannin unlike some parameters including flavonoid, saponin and glycoside which have no standard deviation in some locations.

We observed significant difference in clay and silt content of the soil between the senatorial zones only, the other soil parameters did not vary significantly ($p < 0.05$) between the senatorial zones (Table 3).

DISCUSSION

In nature, the phenotype may change constantly throughout the life of an organism because of environmental changes and the physiological and morphological changes associated with it. Different environments can influence the development of inherited traits and alter expression by similar genotypes. Therefore, the variation indicated in the soil sample parameters measured as shown in Table 3 would have a significant influence on the variation in presence and ranges of the phytochemicals of *Moringa oleifera* seeds from different senatorial zones. Ebonyi South Senatorial Zone has less clay, less silt, less fine sand and more coarse sand. The quality aids porosity of the soil thereby increasing the movement of soil nutrients into the plants [19], hence, facilitating synthesis of phytochemicals by plant. Phytochemicals are present in virtually all plant tissues of *Moringa oleifera* which include leaves, roots, stem and fruits among others (Siddiqui. Several authors have documented the presence of these phytochemicals in *Moringa* plant parts [20]. According to Table 1, all studied phytochemicals were present in samples from Ebonyi South Senatorial Zone, this could be as a result of the textural porous features of the soil (Table 3) which aided plant nutrient absorption. This concurs with [21], who described that, in soil amendment, their plants increased in chlorophyll content due to more nitrogen absorption as porosity of amended soil increased. Acidity

of the soil could also help liberate soil nutrients ion for plants to absorb. Since soil samples from other senatorial zones like Ebonyi North Senatorial Zone are less porous, this will impose limitation on the degree of spread of soil nutrients and its intake into the plants, hence, impairing plant chemical synthesis. Consequently, being the possible cause for the recorded absence of flavonoid in Ebonyi North Senatorial Zone, saponin and glycoside absence in seeds from Ebonyi Central Senatorial Zone and concentration of phytochemicals in *Moringa oleifera* seed samples collected from those locations. Although, the soil from Ebonyi North Senatorial Zone is least in porosity as seen in Table 3, its averagely high acidity would have liberated the available nutrients for plants to absorb; this could have resulted to the presence and selectively high concentration of phytochemicals observed.

The analysis of variance (ANOVA) result showed that the variation in phytochemical constitution observed across the senatorial zones (Table 2 & 3) is not significantly different ($P > 0.05$), which means that the variation is due to chance. This finding concurs with [22], who stated no significance difference on the phytochemical content of seeds carefully collected from geographical locations of Egypt. According to [23], phytochemical study on *Moringa oleifera* flowers from four different locations in India showed that the samples were qualitatively same and quantitatively different, hence, the need for Table 2 & 3 whereby parameters that were present in different locations qualitatively varied quantitatively. Due to high concentration of flavonoid in Ebonyi Central and Ebonyi South Senatorial Zones as shown in Table 3, the seeds from these zones could be used for possible issues relating to antimicrobial, cytotoxicity, anti-inflammatory, antitumor, antioxidants which can protect the human body from free radicals and reactive oxygen species [24]. Across the zones, since the seeds show averagely similar concentration of alkaloid (Table 2) they could be indiscriminately fed to animals to aid their metabolism [25]. The presence of alkaloids are significant for the protection and survival of the plant's seeds in their various locations because they ensure their survival against micro-organisms (antibacterial and antifungal activities), insects and herbivores (feeding deterrents) and also against other plants by means of allelopathically active chemicals [26]. This feeding deterrent of alkaloid is in many cases by directly interacting with molecular targets within the nervous system [27]. Therefore, seeds from all senatorial zones in the Ebonyi State can be stored across seasons and also transported safely. Although, a number of specialized herbivorous species have adapted to either tolerate or sequester alkaloids from their host plant. Due to their alkaloid content, the seeds from research locations can be used as a stimulant (like caffeine and nicotine), analgesic and anti-malarial drug [28]. The absence of saponin (Table 2 & 3) in seeds from Ebonyi Central Senatorial Zone limits its use as adjuvant in the production of vaccines [29] unlike Ebonyi North and South Senatorial Zones where its concentration is high and low respectively. Seeds from Ebonyi North Senatorial Zone will experience highest survival and viability among the zones because of its high concentration of Saponin. Saponins is considered a part of plants' defence systems and as such have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants [30]. Saponin from the seeds can also be used as chemical reagents (e.g. for soap) and biomedical reagents [31]. The defence trait of saponin could be attributed to its mechanism of action whereby it disrupt cellular membranes [32], the type of aglycone, nature and number of sugar chains attached to it [33]. The high and low concentration of glycoside in seeds from Ebonyi South and North Senatorial Zones respectively suggests it could be used for the treatment of cardiovascular diseases [34]. Seeds from Ebonyi Central and Ebonyi South Senatorial Zones have high concentration of tannin and could be employed medicinally in the treatment of diarrhea and hemorrhage [35]. Also for industrialists, seeds from the zones could be of high importance to them since they can source tannin in high quantity for industrial uses like textile dyes, antioxidants in the fruit juice, beer and wine industries and also coagulants in rubber Production [36].

Conclusively, phytochemical study of *M. oleifera* seed from the three senatorial zones of Ebonyi State showed the presence of different phytochemicals of medical and economic importance. The presence and concentration of the phytochemicals were influenced by edaphic factors, which in part varied between the senatorial zones. Further studies are required to assess possible genetic variations in the *M. oleifera* plants from these regions and existence of ecotypes.

REFERENCES

1 Agbo, M.O., P.F. Uzor, U.N. Akazie-Nneji, C.U. Eze-Odurukwe, U.B. Ogbatue and E.C. Mbaoji, 2015. Total Phenolic and Flavonoid contents in Selected Nigerian Medicinal Plants. Dhaka University Journal Pharmaceutical Science, 14(1): 35-41.

2. Cheng, H.Y., C.C. Lin and T.C. Lin, 2002. Antiherpes simplex virus type 2 activity of casuarinin from the bark of *Termina liaarjuna* Linn. Antiviral Research, 55: 447-455.
2. Churchill, F.B., 1974. William Johannsen and the genotype concept". Journal of the History of Biology, 7: 5-30.
3. Costa, M.A., Z.Q. Zia, L.B. Davin and N.G. Lewis, 1999. Chapter Four: Toward Engineering the Metabolic Pathways of Cancer-Preventing Lignans in Cereal Grains and Other Crops. In Recent Advances in Phytochemistry, vol. 33, Phytochemicals in Human Health Protection, Nutrition and Plant Defense, ed. JT Romeo, New York, pp: 67-87.
4. Elango, R. and U. Jadhav, 2010. Phytochemical screening of *Moringa oleifera* using high performance thin layer chromatography. Plant Arch., 7: 49-51.
5. Francis, G., K. Zohar, P.S. Harinder, Makkar and B. Klaus, 2002. The biological action of saponins in animal systems: a review". British Journal of Nutrition, 88(6): 587-605.
6. Garima, M., S. Pradeep, V. Ramesh, K. Sunil, S. Saurabh, K. Jha and R.L. Khosa, 2011. Traditional uses, phytochemistry and pharmacological properties of Moringa oleifera plant: An overview; Der Pharmacia Letter, 3(2): 141-164.
7. Gyamfi, M.A. and Y. Aniya, 2002. Antioxidant properties of Thonningianin A, isolated from the African medicinal herb, *Thonningia sanguine*. Biochemical Pharmacology, 63: 1725-37.
8. Harborne, J.B. and H. Baxter, 1999. The handbook of natural flavonoids, Chichester, UK: JohnWiley and Sons, pp: 1-2.
9. Hasler, C.M. and J.B. Blumberg, 1999. Symposium on Phytochemicals: Biochemistry and Physiology. Journal of Nutrition, 129: 756S-757S.
10. Hassan, B. and A.G. Galal, 2016. Physicochemical properties of Moringa oleifera seeds and their edible oil cultivated at different regions in Egypt. Food and Nutrition Sciences, 7, 472-484. http:// dx.doi.org/ 10.4236/fns.2016.76049.
11. Heta. 2000. Soil physical properties. SSC107- Fall 2000. pp: 3.
12. Inbathamizh, L. and E. Padmini, 2013. Effect of geographical properties on the phytochemical composition and antioxidant potential of *Moringa oleifera* flowers. Journal of Pharmacy Research: Bio. Med. Rx., 1(3): 239-247.
13. Jacobsen, J., C. Jones and A. McCauley, 2005. Soil and water management: Basic soil properties. Montana State University, 1: 2-12.
14. King, A. and G. Young, XXXX. Characteristics and Occurrence of Phenolic Phytochemicals. Journal of the American Dietetic Association, 24: 213-218.
15. Lacaille-Dubois, M.A. and H. Wagner, 2000. Bioactive saponins from plants: An update. In Studies in Natural Products Chemistry; Atta-Ur-Rahman, ed. Elsevier Science. Amsterdam, 21: 633-687.
16. Laxman Rao, P., G. Jayasree, G. Pratibha and T. Ram Prakash, 2017. Effect of soil amendments on texture, porosity of soil and chlorophyll content in maize. Journal of Pharmacognosy and Phytochemistry, 6(3): 435-437.
17. Makkar, H.P., P. Siddhuraju and K. Becker, 2007. Methods in molecular biology: Plant secondary metabolites. Human Press Totowa, pp: 93-100.
18. Molyneux, R.J., R.J. Nash and N. Asano, 1996. Alkaloids: Chemical and Biological Perspectives, Vol. 11, Pelletier SW, ed. Pergamon, Oxford, pp: 303.
19. Mythili, K., C.U. Reddy, D. Chamundeeswari and P.K. Manna, 2014. Determination of Total Phenol, Alkaloid, Flavonoid and Tannin in Different Extracts of *Calanthe Triplicata*. Research and Reviews: Journal of Pharmacognosy and Phytochemistry, 2(2): 40-44.
20. Narender, P.D., R. Ganga, E. Sambasiva, T. Mallikarjuma and V.S. Praneeth, 2012. Quantifiaction of phytochemical consitutents and *in vitro* antioxidant activity of *Mesua ferrea* leaves. Asian Pac Journal of Tropical Biomed., 2(Suppl 2): S539-S542.
21. Oda, K., H. Matsuda, T. Murakami, S. Katayama, T. Ohgitani and M. Yoshikawa, 2000. Adjuvant and haemolytic activities of 47 saponins derived from medicinal and food plants. Biol Chem., 381: 67-74.
22. Osbourn, A., 1996. Saponins and plant defence- a soap story. Trends plant Science, 1: 4-9.
23. Radovich, T., 2009. Farm and Forestry Production and Marketing Profile for *Moringa* (*Moringa oleifera*). In: Specialty Crops for Pacifc Island Agroforestry, Elevitch, C.R. (Ed.). Permanent Agriculture Resources (PAR), Holualoa, Hawai'I.
24. Rao, R.V.K., N. Ali and M.N. Reddy, 1978. Occurrence of both sapogenins and alkaloid lycorine in Curculigo orchioides. Indian Journal Pharma Science, 40: 104-105.

25. Robbers, J.E., M.K. Speedie and V.E. Tyler, 1996. "Chapter 9: Alkaloids". Pharmacognosy and Pharmacognosy biotechnology. Philadelphia: Lippincott, Williams & Wilkins, pp: 143-185.
26. Sarkar, S., 1996. Medicinal Plants and the Law. Occassional Paper Series (3). World Wildlife Fund, New Delhi, India.
27. Senguttuvan, J., S. Paulsamy and K. Karthika, 2014. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. Asian Pacific Journal of Tropical Biomedicine, 4(Suppl 1): S359-S367.
28. Siddiqui, S., A. Verma, A.A. Rather, F. Jabeen and M.K. Meghvansi, 2009. Priliminary phytochemicals analysis of some important medicinal and aromatic plants. Advan Biol. Res., 3(5-6): 188-195.
29. Singh, B. and R.P. Rastogi, 1970. Cardenolides-glycosides and genins. Phytochemistry, 9: 315-331.
30. Tapas, A.R., D.M. Sakarkar and RB. Kakde, 2008. Flavonoidsas Nutraceuticals: A Review. Tropical Journal of Pharmaceutical Research, 7: 1089-1099.
31. Teklit, G.A. and M.T. Firehiwot, 2016. Phytochemical and antibacterial activity Moringa oleifera available in the market of Mekelle. Journal of Analytical & Pharmaceutical Research, 2(1): 00011.
32. Trease, G.E. and W.C. Evans, 1989. Pharmacognosy,13th edithion. London: Bailliere Tindall, pp: 176-180.
33. Tsaknis, J., S. Lalas, V. Gergis, V. Dourtoglou and V. Spiliot, 1999. Characterization of *Moringa oleifera* variety Mbololo seed oil of Kenya. Journal of Agricultural Food Chemistry, 47(11): 4495-9.
34. Van Reeuwijk, L.P., 2002. Procedures for Soil Analysis. 6th edition. Technical Paper/International Soil Reference and Information Centre,Wageningen, The Netherlands.
35. Wink, M., 2000. Interference of alkaloids with neuroreceptors and ion channels. Stud Nat Prod Chem., 21: 3-122.
36. Xu, R., W. Zhao, J. Xu, B. Shao and G. Qin, 1996. "Studies on bioactive saponins from Chinese medicinal plants". Advances in Experimental Medicine and Biology. Advances in Experimental Medicine and Biology, 404: 371-82.