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PIXE Analysis Of Some Nigerian Biologically Active Plants

S.O. Olabanji[(*, a, b)], O.R. Omobuwajo[(c, d)], A.A. Elujoba[c], E.A. Sofowora[c], D. Ceccato[(a, e)], M.C. Buoso[(a)], and G. Moschini[(a, e)]

[(a)]*Istituto Nazionale di Fisica Nucleare (INFN), Laboratori Nazionali di Legnaro (LNL), I-35020 Legnaro, Padova, Italy.*
[(b)]*Centre for Energy Research and Development (CERD), Obafemi Awolowo University, Ile-Ife, Nigeria.*
[©]*Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria,*
[d]*Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Nigeria.*
[(e)]*Dipartimento di Fisica, Universita di Padova, I-35131 Padova, Italy.*

Abstract. PIXE analyses of some Nigerian biologically active plants were carried out. Two groups of plants were analyzed. These groups of plants are commonly used as medicinal plants in Nigeria for which pharmacopoeia standards are being established to enable their use for pharmaceutical purposes. These are Jatropha curcas Linn (Euphorbiaceae) – antimicrobial, Bridelia ferruginea Benth (Euphorbiaceae) – astringent and hypoglycemic agent, Momordica charantia Linn (Cucurbitaceae), Senna occidentalis Linn (Caesalpinaceae)- laxative, and Chromoleana odorata (L) R.M. King & Robinson (Asteraceae) antimicrobial. PIXE measurements were carried out using collimated proton beams produced by the 2.5 MV AN 2000 accelerator at Istituto Nazionale di Fisica Nucleare (INFN), Laboratori Nazionali di Legnaro (LNL), Padova, Italy. Twenty elements were detected in the samples. The results showed that none of the plants contains any toxic heavy metals such as Pb, As, Cd and Hg. Three plants Jatropha curcas (Nsukka) (4.6 ± 3.1 ppm), Chromoleana odorata (Ife) (11.6 ± 7.4 ppm) and Senna occidentalis (Ife) (5.7 ± 4.7) showed detectable levels of selenium. The plant samples obtained from Jos (Guinea Savannah) generally showed higher concentrations of some elements compared to the other three locations Nsukka (Derived Savannah), Ile-Ife (Rain forest) and Zaria (Sudan Savannah) from which plants were collected. The elements were relatively more highly concentrated in the leaves than the stem and bark. The results give a good picture of the distribution of elements in the plants from different locations, with evidence that environment has effect on the constituents of the plants.

Keywords: PIXE, Medicinal plants, Pharmacopoeia standards, Trace elements, Nigeria.

INTRODUCTION

The need to document medicinal plants commonly used in Nigeria and indeed in some regions of Africa continues to increase daily as even base line data on their major constituents including their mineral and elemental analysis are not available. These groups of plants (Table 1) are candidate plants on the list II of the African Pharmacopoeia (A. P. 1985) [1]. The medicinal plants namely *Jatropha curcas* Linn (Euphorbiaceae) – antimicrobial, *Bridelia ferruginea* Benth (Euphorbiaceae) – astringent and hypoglycemic agent, *Momordica charantia* Linn (Cucurbitaceae), *Senna occidentalis* Linn (Caesalpinaceae)- laxative, and *Chromoleana odorata* (L) R. M. King & Robinson (Asteraceae) antimicrobial are commonly used in Nigeria and are some of the plants for which pharmacopoeia standards are being set in their preparation for pharmaceutical use.

TABLE 1. The medicinal plants in Nigeria for which pharmacopoeia standards are being established.

Plant Species	Family	Plant's Part
Jatropha curcas Linn	Euphorbiaceae	Leaves
Bridelia ferruginea Benth	Euphorbiaceae	Leaves, Stem barks
Momordica charantia Linn	Cucurbitaceae	Leaves and Stems
Chromoleana Odorata (L) *R. M. King & Robinson*	Asteraceae	Leaves
Senna occidentalis Linn	Caesalpinaceae	Leaves, Root's bark, Fruits

Many parameters must be examined in the process of preparing medicinal plants for commercialization. A few of such important parameters are the mineral, trace element and heavy metal contents. The investigation of the chemical composition of herbs is of significance with regards to the development of naturally occurring resources. It is therefore important

to have base line data of the elemental composition of medicinal plants to be used as food or medicine. The standardization of Nigerian medicinal plants has been on in a bid to set appropriate pharmacopoeia standards for commonly used medicinal plants and obtain base line data on the quality of plants available especially in the wild (natural environment), in readiness for their inclusion in subsequent volumes of the Nigerian Herbal Pharmacy. The limit of toxic elements is of great concern as plants are known to take up from their environment many nutrients and such constituents that are taken up can be very important. The data obtained form the base line information for the analysis of these plants for their further use as pharmaceutical raw materials.

EXPERIMENTAL

Sampling and Plant Collection

The medicinal plants were collected from four representative geographical zones of Nigeria; the zones chosen were Ile-Ife (Rain forest), Zaria (Sudan Savannah), Jos (Guinea Savannah) and Nsukka (Derived Guinea Savannah), where the plants are known to exist naturally according to information obtained from the Forestry Research Institute of Nigeria (FRIN). Ile-Ife is in the Southwestern part of Nigeria, Nsukka is in Southeastern part, Zaria is in the Northern part and Jos is in the middle belt of Nigeria. Two to three plants of the same specie were collected in each location and combined together. All the collections were done during the rainy season, April to October, 2003. About 2 kg of each plant material was collected. All samples were thoroughly cleaned in de-ionized water and then air-dried. About 1g of the dried sample was homogenized by thoroughly mixing it together and grinding it into powder. About 260 mg of each sample was pelletized.

Analysis

The work was done at Istituto Nazionale di Fisica Nucleare (INFN), Legnaro (Padova), Italy with 2.5 MV AN – 2000 Van de Graaff accelerator. The proton beam of 6 mm diameter and energy 1.8 MeV was used to bombard the pelletized samples, which were coated with 5μg/cm^2 carbon to ensure good electrical contact. The emitted X-rays were detected using a 30 mm^2 Si (Li) detector with energy resolution of 180 eV (FWHM) at 5.9 keV. Homogenizing the beam on target samples was important and this was achieved with a 200μg/cm^2 gold diffuser. High dead times can constitute serious problems to good measurements hence a 150 μm thick Mylar was used to reduce the count rates, making the dead time to be generally less than 10% with beam currents of 5 – 20 nA. The details of the PIXE setup used in this work are given in Ref [2]. The generated X-ray data were stored and analyzed using the computer code GUPIX [3]. Two standards namely, the National Institute of Standard Reference Material SRM 1515 (Apple leaves) and NBS, SRM 1547 (Peach leaves) contain the elements of interest and were prepared in the same ways as the samples and run.

RESULTS AND DISCUSSIONS

The results of the analysis are presented in Table 2 which shows the detection of twenty elements in the medicinal plants. Many of these elements are very useful and are needed by the human body. The major elements detected include magnesium, aluminum, silicon, phosphorus, sulfur, chlorine, potassium and calcium. Some of the minor elements detected include manganese, iron, zinc, strontium and barium while the trace elements include titanium, vanadium, chromium, nickel, copper, bromine and rubidium. The comparison of our PIXE measurements with the certified values from the two standards was reasonably good. Although the roles of some of these elements have not been fully elucidated, it is becoming clear that many have effect on the biochemical systems in the body. There were no toxic heavy metals detected in all the medicinal plant species from different areas. The results serve as a good base line data.

Concentrations of elements in different plant parts differ also. From our results it can be observed that in some cases, the stem compared to other parts of the plant has the lowest amount of elements. Researchers have demonstrated that high levels of essential elements such as Fe, Mn, Zn, and Ca could influence the retention of toxic elements in humans [4]. From the results (Table 2) it is clear that location has an effect on chemical constituents as it is observed that all the plants samples obtained from Jos generally had the highest quantity of elements compared to those from other areas. Potassium, calcium, magnesium and silicon are the elements of greatest concentration in all the plants. In *Jatropha curcas* – a plant used for its antimicrobial properties and locally for treatment of skin diseases, certain elements appear to occur in the similar concentration range for the different samples. For this plant, only the Jos sample has significantly relative higher concentration of many of the elements. The concentrations of calcium and titanium for some plants are fairly in better agreement for Jos and Ife than for Zaria and Nsukka. The plant *Bridelia ferruginea* is used in the preparation of an astringent (Stem bark) and as a hypoglycemic agent (leaves). The *Bridelia ferruginea* leaves show relatively higher concentration of potassium for plants taken from

TABLE 2. Concentrations (ppm) of medicinal plants in Nigeria for which pharmacopoeia standards are being established.

PLANT SPECIES	Mg	Al	Si	P	S	Cl	K	Ca	Ti	V
Jatropha curcas (Ife)	9193 ± 130	1654 ± 107	7149 ± 78	4539 ± 63	2389 ± 41	5122 ± 42	30455 ± 48	24366 ± 82	13.1 ± 1.9	
Jatropha curcas (Zaria)	13073 ± 141	2464 ± 115	7583 ± 78	4146 ± 61	1960 ± 39	4421 ± 40	33850 ± 47	21304 ± 87	29.6 ± 2.4	
Jatropha curcas (Jos)	13278 ± 155	2903 ± 150	11955 ± 104	5980 ± 80	2191 ± 46	3233 ± 43	48451 ± 62	29165 ± 116	22.3 ± 2.4	
Jatropha curcas (Nsukka)	12257 ± 142	2660 ± 137	9647 ± 93	6933 ± 67	2480 ± 42	5052 ± 42	46836 ± 56	12223 ± 100	24.1 ± 2	
Bridelia ferruginea (Ife)	6240 ± 111	5325 ± 87	10208 ± 66	2458 ± 51	2506 ± 32	2679 ± 29	8295 ± 27	20822 ± 45	55.8 ± 2.9	
Bridelia ferruginea (Zaria)	5545 ± 96	3711 ± 65	4357 ± 45	1394 ± 36	1166 ± 23	545 ± 18	6793 ± 21	14497 ± 36	31.1 ± 3.2	
Bridelia ferruginea (Jos)	7422 ± 356	4848 ± 221	5082 ± 157	1842 ± 117	2083 ± 93	608 ± 72	5771 ± 74	18413 ± 119	55.8 ± 8.1	
Bridelia ferruginea (Ife)	5068 ± 92	709 ± 58	1306 ± 48	948 ± 59	532 ± 32	1906 ± 29	4567 ± 29	49710 ± 54	21.8 ± 2.6	5.4 ± 2.08
Bridelia ferruginea (Zaria)	8069 ± 97	1381 ± 63	2125 ± 46	1173 ± 53	459 ± 28	332 ± 23	10852 ± 33	41766 ± 62	39.6 ± 4.6	9.8 ± 3.47
Bridelia ferruginea (Jos)	5819 ± 94	753 ± 59	1229 ± 51	1088 ± 62	436 ± 33	2811 ± 32	5046 ± 31	53085 ± 58	17 ± 2.4	
Momordica charantia (Ife)	7448 ± 137	2264 ± 142	7037 ± 104	8750 ± 85	3923 ± 51	2157 ± 43	56690 ± 68	34270 ± 130	35 ± 2.6	
Momordica charantia (Zaria)	8533 ± 136	3948 ± 139	10093 ± 98	7242 ± 79	3960 ± 49	6176 ± 49	51964 ± 62	30386 ± 121	77.7 ± 3.2	8.4 ± 2.7
Momordica charantia (Jos)	8989 ± 142	3958 ± 151	13920 ± 109	7287 ± 91	3894 ± 53	4984 ± 48	43496 ± 65	39917 ± 115	46 ± 3.5	10.3 ± 2.8
Chromoleana Odorata (Ife)	7770 ± 111	2787 ± 105	11048 ± 86	6305 ± 75	5857 ± 54	12817 ± 53	26935 ± 43	19468 ± 73	22 ± 2	
Chromoleana Odorata (Nsukka)	12570 ± 181	4024 ± 144	9490 ± 97	6751 ± 83	5373 ± 59	12156 ± 54	28341 ± 45	23510 ± 79	30.1 ± 2.3	5.7 ± 1.87
Senna Occidentalis (Ife)	2984 ± 79	817 ± 56	1629 ± 42	2598 ± 38	1485 ± 25	2469 ± 24	12627 ± 27	11463 ± 42	14.9 ± 1.5	
Senna Occidentalis (Zaria)	8550 ± 123	2693 ± 103	3928 ± 73	5483 ± 65	5266 ± 47	5099 ± 42	34825 ± 48	26555 ± 90	44.1 ± 2.4	
Senna Occidentalis (Jos)	11041 ± 132	4066 ± 101	5460 ± 74	6715 ± 69	5762 ± 47	5991 ± 43	27319 ± 43	31278 ± 81	59.6 ± 3.3	
Senna Occidentalis (Zaria)		2853 ± 42	776 ± 29		6443 ± 39			24412 ± 31	370 ± 9	
Senna Occidentalis (Jos)	16541 ± 1523	14680 ± 849	11923 ± 532	16100 ± 447	7059 ± 444	13958 ± 221		51005 ± 71		
Senna Occidentalis (Nsukka)	6652 ± 101	2507 ± 82	1649 ± 63	9376 ± 61	3272 ± 38	2972 ± 31	29006 ± 40	19028 ± 74	10.9 ± 1.7	

Table 2 (Continued)

PLANT SPECIES	Cr	Mn	Fe	Ni	Cu	Zn	Br	Rb	Sr	Ba
Jatropha curcas (Ife)		70.4 ± 2.8	181 ± 4	9.09 ± 2.62	9 ± 2.07	28.6 ± 3.3			59 ± 17.6	
Jatropha curcas (Zaria)		49 ± 2.3	257 ± 4			9.59 ± 2.59				59.5 ± 9.3
Jatropha curcas (Jos)	6 ± 2.23	123 ± 3	176 ± 4			19.3 ± 3.7			51 ± 18	
Jatropha curcas (Nsukka)		101 ± 3	231 ± 5			15.5 ± 3.1	21.5 ± 7.4	37.2 ± 14.7		
Bridelia ferruginea (Ife)	7.5 ± 2.11	858 ± 7	426 ± 7		4.7 ± 1.83	43.7 ± 3.2	15.4 ± 5.8		192 ± 21	121 ± 11
Bridelia ferruginea (Zaria)		153 ± 3	196 ± 3	3.89 ± 1.49	10.1 ± 1.7	31.3 ± 2.7			38.6 ± 13.8	279 ± 9
Bridelia ferruginea (Jos)		379 ± 17	426 ± 22			60.7 ± 14.3				
Bridelia ferruginea (Ife)		187 ± 3	151 ± 4	16.6 ± 4.1		20.3 ± 2.8			331 ± 28	109 ± 9
Bridelia ferruginea (Zaria)		54.1 ± 2.6	333 ± 4		7.4 ± 2.26	20 ± 2.9			214 ± 23	507 ± 13
Bridelia ferruginea (Jos)		219 ± 4	123 ± 3	29.6 ± 4.6		29.8 ± 3.4			240 ± 24	56.8 ± 9.1
Momordica charantia (Ife)		38.1 ± 3	363 ± 6	26.1 ± 4.1	9 ± 2.94	29.3 ± 3.9				
Momordica charantia (Zaria)	13.5 ± 2.4	28.6 ± 3.1	781 ± 8			148 ± 6		69.2 ± 16.7		58.7 ± 13.6
Momordica charantia (Jos)		52.8 ± 3.6	769 ± 8		15.5 ± 3.1	96 ± 5		87.2 ± 20.6	87 ± 24.8	37.1 ± 14.3
Chromoleana Odorata (Ife)		70.2 ± 2.8	326 ± 5		37.3 ± 3	53.6 ± 3.9	52.2 ± 10.7			
Chromoleana Odorata (Nsukka)	6.59 ± 1.91	64.2 ± 2.9	420 ± 5		25.5 ± 3.1	90.4 ± 4.4	25.5 ± 7.3		39.7 ± 15.6	
Senna Occidentalis (Ife)		99 ± 2.4	107 ± 3		5 ± 1.59	18.8 ± 2.4	23.1 ± 6.4		50.7 ± 15.2	29.1 ± 5.6
Senna Occidentalis (Zaria)		48.7 ± 2.6	450 ± 6	12 ± 2.9	10.5 ± 2.4	68.5 ± 4.5		37.6 ± 12.9		
Senna Occidentalis (Jos)		86 ± 3.2	744 ± 7	8 ± 3.05	12.9 ± 2.3	80.7 ± 4.3		45.2 ± 15.8	61.8 ± 20	184 ± 12
Senna Occidentalis (Zaria)	7.79 ± 1.26	8.9 ± 1.46	45.8 ± 1.9			13.5 ± 2.4				
Senna Occidentalis (Jos)	157 ± 6	22.6 ± 7.8	59.5 ± 9.8		81.7 ± 16.2	94.5 ± 12.4				
Senna Occidentalis (Nsukka)		18.6 ± 1.8	175 ± 3		8.19 ± 2.1	59 ± 3.6		67.7 ± 13.2	40.2 ± 13.7	

Ile-Ife than for Zaria and Jos. However, for stem bark, the concentrations of potassium for samples taken from Ife are relatively lower than the ones from Zaria and Jos. Similarly, the *Bridelia ferruginea* leaves show a relatively higher concentration of Ca for plants taken from Ife than from Zaria and Jos. But, for the stem bark, the concentrations of calcium are fairly close for all the samples taken from Ife, Zaria and Jos. A similar close concentration for Ca is observed for *Momordica charantia* for leaves and stems taken from Ife, Zaria and Jos.

For *Jatropha curcas,* the concentrations of leaves for magnesium and aluminum for Zaria, Jos, and Nsukka are fairly close, but the concentrations of magnesium and aluminum from Ife are relatively lower than the ones from these regions. For *Momordica charantia,* the concentrations of magnesium and aluminum for leaves and stem from Zaria and Jos are in close agreement but higher than the ones from Ife. Generally, the concentrations of various elements from different plants and from different locations do not form a particular pattern.

Momordica charantia is an herb used as hypoglycemic agent locally. The same trend of high elemental content was observed in some samples from Zaria and Jos than the samples from Ile-Ife. Some of the plant samples did not show any contents of selenium. *Chromoleana odorata* is a plant used for its anti microbial properties. It is especially used to treat fresh wounds. No sample was obtained from Zaria and Jos. The results show that the samples from Nsukka generally show the higher content of elements than the ones from Ife.

Senna occidentalis (Leaves and Fruit) are used as laxative locally. Almost all the samples show no content of selenium, except the samples collected from Ife and Nsukka. Generally the results give a good picture of the distribution of elements in the plants from the different locations, with evidence that environment has effect on the constituents of the plants. It is therefore important in the collection of plants for preparation of herbal medicine to take note of the area of collection, to ensure uniformity of quality of drugs.

CONCLUSION

PIXE technique was employed for the elemental compositions of some listed medicinal plants that are commonly used in Nigeria. As the use of herbs increases in Africa and some other countries in the world, it is necessary to determine the quality of these herbs to ensure that safe standards are maintained. This work has made a modest contribution along that line and has also provided some base line data on the quality of some medicinal plants in their natural environment which is a gallant step towards setting the appropriate Pharmacopoeia standards for commonly used medicinal plants in Nigeria. The base line data of these plants provide information for their further use as pharmaceutical raw materials.

Twenty different elements were detected in the medicinal plants at different concentrations. Our results show that three medicinal plants namely Jatropha curcas [Nsukka], Chromoleana odorata [Ife], and Senna occidentalis [Ife] contain selenium with concentrations of (4.6 ± 3.1 ppm), (11.6 ± 7.4 ppm) and (5.7 ± 4.7 ppm) respectively. Selenium is a very important element in relation to cancer. Our results also showed that there were no toxic elements in all the medicinal plants and that location of plant species and their environments have effects on the chemical constituents of the plants. Thus in the preparation of the herbal medicine, care must be taken about the location of the plants to ensure uniformity and quality of the drugs to be produced from the medicinal plants.

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REFERENCES

1. African Pharmacopoeia. Organization of African Unity/STRC *Publication No.2, First Edition*, Lagos Nigeria 1985; pp 247-249.

2. Olabanji S.O., Buoso M.C., Ceccato D., Haque A.M.I., Cherubini R. and Moschini G. *Nucl. Instr. and Meth. In Phys. Res. B* 1996; **109/110:** 258.

3. Maxwell J.A., Teesdale W.J. and Campbell J.L., *Nucl. Instr. and Meth. Phys. Res. B* 1995; **95:** 407.

4. M. Ivey and G. Elmen (1986) Nutritional Supplements, Mineral and Vitamin Production in: Handbook of Non prescription Drugs, 8th Edn, American Pharmaceutical Association, The National Professional Society of Pharmacists, 2215 Constitution Avenue, N.W Washington, D.C. 20037, USA, 1986; 215.