

Phytochemical Analysis and Cholinesterase Inhibition of *Cyperus platycaulis*.

J.O. Echeme, Ph.D.² and M.E. Khan, M.Sc.¹

¹Department of Chemistry Gombe State University, Gombe, Nigeria.

²Department of Chemistry, University of Agriculture, Umodeke, Nigeria.

E-mail: ememulu@yahoo.com¹
johnbullecheme@yahoo.com²

ABSTRACT

Cyperus platycaulis is used in folk medicine in Kano, northern Nigeria, for the treatment of cold, cough, (tuberculosis), chest congestion, asthma, sneezing, and catarrh. The tuberous fragrant herb (black species) was extracted using ethanol, chloroform, n-butane, and n-hexane, to determine the physico-chemical properties of the plant and the cytotoxicity of the extracts with reference to its anti-bacterial (*anti-tuberculea*) properties. Fractionation of the crude compounds followed by phytochemical screening, anti-cholinesterase (enzyme estimation) activity, and inhibition of sheep liver by the extracts are reported. Ethanol and n-butane extracts were positive for nearly all bioactive compounds tested. Chloroform and n-hexane extracts were both negative for carbohydrates, tannins, and glycosides. Alkaloids were completely absent. Cholinesterase inhibition of the extracts was encouraging. The order of ChE inhibition, (percentage inhibition of ChE over control) from highest to least was F_E001 (72.4%), > F_C001 (66.5%), > F_B001 (66.2%), and >F_H001 (60.3%). This correlates ethnomedical claims and thus provides for the first time, the pharmacological basis for the folkloric usage of *Cyperus platycaulis* as a potential cure for tuberculosis in Kano State, Northern Nigeria.

(Keywords: physicochemical, ChE, cytotoxicity, antibacterial properties, tannins, ethnomedicine, folk medicine, active ingredients, tuberculosis, extracts)

INTRODUCTION

Plants constitute an indispensable constituent of human existence such that finding medicinal value in them is an old idea. Plant-based drugs are gaining popularity because of several advantages such as lessened side effects, better patient tolerance, less expensive, and acceptance

due to a long history of use. Herbal medicines provide rational means for the treatment of many diseases that are incurable in other systems of medicine. Their bio-molecules appear as alternatives for the control of even resistant species of bacteria and human pathogens and their uses have been shown to have a scientific basis (Mathias *et al.*, 2000; Ganguly *et al.*, 2001; Martino *et al.*, 2002). The medicinal value of these bio-molecules (secondary metabolites) is due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of which include: alkaloids, glucosides, glycosides, steroids, flavanoids, fatty oils, phenols, resins, phosphorus, and calcium for cell growth, replacement, and body building (Chidambara *et al.*, 2003).

Basic phytochemical investigation of these bio-molecules for their major phytoconstituents for addressing medical disorders, pharmaceutical, and nutraceuticals requirements for the betterment of human existence is vital. In Kano, northern Nigeria, tuberculosis is a serious health challenge (FHI, 2001; Nwankwo *et al.*, 2005; Emokpae *et al.*, 2006). The plant family Cyperaceae possesses medicinal properties that are effective in the management of tuberculosis, cough, swellings and other ailments (Kubmarawa *et al.*, 2007). This underscores the usage of *Cyperus platycaulis*, by the Hausa and the Fulani tribes as a remedy for several human and animal ailments and even as an insect repellent (Dalziel, 1916, Deeniand and Sadiq, 2002). The methanol and pet. ether fractions have been shown to have anti-feedant properties (Dalziel, 1994).

In Sudan, the perfume obtained from *Cyperus ptyticaulis* is used to repel insects such as houseflies and mosquitoes. The light petroleum and methanol extracts of the plant rhizomes were evaluated against *Tribolium castaneum* (Herbst)

(the red flower beetle) (Akatsuka, *et al.* 1994). The dried trunk bark in Nigeria (i.e. methanol-water (1:1) extract) is found to be active on *Salmonella typhii* at 10 mg/ml. The petroleum ether fraction (1.0 mg/ml) is active on *Escherichia coli*, *Vulgaris species*, *Staphylococcus sp.*, and *Streptococcus sp.*, at MIC of 1.0mg/ml and LC₅₀-100 mg/ml (Fatope *et al.*, 2001).

The hexane extract is active on *Spodotera litura* at 1.0 mg/ml in Thailand and the hot extract kills cell cultures at 500 mg/ml in China with an inhibitory rate of 69%. It is also an anti-malaria herb with the ethanol-water extract 50%, active on mouse (intragastric activity), with a daily dosing for 4 days, at 500 mg/ml having a 49% inhibition.

In India, (NAPRALERT 2002) *Cyperus platycaulis* (*Kajiji* in Hausa) is an annual or perennial herb, often tufted, with leaves basal or absent. It is an inflorescence umbellate (simple or compound) subtended by leaf-like bracts with glumes alternating in two rows; glumes falling at the base and rachilla persistent. (Burkill1985).

The wide spread drug resistance to bacterial strains are also a main hurdle that forces researchers to discover new plant-based antimicrobial against for these strains. Thus this paper reports the phytochemical screening of *Cyperus platycaulis* and its potentials against microbial (*anti-tuberculae*) agents, with the view to making sure that their claimed curative property on tuberculosis is ascertained. Our research is aimed at sourcing natural therapeutics whose chemotherapeutic index surpasses that of the four TB-drugs, INH, PZA, EMB, and RMP, used in combination, and as such provide a natural chemotherapy for MDR-TB.

MATERIALS AND METHODS

Sampling: Fresh samples of *Cyperus platycaulis* were collected at the peak of the dry season (April 2004) in Gwoza (LGA), Borno State, Northern Nigeria. They were identified and authenticated in the Department of Biological Sciences, Bayero University, Kano. A voucher specimen of the sample with number BUK 042 was deposited in the herbarium of the Department. The samples were air dried in the laboratory before pounding to a fine powder using a 600W DAB4 Vivacio blender (Moulinex, France)

to a mesh size of about 70 and then stored in a dry container.

Extraction: 530g of the plant were percolated with 4L of 95% ethanol for two-weeks. After which, there was decantation, filtration, and concentration on rota-vapour (Stuart RE 300 series, UK, R110) at 40°C to obtain ethanol soluble fractions, (F_{E01}), labeled, F_{E0C} (29.42g), crude.

The above fraction was macerated with solvents of different concentrations, chloroform, [C] (10.7g, 36.4%), n-Butanol, [n-B] (06.6g, 22.4%), and n-Hexene, [n-H] (4.9g, 6.6 %), to obtain the fractions that were used for phytochemistry and bioassay.

Phytochemical Screening: Phytochemical analysis of all the evaporated solvent extracts was conducted in accordance with the standard procedure (Harborne, 1992, Kokate 1994, Abulude 2001 and 2007). Tests for saponins, tannins, flavanoids, volatile oils, glycoside, alkaloids, phenols, and resin were carried out in all the fractions

Preparation of Stock Solution: Six different extracts (F_{E0R}), (F_{E0S}), (F_{E0L}), (F_{W0R}), (F_{W0S}), and (F_{W0L}) were used for both analyses. Concentrations (in triplicates) were produced from each extract at 2000, 1500, 1000, and 500µg / (cm³), respectively using the dilution formula (Almagboul, 1985).

CHOLINESTERASE INHIBITION

Cholinesterase is one of many important enzymes needed for the proper functioning of the nervous system of humans and animals. Certain classes of pesticides, such as organophosphates (OPs), carbamates (CMs), and chlorinated derivatives of nicotine work against undesirable insects by interfering with or inhibiting cholinesterase. While the effects of cholinesterase inhibiting products are intended for insect pests, these chemicals can also be poisonous or toxic to humans by exposure through inhalation, ingestion, eye, or skin contact during the manufacture, mixing, or application of these pesticides (Davies, 1981).

Mode of Action: Electrical switching centers, called 'synapses' are found throughout the nervous systems of humans, other vertebrates,

and insects. Muscle, glands, and neurons are stimulated or inhibited by the constant firing of signals across these synapses (Goh, *et al.*, 1985).

The focus is on a typical synapse in the nervous system, in which a muscle is being directed by a nerve to move. An electrical signal, or nerve impulse, is conducted by acetylcholine across the junction between the nerve and the muscle. Normally, after the appropriate response is accomplished, cholinesterase is released which breaks down the acetylcholine terminating the stimulation of the muscle. The enzyme cholinesterase accomplishes this by chemically breaking the acetylcholine into other compounds and removing them from the nerve junction. If cholinesterase is unable to breakdown or remove acetylcholine, the muscle can continue to move uncontrollably.

Mechanism of Inhibition: Cholinesterase enzyme (ChE) is a polypeptide with an aggregate of four sub-units and with four active sites per molecule (one active site/sub-unit). Each active site serves to hydrolyze acetylcholine and has one anionic (-ve) site, corresponding to the carboxylic group of either aspartic or glutamic acid. The anionic site of ChE draws the (+) charge of the +N (CH₃) of acetylcholine to form an ionic bond with carboxylic acid of its own, while the esteratic site cleaves acetic acid of acetylcholine.

Anticholinesterase compounds act as substrate analogues of ChE thus forming covalent bond with esteratic, while the ChE becomes a phosphorylated intermediate which is unstable and easily hydrolyzed as the acetic acid in acetylcholine. The enzyme becomes unreactive since the ester remains to block the active site.

Relationship between ChE and Huperzine A: In our effort to isolate naturally occurring ChE inhibitors, Huperzine A, an effective and acknowledged plant product, is used as a reference standard. Huperzine A is an alkaloid isolated from *Huperzia serrata*, which prevents the breakdown of acetylcholine, (Cheng, *et al.*, 1996), an important substance needed by the nervous system to transmit information from cell to cell (Cheng, 1998). Animal research has suggested that Huperzine A's ability to preserve acetylcholine may be greater than that of some prescribed drugs (Kozikoski, *et al.*, 2000).

Enzyme Estimation (Cholinesterase Inhibition): In this investigation, four extracts F_E001, F_C001, F_B001, and F_H001 are selected for testing cholinesterase inhibition activity and appropriate concentrations of (5, 10, and 20 µg) *Cyperus platycaulis* extracts prepared in acetone were tested for cholinesterase inhibition in relation to a standard and established cholinesterase inhibitor, Huperzine A. The cholinesterase inhibition caused is discussed in relation to the secondary metabolites found in the extracts. Fresh sheep liver is employed as cholinesterase enzyme source.

Enzyme Preparation: Fresh sheep liver was procured from healthy sheep immediately after slaughter. 1% Homogenate (w/v) of sheep liver was prepared in distilled water at 0 °C as per the method reported for mammalian livers. Cholinesterase inhibition by colorimetric method was made by the method (Nandakuma, *et al.*, 1976), but with the following modifications:

Appropriate amounts of extracts in 5, 10, or 20 µg / 0.1 ml of acetone were separately dispensed in the test tubes and the acetone allowed to evaporate. The 0.1 ml of sheep liver homogenate is pre-incubated with 5, 10, or 20 µg of extract separately for 15 minutes at 37°C in a thermostatic water bath. Standard Huperzine A, technical grade obtained from Sigma Chemical Company, USA, is also pre-incubated in the manner described above, with 0.1ml of (w/v), homogenate at 5, 10, or 20, µg to allow the inhibition of liver cholinesterase by the extracts. The experiment was carried out in triplicates at all concentrations.

After pre-incubation, 0.2 ml of 0.2% azo-dye in water is added, followed by 0.1 ml of ethylacetoacetate substrate in acetone and the reaction mixture made to a total of 1.0 ml with distilled water prior to addition of substrate.

The enzyme incubation time of 1.min. after addition of substrate is not exceeded. Enzyme activity is arrested at the end of exactly one minute by the addition of 4 ml of glacial acetic acid. The magenta color developed is read at 540 nm in a spectrophotometer.

The control enzyme reaction mixtures are without extracts and are also made up of a total of 1 ml with distilled water.

However, the pre-incubation of 15 minutes with enzyme alone and enzymatic incubation for one minute with addition of ethylacetoacetate and azo-dye was carried out with extracts. The control optical density was normalized to 100 % and the percent inhibition calculated as below (Trivedi, *et al.*, 1986).

$$\frac{C - E}{C} \times 100 = \% \text{ inhibition.}$$

where C = Control absorbance unit

E = Exp`tal absorbance unit (with extract).

OR

From the enzyme activity units,

$$\frac{C - E}{C} \times 100 = \% \text{ ChE. inhibition.}$$

where C = amount of Huperzine A formed in control.

E = amount of Huperzine A formed in experimental.

A standard graph for Huperzine A vs. absorbance at 540 nm with an azo-dye is employed for calibrating enzyme activity units/mg wet weight of tissue.

Calibration Curve: A calibration (straight line), curve was plotted for ChE inhibition vs Huperzine A amount (Figure 1 and Table 1), which obeys Beer-Lambert's law. Least square fitting of the best straight line gave the equation:

$$\text{ChE inhibition (\%)} = 1.25 \times \text{Huperzine A (\mu g)} + 0$$

for the general equation $Y = mx + c$, were m and c are the slope and intercept, respectively.

RESULTS

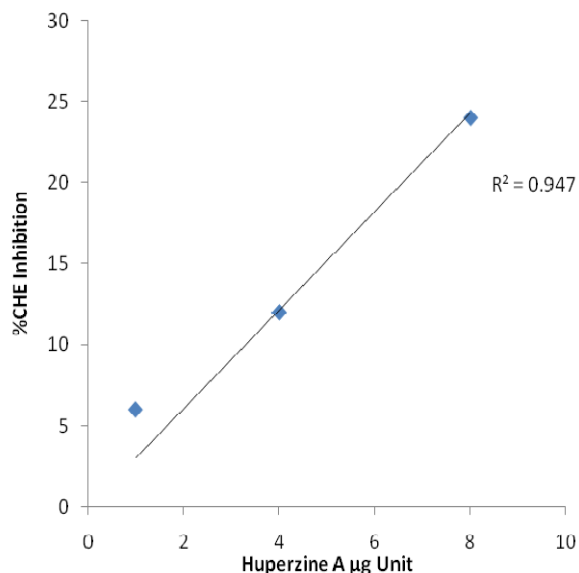


Figure 1: Standard Calibration Curve with Huperzine A as a Reference Standard for Deducing ChE Inhibition.

Table 1: Results of Phytochemical Analysis of Extracts from Black species of *Cyperus platycualis*.

<i>Cyperus platycualis</i> Extract				
Bioactive compounds	F _E 001	F _C 001	F _B 001	F _H 001
Saponnins	-	+	-	+
Tannins	+	-	+	-
Flavanoids	+	+	-	+
Alkaloids	-	-	-	-
Glycoside	+	-	-	-
Carbohydrates	+	-	+	-
Sterols	+	+	+	+
Resin	+	+	+	+

(- = Absent), (+ = Present)

Table 2: Percent Liver ChE Inhibition by the Extracts from *Cyperus platycaulis* in Comparison with Standard Huperzine A. Reference Standard (degree of inhibition) Expressed in terms of Huperzine A Units (HAU).

Fraction	Amount of fraction µg/ml	Percent Inhibition of ChE over control (Y)	% Decrease or Increase compared to standard Huperzine A % inhibition	Equivalent Huperzine A deduced from standard graph (X) HUA	Remark ChE Inhibition.
F _E 001	5	15.21	10.32	11.11	Trace of inhibition at low conc. And high inhibition at high conc.
	10	47.78	33.78	38.22	
	20	72.42	48.42	57.94	
F _C 001	5	20.53	17.81	20.24	“
	10	57.59	43.59	46.07	
	20	66.48	42.48	53.18	
F _B 001	5	21.52	15.22	17.20	“
	10	46.83	27.83	37.46	
	20	66.22	47.22	52.98	
F _H 001	5	27.13	19.54	22.65	“
	10	50.63	36.63	40.50	
	20	60.28	36.28	48.22	

DISCUSSION

In our attempt to uncover more effective and more affordable drugs from plant sources against MDR-TB, the ancient medical uses of *Cyperus platycaulis* proved authentic as the various phytochemicals tested for were present, and the extracts indicated considerable activity against the selected micro-organisms. Resin and sterols were present in all the fractions tested. Saponins, tannins, and carbohydrates were found in two out of the four fractions subjected to test while alkaloids were completely absent in all fractions. This shows the generality of secondary metabolites in medicinal plants.

To a large extent, the phonological age of the plant, percentage humidity of the harvested material, geographical location, climatic conditions, soil condition, time of harvest, and the method of extraction are possible sources of variation for the chemical composition, toxicity, and bioactivity of the extracts (Felix, 1982).

The anti-infective potential of plants is mainly due to a variety of antimicrobial compounds that are produced by the plants for their own purposes. These are either produced in the plant or induced after infection. These can also be induced by abiotic factors such as UV irradiation and they have been defined as antibiotics, formed in plants via a metabolic sequence induced either biotically

or in response to chemical or environmental factors (Cos *et al*, 2006).

Enzyme Estimation: ChE inhibition of crude fraction is being tested in the present investigation. Table 2 gives the fractions and the percentage inhibition, of sheep liver ChE against a standard of Huperzine A. The degree of inhibition is expressed in terms of Huperzine A units (HAU). For all the fractions, the order of ChE inhibition, from the highest to the lowest, is F_C001 > F_B001 > F_E001 > F_H001.

The order of ChE, inhibition indicates that the chloroform soluble fraction F_C001 showed greater inhibition at 20 µg/ml than all the other fractions. Thus, Table 2 presents the inhibition of sheep liver ChE of extracts (values obtained are the mean of triplicate). The degree of toxicity and degree of inhibition seem to correlate and support the hypothesis that the higher the concentration, the higher the toxicity and the more the inhibition and vice versa.

A calibration curve plotted for the % ChE inhibition vs Huperzine A units (Figure 1) obeys Beer Lambert's Law. Least square fitting of the best straight line gave the equation: % ChE inhibition = 1.25 X Huperzine A (µg) + 0, for the general equation Y= mx+ c, where, m is the slope and c the intercept.

CONCLUSION

Histochemical and phytochemical studies performed in this investigation confirmed the presence of various plant metabolites in the plant tissue and the percentage inhibition of sheep liver ChE by the extracts. The evidence reported in this paper supports that *Cyperus platycaulis* has relatively high bioactive secondary compounds (metabolites) and thus likely to hold promise for drug discovery.

ACKNOWLEDGEMENTS

With love and immense respect, we appreciate the support of the Central Drug Research Institute (RSIC-CDRI) Lucknow, India; Wayne State University, Detroit Michigan; and Sigma Chemical Company, USA for the Standard Huperzine A Technical Grade.

REFERENCES

1. Abulude, F.O. 2007. "Phytochemical Screening and Mineral Contents of Leaves of Some Nigerian Woody Plants". *Research Journal of Phytochemistry*. 1(1): 33-39.
2. Abulude, F.O. 2001. "Mineral and Phytate Contents of 10 Vegetables grown in Nigeria and Calculation of their Phytate; Zn, and Ca; Phytate Molar Ratios". *Adv. Food Sci.* 23:36-39.
3. Akatsuka, T.O., Kodama, H., Kato, S., Suzuki, S. Ishii, and Birai, S. 1994. "Insecticides from *Cyperus rotundus*". *Kokai Tokyo koho*. 6(16): 516.
4. Almagboul, A.Z., Bashir, A.K., Farouk, A., Karim, A., and Salih. 1985. "Anti-Microbial Activity of Certain Sudanese Plants used in Folkloric Medicine: Screening for Anti-Bacterial Activity (IV)". *Fitoterapia*. 56:331-337.
5. Burkill, H.M. 1985. *The Useful Plants of West Tropical Africa*. Edition 2, Vol. 1. Royal Botanic Gardens: Kew, UK.
6. Cheng, D.H., Ren, H., and Tang, X.C. 1996. "Huperzine A, a Novel Promising Acetylcholinesterase Inhibitor". *Neuroreport*. 8:97-101.
7. Cheng, D.H., and Tang, X.C. 1998. *Mechanism of Inhibition of Cholinesterase by Huperzine A and Comparative Studies of Huperzine A*. 3rd Edition, Humana Press: Totowa, NJ. 64 – 67.
8. Chidambara, K., Vanitha, A., Mahadeva, M., and Ravishankar, G. 2003. "Antioxidant and Antimicrobial Activity of *Cissus quadrangularis* L". *Journal of Medicinal Food*. 6(2).
9. Cos, P., Vlietinck, A.J., Berghe, D., and Maes, L. 2006. "Antiinfective Potential of Natural Products: How to Develop a Stronger *in vitro* 'Proof-of-Concept'". *J. Ethnopharmacol.* 106:290-302.
10. Dalziel, J.M. 1916. "The Useful Plants of West Tropical Africa. Crown Agents for Overseas Government and Administration". *J. Clinical path.* Millbank: London, UK. 45; 493 – 496.
11. Dalziel, J.M. 1994. *The Useful Plants of West Tropical Africa*. Wartmoughs Ltd.: London, UK. 188 -190.
12. Davies, J.E. and Freed, V.H. 1981. *An Agro-Medical Approach to Pesticides Management-Some Health and Environmental Considerations*. Consortium for International Protection: Berkeley, CA.
13. Deeni, Y.Y. and Sadiq, N.M. 2002. "Antimicrobial Properties and Phytochemical Constituents of the Leaves of African Mistletoe (*Tapinanthus dodoneifolius* (DC) Danser) (Loranthaceae): An Ethno-medicinal Plant of Hausa land, Northern Nigeria". *Journal of Ethnopharmacology*. 83:235-240.
14. Emokpae, M.A., Nwokedi, E.E., and Dutse, A.I. 2006. "Biochemical Changes in Adult Nigerians with Pulmonary Tuberculosis in Kano-Nigeria". *Highland Medical Research Journal*. 4(1):15-21.
15. Fatope, M.O. 2001. *Looking Back and Looking Forward*. Natural Product Science: Washington, DC.
16. Felix, M.T. 1982. *Medical Microbiology*. Churchill Livingstone (Publishers): London, UK. 445-459.
17. FHI (Family Health International). 2001. "Kano State, Nigeria. Report of the In-Depth Assessment of the HIV/AIDS Situation". Institute for HIV/AIDS: Arlington, VA.
18. Ganguly, R., Mishra, P., and Sharma, A. 2001. "Microbes and Infection". *Indian J. Microbio.* 41: 211–213.
19. Goh, K.W. and Smith, R.F. 1985. "Pesticide Safety for IPM field Scouts". Chemicals Pesticide Programme. Cornell University: Ithaca, NY.
20. Harborne, J.B. 1992. *Phytochemical Methods*. Chapman and Hall Publications: London, UK. 7–8.

21. Kokate, C.K. 1994. *Practical Pharmacognosy*. Vallabh Kubma: Prakashan, India.
22. Kozikowski, A.P. and Tuckmantel, W. 2000. "Chemistry, Pharmacology, and Clinical Efficacy of the Chinese Nootropic agent Huperzine A". Lucknow, India. 142.
23. Kubmarawa, D., Ajoku, G., Enwerem, M., and Okorie, D. 2007. "Preliminary Phytochemical and Antimicrobial Screening of 50 Medicinal Plants from Nigeria". *African Journal of Biotechnology*. 6 (14):1690-1696.
24. Martino, P.D., Gagniere, H., Berry, H., and Bret, L. 2002. "Anti-Microbial Agents". *Microbes and Infection*. 4:613–620.
25. Mathias, A.J., Somashekar, R.K., Sumithra, S., and Subramanya, S. 2000. "Anti- Microbe Agents". *Indian J. Microbio*. 40:183–190
26. Nandakuma, N., Visweswariah, V., and Majunder, S.K. 1976. "Third Epidemic Multi-Drug Resistant Tuberculosis". *J. Asso. of Anal Chem*. 50: 641
27. NAPRALERT. 2002. "National Programme for the Collaborative Research in the Pharmaceutical Science". College of Pharmacy, University of Illinois: Chicago, IL. (Unpublished).
28. Nwankwo, E.K., Kwaru, A., Ofulu, A., and Babashani, M. 2005. "Haematological Changes in Tuberculosis in Kano, Nigeria". *Journal of Medical Laboratory Sciences*. 14(2):35-39.
29. Trivedi, S.S. and Desai, S.C. 1986. "Primary Anti-Tuberculosis Drug Resistance and Acquired Rifampicin Resistance in Gujarat, India". *Tubercle*. 69: 37.

SUGGESTED CITATION

Echeme, J.O. and M.E. Khan. 2009. "Phytochemical Analysis and Cholinesterase Inhibition of *Cyperus platycaulis*". *Pacific Journal of Science and Technology*. 10(1):350-356.



[Pacific Journal of Science and Technology](http://www.akamaiuniversity.us/PJST.htm)

ABOUT THE AUTHORS

Professor JohnBull O. Echeme, is the Head of the Department of Chemistry, University of Agriculture Umodeke, Nigeria. He holds a Ph.D. and M.Sc. from the University of Lucknow, India.

Muluh Emmanuel Khan, serves as an Assistant Lecturer in the Department of Chemistry, Gombe State University, Gombe, Nigeria. He holds a B.Sc. and M.Sc. from Bayero University Kano (B.U.K.) Nigeria. He is presently undertaking a Ph.D. at Ahmado Bello University (A. B. U), Zaria Nigeria.