ChemSearch Journal 7(2): 18 – 23, December, 2016
Publication of Chemical Society of Nigeria, Kano Chapter

Received: 21/10/2016 Accepted: 06/11/2016
http://dx.doi.org/10.4314/csj.v7i2.4

Levels of Lambda-Cyhalothrin and Polychlorinated Biphenyls Residues in some Herbal Remedies from Northwest Nigeria

Bello A. A.[1], Uzairu A.[2], Okunola O. J.[3], Sallau M. S. [2] and Uba S.[2]
[1]Department of Chemistry, Sa'adatu Rimi College of Education, Kumbotso, Kano State, Nigeria
[2]Department of Chemistry, Ahmadu Bello University Zaria, Nigeria
[3]Department of Applied Chemistry, Federal University Dutsin-Ma, Katsina State, Nigeria)
Email: abbelbich@gmail.com

ABSTRACT

The study determined the presence and concentration of chloride, phosphate, lambda-cyhalothrin and polychlorinated biphenyls PCBs by Gas Chromatography-Mass Spectrometry method to discern contamination of selected herbal remedies. Sampling was carefully carried out in Northwest Nigeria. The chloride and the phosphate were found to be in the concentration ranges of non-detected – 141.8mg/kg and 4.6 – 40.2mg/kg, respectively. The congeners 2,2',5,5'-tetrachlorobiphenyl and 2,2',3,4,4',5,5'-heptachlorobiphenyl were not detected in any sample while the highest concentration of the PCBs was recorded for 2,3,3',4,4',5-hexachlorobiphenyl in one sample. Other three congeners were detected at varied concentrations in different percentages of the samples. The pesticide lambda-cyhalothrin was not detected in any sample.

Keywords: Herbs, contamination, polychlorinated biphenyls, lambda-cyhalothrin, concentration

INTRODUCTION

Studies have shown that people around the globe used herbal remedies because of their accessibility, affordability, good efficacy and safety (Gyasi *et al*, 2011) However, often the safety is questioned, as herbs may be contaminated by the environment in the course of cultivation, transportation, processing or storage. The contaminants may comprise of toxic metals and non-metals, radio-actives materials, micro-organisms, persistent organic pollutant such as polychlorinated biphenyls and pesticides residues (WHO, 2007).

Polychlorinated biphenyls (PCBs) are a group of 209 synthetic isomers (known as congeners). Structurally, they are two benzene rings joined together by a single bond. The positions of substituents are designated by a number and number prime eg 1 or 1'. PCBs general formula is $C_{12}H_{10-n}Cl_n$. PCBs are toxic chemicals that persist in the environment to the extent that about 10% of the PCBs produced since 1929 still remain in the environment today, although their production was banned in 1980 (Greenfact, 2015). In the human body, PCBs cause adverse effects on the nervous, immune and endocrine systems, impair reproductive function and may be carcinogenic (EFSA, 2012).

Scientists cannot say for sure that there is ever a "safe" level of pesticide residues in food because many of the chemical messengers in our bodies function at precisely minute quantities of ppm or even ppb (Boobis, *et al*., 2008). Incidents of pesticide toxicity are abounding. (Oluromíniyi and Emily, 2011). Pesticide residue analysis is posed with many difficulties because commercial products are mostly a mixture of different compounds and many are readily oxidized or hydrolyzed to form derivatives or metabolites (Allen, 1974). Therefore, it may be desirable to test herbal drugs for broad groups of residual compounds in general, rather than for individual pesticides, even though there is no specification on the pesticide residue allowed (Jacobs *et al.*, 1997). Many pesticides contain chlorine in the molecule, which, for example, can be measured by analysis of total organic chlorine. In an analogous way, insecticides containing phosphate can be detected by measuring total organic phosphorus. Several studies were done about pesticide residue (Mosleh *et al.*, 2014) based on history of application of a given pesticide and availability of standard compounds for determination. This work was carried out based on the availability of standard compound of analysis for lambda cyhalothrin [(RS)-α-Cyano-3-phenoxybenzyl-(Z)-(-1RS,3RS)-(2-chloro-3,3,3-trifluoropropenyl)-2,2 dimethylcyclopropanecarboxylate] in the laboratory and the fact that it is a common active ingredient in pesticides.

Herbal remedies are a common commodity in Northwestern Nigeria and many of these are not registered with the regulatory body. This means that safety of use or safety after use is uncertain. This study is aimed at determining concentration of six congeners of PCBs and Lambda-cylohathrin in the herbal remedies produced in the Northwestern region of Nigeria.

Materials and Methods

Reagents

The following analytical grades reagents and distilled deionized water were used. 98% H_2SO_4, ammonium molybdate dehydrate (Merck), $AgNO_3$, $Mg(NO_3)_2.4H_2O$, $SnCl_2.2H_2O$, 99.8% acetonitrile, cyhalothrin standard (Sigma-Aldrich), 99.5% benzene, isobutanol, 99.9% methanol, diethyl ether (JHD).

All glassware were rinsed with acetone\hexane mixture, heated at 400°C for 4 hrs and cooled to 22°C before use. A total of 29 samples were purchased based on their claimed therapeutic effect from wholesaler herbalists in Kano, Chiranci, Maigatari, Kawo Kaduna and Zaria of Kano, Katsina, Jigawa and Kaduna States, Nigeria, respectively. The samples were analyzed for chloride and phosphate content. 12 of the samples (i.e samples 5, 8, 9, 10, 11, 17, 18, 19, 22, 23, 28 and 29) which were with relatively high chloride content were analyzed for lambda-cyhalothrin and PCBs.

Figure 1: Sampling area for the study

Determination of Chloride

1.0g sample was extracted in 50 ml 0.75M H_2SO_4. After neutralization, the extract was titrated against 0.0282M $AgNO_3$ (Edward *et al*, 1981).

$$\text{Cl (mg/l)} = \frac{(A-B)Mx\ 70900}{Volume\ of\ sample}$$

A = ml of $AgNO_3$ used for titrating sample B = 0.2ml of $AgNO_3$ used for titrating blank
M = molarity of $AgNO_3$

Determination of Phosphates

1.0g of each sample was digested by treatment with 5 ml 1.0M $Mg(NO_3)_2.4H_20$ and ignited at 650°C using Electric muffle furnace TT-EF Techmel model and the ash been dissolved in 15ml 2 M H_2S0_4 and 20ml deionized water (Bertramson, 1942). The sample solution was extracted in 50 ml 1:1 benzene-isobutanol solvent and 15 ml ammonium molybdate reagents. The extract was reacted with 15ml 49:1 methanol-sulphuric acid and 10 drops 25g/dm^3 stannous chloride for colour development. Absorbance reading of the sample together with calibration standards and blank were taken at wavelength of 652nm using UNICAM Winelight UV/Vis spectrophotometer.

$$\text{Phosphate (mg/l)} = \frac{\text{Reading from curve x 1000 x D}}{Volume\ of\ sample}$$

Where D = dilution factor

PCB's analysis

50ml of 1:2 acetone:hexane mixture was added to 10g of the air dried, powdered sample. The mixture was blended and centrifuged at 100rpm for 5 minutes. The supernatant/extract was decanted and the residue was washed with another 20 ml 1:2 acetone:hexane and combined with the first extract. The extract was evaporated to about 10 ml using vacuum rotary evaporator. The solution was cleaned with H_2SO_4. The acid layer was then discarded and ether layer was dehydrated by anhydrous sodium sulphate, transferred to 50 ml flask and concentrated at 40 °C. The remnant was then dissolved in petroleum ether in a 10 ml measuring flask followed by the addition of 1 ml internal standard solution. Extracts were ran into Gas chromatography-Mass spectrometry (QP2010 PLUS SHIMADZU,JAPAN) together with calibration standards according to the instrument's operating conditions (Allen, 1974, Du *et al*, 2007).

Lambda-cyhalothrin determination

10.0g of air dried powdered sample was weighed into a 400ml beaker. 60 ml acetone was added and the mixture was homogenized for 3 min. The mixture was then filtered through 12 cm Buchner funnels connected to a suction pump, with filter paper. The beaker and residues were rinsed with 20 ml of acetone and the washings added to the filtrate. The filtrate was transferred to a separating funnel where 60 ml of hexane was added. This was then shaken for 5 min. In addition 4.0 % aqueous NaCl solution was added to the mixture and manually mixed vigorously for 1 min. On separation, the aqueous layer was discarded while the hexane layer was passed through glass wool containing 15g Na_2SO_4 into 250 ml round bottom flask. The content of the flask was evaporated to dryness.

The residue was re-dissolved in 10ml hexane and then transferred together with the 5ml hexane used in rinsing the round bottom flask to 125ml separating funnel. 30 ml acetonitrile-saturated hexane (300ml acetonitrile +100ml hexane shaken in separating funnel, discarding the acetonitrile layer after separation) was added and shaken. After separation, the acetonitrile phase was transferred to 250ml round bottom flask. Addition of 30ml acetonitrile- saturated hexane was repeated twice and each time transferring the acetonitrile to the round bottom flask. The acetonitrile extract was dried on rotary evaporator at 60°C and the residue dissolved in 5ml hexane (Food Safety and Standards Authority of India FSSAI, 2012). The sample obtained and the calibration standards were studied using GCMS- instrument (QP2010 PLUS SHIMADZU,JAPAN) under the operating conditions;

GC-2010]

Column oven temp. :60.0 °C
Injection Temp. :280.00 °C
Injection Mode :Splitless
Sampling Time :1.00 min
Carrier Gas: Helium
Flow Control Mode :Linear Velocity
Pressure :51.0 kPa
Total Flow :2.8 mL/min
Column Oven : Yes
SPL2 : Yes
MS :
IonSourceTemp :200.00 °C
Interface Temp. :260.00 °C
Solvent Cut Time :1.50 min
Detector Gain Mode :Relative
Detector Gain :0.00 kV

RESULTS AND DISCUSSIONS

Table 1 shows the result of phosphate content and chloride content in the various samples. Result from the chloride analysis shows that only two samples were in below detection limit while the rest ranged from 14.2mg/Kg in sample No 6 to 141.8mg/kg in sample No 29. All of the samples analyzed showed phosphate content at between the range of 4.6 mg/Kg in sample No 19 – 40.2mg/Kg in sample No13. However, sample 13 is an outlier, that the other 28 samples phosphate range between 4.6 -19.1mg/Kg. Chloride and phosphate generally occurs in the range of 0.04 – 0.4% and 0.05 – 0.3% in plants (Allen, 1974). The observed chloride concentration was in the range of nd – 141.8 mg/kg translates to nd – 1.418%. Therefore these levels are greater than the highest average percent in plants indicating a probable contamination by chloride in some of the samples. Similarly, the phosphate ranged 0.023 – 0.201% which is within the general range of phosphate in plants declaring free of phosphate contamination. The phosphate content of sample No 13 is also within the general range of phosphate in plants.

Table 1: Concentration of chloride and phosphate in the herbs.

Sample	Cl^- (mg/Kg)	PO_4^{3-}(mg/Kg)
1	44.3	5.7
2	31.9	16.6
3	46.0	11.6
4	40.7	11.0
5	56.7	13.7
6	14.1	9.8
7	46.0	12.3
8	63.8	10.5
9	63.8	18.8
10	102.2	17.2
11	67.0	16.0
12	33.6	13.2
13	ND	40.2
14	30.4	8.6
15	49.0	13.1
16	42.5	8.4
17	55.0	18.4
18	111.0	15.4
19	63.8	4.6
20	40.7	17.1
21	51.4	18.1
22	51.4	10.6
23	67.0	13.2
24	ND	19.1
25	23.0	10.6
26	46.0	9.9
27	27.8	10.8
28	53.1	17.5
29	141.8	11.3
Range	ND-141.8	4.6-40.2

ND: Not detected

Table 2 shows the concentration of the PCB congeners in the samples. Congener c and f were not detected in any of the samples. However, other congeners were found at varied percentages in the samples, with congener d occurring in 50% of the total samples. Only sample No. 3 is contaminated by three different congeners. Sample No. 4, 10 and 11 were contaminated by two different congeners, while seven samples were containing one kind of congener or another and sample No.9 is uncontaminated by any of the congeners. Congener (e) recorded highest concentration in sample No 1. Mosleh (2014) found some medicinal plants to be free of the PCBs, while another study reported PCBs concentrations of 2.75 ng/g in rosemary and 2.39 ng/g in oregano plants (Storelli, 2013). These concentrations are both below the minimum detected level of 17ng/g in this study. Nevertheless, detection of any of the PCBs shows that care must be exercised in consuming the herbal formulations because of the complexities of the PCBs in many respects giving rise to different toxicities which could potentially lead to clinical conditions of cancer, neurological disturbance, reduced fertility and so on (EFSA, 2012).

Table 2 : Concentrations (ng/g) of the six PCBs congeners in herbal remedies.

Sample	PCB Congeners						Number of detected congeners
	a	b	C	D	e	f	
1	N.D	N.D	N.D	N.D	323	N.D	1
2	N.D	N.D	N.D	N.D	212	N.D	1
3	36	30	N.D	176	N.D	N.D	3
4	N.D	28	N.D	N.D	118	N.D	2
5	N.D	N.D	N.D	195	N.D	N.D	1
6	N.D	N.D	N.D	83	N.D	N.D	1
7	N.D	N.D	N.D	82	N.D	N.D	1
8	ND	ND	ND	ND	20	ND	1
9	ND	ND	ND	ND	ND	ND	Non
10	N.D	N.D	N.D	83	69	N.D	2
11	N.D	28	N.D	170	N.D	N.D	2
12	17	N.D	N.D	N.D	N.D	N.D	1
Range	17-36	28-30	N.D	82-195	20-323	N.D	
% Sample	16.67	25	0	50	41.67	0	

ND: Not detected

a) 2,4,4'-trichloro-1,1'-biphenyl b) 2,2',5,5'-tetrachloro-1,1'-biphenyl c) 2,3',4,4',5-pentachloro-1,1'-biphenyl
d) 2,2',4,4',5,5'-hexachloro-1,1'-biphenyl e) 2,3,3',4,4',5-hexachloro-1,1'-biphenyl
f) 2,2',3,4,4',5,5'-heptachlo-1,1'-biphenyl

Lambda-cyhalothrin residue was not detected in any of the samples covered by this study. 1.0mg/kg is the limit of cyhalothrin in herbs set by European pharmacopoeia (2006). However, Farag *et al.,* (2011) detected 0.073mg/Kg lambda-cyhalothrin among the 17 pesticides analyzed in onion samples and orange samples in Egypt.

Even though, the chloride content in some of the samples reported by this study may be attributable to pesticides contaminations, the pesticide may be of different type other than cyhalothrin. A study on Chinese herbal products reported that only 48 of 65 samples tested positive for at least one pesticide residue out of 51 different types tested [Greenpeace wwwgreenpeace.cn. (6/24/2013 12:16:16 pm)].

CONCLUSION

This study has successfully investigated the presence of residual species of lambda-cyhalothrin and polychlorinated biphenyls or their possible contamination of some herbal remedies from Northwest Nigeria. The observed chloride content above average in the plants samples may indicate possible contamination of some of herbs by chlorine-containing substances. The substances could be the detected PCBs or pesticides other than the lambda-cyhalothrin. The samples could potentially lead to health hazards. Producers should regulate the herbal products and consumers be cautioned about its use.

ACKNOWLEDGEMENT

The authors are grateful to Mr Gero Muhammad and his colleagues at the Chemistry Laboratory Section, NARICT Zaria for their assistance with the analysis.

REFERENCES

Allen, S.E. (1974) Chemical analysis of ecological materials. Blackwell Scientific Publications, Oxford. 340-356.

Bertramson, B. R. (1942) Studies on the ceruleomolybdate determination of phosphorus. Soil Sci. 53: 135-141. www.plantphysiol.org on July 9, 2015

Boobis, A.R., Ossendorp, B.C., Banasiak, U., Hamey, P.Y., Sebestyen, I. and Moretto, A. (2008) Cumulative risk assessment of pesticide residues in food. Toxicology Letter 15:137-150.

Du, B., Li, X. and H. Liu, H. (2007). Determination of organochlorine pesticide residues in herbs by capillary gas chromatography *Life Science Journal* 4(1): 40-42

Edward, I.K., Kalra, Y.P. and Radford, F.G. (1981). Chlorine determination and levels in the soil – plant environment. Environmental Pollution. Series B 2 109-117.

European Food Safety Authority EFSA (2012) Update of the monitoring of dioxins and PCBs levels in food and feed. *EFSA Journal*, 10(7):2832.

European Pharmacopoeia (2004), European Directorate for the Quality of Medicines, Council of Europe 5th ed. Strasbourg.

Farag, R.S., Abdel Latif, M.S., Abd El-Gawad, A.E. and Dogheim, S.M. (2011) Monitoring of pesticide residues in some Egyptian herbs, fruits and vegetables. *International Food Research Journal 18: 659-665*

GreenFacts, (2015) Facts on health and environment, http://www.greenfacts.org/favicon.ico Thursday, 30 July, 2015 12:59:54 PM

Greenpeace wwwgreenpeace.cn. (6/24/2013 12:16:16 pm

Gyasi, R.M., Mensah, C.M., Adjei, C.O. and Seth Agyemang, S. (2011) Public perception of the role of traditional medicine in the health care delivery system in Ghana. *Global Journal of Health Science*. **3**(2).

Jacobs, M. N., Johnston, P. A., Wyatt, C. L., Santillo, D., and French, M. C. (1997). Organochlorine pesticide and PCB residues in pharmaceutical, industrial and food grade fish oils, *International Journal of Environment and Pollution*, 8 (1-2): 74-93.

Mosleh, Y. Y., Mofeed, J., Almaghrabi, O. A. Kadasa, N. M., El-Alzahrani H. S., Fuller, M. P. (2014). Residues of heavy metals, PCDDs, PCDFs, and DL-PCBs some medicinal plants collected randomly from the Jeddah, central market, *Life Science Journal* 11(7): 1-8.

Oluromíniyi I, and Emily M . (2011). Agricultural pesticide contamination". In: Encyclopedia of Earth. Eds. Cutler J. Cleveland (Washington, D.C.: Environmental Information Coalition, National Council for Science and the Environment).

Storelli, M. M. (2013) PCBs and pesticide (DDTs) levels in aromatic herbs collected in selected areas of Southern Italy, *Environmental Science and Pollution Research* , 21(2)

WHO (2007) guidelines for assessing quality of herbal medicines with reference to contaminants and residues.