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FROM NATURE TO DRUGS:
Theories and Realities

by

Saburi Adejinmi Adesanya
Professor of Pharmacognosy

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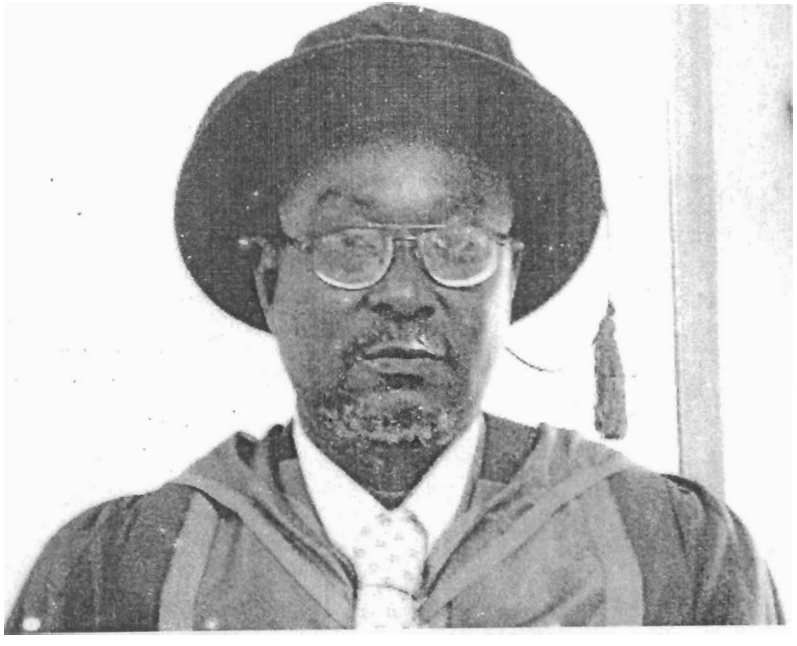

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INTRODUCTION

Mr. Vice-Chancellor Sir, permit me to start this lecture with a postscript.

In the past, it was the spirit of adventure which led the whites to Africa and beyond and the claim to have discovered River Niger even though Nigerians have been living with it for centuries. However, their curiosity did lead to discovery and development of drugs such as eserine/phystostigmin now in use in ophthalmic medicine. This was after observing the use of Calabar bean (*Physostigma venenosum* Balf.; Fam. Leguminosae) by Nigerians in Akwa Ibom/Cross River as ordeal poison (Sofowora, 1978). They discovered reserpine, the potent sedative from observing traditional medical practitioners in the treatment of psychiatric cases (Kokwaro, 1976). They did search other worlds and discovered quinine from Cinchona bark used by natives in Peru, for the treatment of malaria, this they used for a period, rejected it and came back to it for the treatment of special conditions of malaria like cerebral malaria (Trease and Evans, 1978). After independence, they also trained us in the use of their 'magic' which is research and let us loose in our various countries with the assumption that we can do everything ourselves and contribute to the new world order, but what has happened since then? Did the Europeans give us the goat without leaving the rope with which it was tied?

The early days of research in Nigeria with particular reference to this University witnessed some remarkable research breakthroughs that led to the 'Ife Brown' beans, the production of the yam pounder, Fagara in sickle cell management and mother tongue education. The Europeans have since moved ahead as nothing is static but how far have we changed in our ideas and action? Are we moving with the world? Are we apostles of the Yoruba saying '*eje ka se bi a tin se, kio le ri bio ti nri*' translated as 'lets do it the usual way so that we can keep on getting the usual results'.

This lecture attempts to put my work of over 25 years and me at the centre stage for your appraisal. After all, the great D.O Fagunwa did state in that classic '*Ogboju ode ninu igbo irunmale*' and I quote "*Lowe lowe la n'lulu agidigbo, Ologbon nii jo. Omoran nii mo*" translated by the Nobel Laureate Prof. Wole soyinka as "like the sonorous proverb do we drum the *agidigbo*, it is the wise who dance to it and the learned who understand the language" (Fagunwa, 1949; Soyinka, 1968). My lecture will be the veritable *agidigbo*, I will present it and it is you, the wise-heads who will interpret it.

Mr Vice-Chancellor Sir, ladies and gentlemen, it is in this light that I call you to listen to this lecture: From Nature to Drugs-theories and realities.

Nature in the context of this lecture is defined as all things not made by man which includes plants, animals, fungi, bacteria, marine fauna and flora, rocks, and others which are exploitable by man in the development of drugs. Drugs on the other hand, are 'preparations' developed to aid the recovery of man from a state of chemical and structural imbalance. The discovery of these preparations from nature involves many steps which in our context include the historical use of plants in ethno medicine and traditional medical practice to research into active plants and their co-generic species. It also includes identification and isolation of bioactive compounds, evaluation of their effects in animals and man and their management and rational use in medical practice. It is my belief that Pharmacognosy stands firmly in the middle of this chain and it is in this middle that my work stands as link between nature and drugs. This lecture supports earlier definitions of Pharmacognosy and its relationship with traditional medicine as presented at the two previous inaugural lectures in the field by the first two Professors in my department. These are 'Man, Plants and Medicines in Africa: some fundamental perspectives' by Prof A. Sofowora (Sofowora, 1981), (my supervisor and guru in traditional medicine research in Africa) and 'Pharmacognosy for health and culture - the PHC jungle connection' by Prof A. A. Elujoba (Elujoba, 1999). It also represents another block laid on the foundation made by these eminent scholars and other workers like Prof S. K. Adesina in 'Understanding the chemical nature of plants for better health' (Adesina, 1995), in the development of traditional medicines in Africa.

I started my career at the University of Ibadan where in my final year at the Department of Biochemistry I was introduced to thin layer chromatography and like most undergraduate students, I looked at colours separating on plate without any comprehension. Little did I know that this procedure will rule my research life? It was when I came over to the Faculty of Pharmacy, University of Ife (now Obafemi Awolowo University) to study for a Master of Philosophy degree in the Department of Pharmacognosy, at the direction of my brother and mentor, Prof A. Caxton-Martins, and later Ph.D at the School of Pharmacy, University of London, that I appreciated what this simple procedure coupled with spectroscopic techniques meant to drug discovery and development. These exposures together with opportunities to serve on the University Research Committee, the Board of Postgraduate College and as Editor-in-Chief of *The Nigerian Journal of Natural Products and*

Medicine (published by the Nigerian Society of Pharmacognosy) gave me a unique platform to evaluate the level of research in natural products in Nigeria.

Chromatographic separations have since progressed from the use of paper and silica gel to various reversed phase systems. High performance liquid chromatographic (HPLC) systems are now being coupled to several spectrometers for online measurements and structural determinations. Several medium pressure chromatographic systems are now being used to extract a plant and separate its constituents concurrently. This is in addition to several new techniques like lobar, droplet counter current chromatography (DCCC), and so on. Many non-destructive spectroscopic techniques like one dimensional and two dimensional nuclear magnetic resonance spectroscopy (1D- and 2D-NMR), Fourier-transform infra red spectroscopy (FT-IR), mass spectrometry (MS and MS-MS) are now used in the characterization and identification of compounds. The technology is advancing in leaps and bounds. However, the expertise in handling the instruments and teaching these methods is diminishing in our University system. This has greatly affected the capacity of local researchers to produce fruitful results in this field. In reality, separation science and spectroscopy is fast becoming a specialist field. Thus in these days of multidisciplinary research and diffuse boundaries between disciplines, research papers from Nigeria are particularly deficient in chromatographic analysis and structural elucidation of compounds.

I therefore, at this juncture commend the effort of the University administration in establishing the Central Science Laboratory to expose the students and researchers to modern instruments in many fields of science.

INFORMATION ON ETHNOMEDICINAL PREPARATIONS

It is an accepted fact that a large segment of the population in tropical countries rely on traditional medicines for their health needs. It is also known that many of these ethnomedicines have given up drugs to modern medical practice. The practices are well established and regulated in China, India and Europe. The regulation has led to a systematic scientific evaluation of these herbs leading to new medicines. A recent example is artemisine from *Artemisia annua* Linn. (Compositae) (Murray and Perkins, 1996). However, such structure and regulated use is still absent

in many African countries inspite of the widespread and heavy use of herbs by the local populace. This situation was recognized by early workers in the field. Sofowora (1984) in his book, *Medicinal Plants and Traditional Medicine in Africa*, highlighted the problems of getting information from traditional medical practitioners and associated this with problems encountered in carrying out research into such medicines. These problems include

i. Cost of purchasing information;
ii. Distrust of researchers by herbal practitioners;
iii. The desire to pass down information only to offsprings,
iv. Restrictions due to cult/trade membership;

These restrictions actually affected the level and type of information revealed to researchers such that in most books, only the plants are mentioned, the methods of preparation are often missing. Only in recent ethnobotanical surveys were records of mode of preparation included (AU/STRC 2004). There have also been various efforts in organizing herbal practitioners into associations, then registering them but these have not been successful. A viable structure would have provided a forum for the education of the traditional medical practitioners on benefits of research and intellectual property right. It is also a fact that these people are getting older and vital information is being lost (Sofowora, 1984 and Elujoba, 1999). What then is the current situation? How far have we gone in breaking down these barriers to information?

I was able to reassess this problem during my sabbatical leave at the University of Uyo between 2000 and 2001. In a survey of 255 herbalists spread throughout the 30 local government areas in Akwa Ibom State, it was found that the majority of the practitioners were illiterates (89%) and did not keep records on their patients. Moreover, most of these practitioners, who were between 50-59 years old, acquired the experience from their parents. They also had trained apprentices who were mainly their children. However, in urbanized local government areas, like Uyo and Eket, many of the children were in school. Thus, as child education progressed, information about traditional medical practice was being lost as they were not apprenticed. The inference from this, is that information is being lost rapidly in Africa due to lack of organization and the situation has not changed dramatically; three decades after research started in this field.

Thus, a researcher is left with no choice than to second guess the information found in literature, rely on accidental discovery or conduct

random investigations. The most reliable information is still that obtained from the trained practitioner. An example is the knowledge of the abortifaecient property of *Lagenaria brevifolia* Robert (Cucurbitaceae) given to Prof. A. Elujoba by his uncle who was a practitioner. Another veritable source of information is apprenticeship and joining the trade association, this is only for the brave due to perceived association of traditional medicine with cultic practices. I commend my senior colleague for successfully undergoing the apprenticeship and using the information acquired to start the 'Village Chemist' project. At least now, there is scientific observation of the effects of herbal preparations and a pragmatic approach towards development of local formulations. Those other scientists, who are not bold enough to take this route, are left with the other options mentioned above.

In our search for information and possibly organizing the traditional medical practitioners into manageable organizations with training curricula, we must not forget that the best discovery will have to come from those who are co-pilots in the management of diseases: the orthodox doctors. The patients that go to the herbal practitioner invariably end up in the hospital. In most cases these people use both the herbal practitioner's prescription or openly available recipes and the doctor's concurrently or one after the other. In some cases the patient comes to the doctor and confesses to having been cured or using some herbs before coming to the hospital. A careful investigation of these claims might lead to an even more credible discovery as was obtained for *Rauvolfia vomitoria* (L) Benth (Apocynaceae) and others. There must be a way of reporting these observations for researchers to work on.

JUSTIFICATION OF ETHNOMEDICINAL PREPARATIONS

The World Health Organisation (WHO), supports the use of traditional medicines provided it is proven to be efficacious and safe. The National Agency for Food, Drugs, Administration and Control (NAFDAC) has also started listing ethnomedicinal preparations on the market. The assumption is that since the African population has not drastically decreased over the centuries, then these preparations must have some truth about them. Perhaps the most prominent of the doubts about herbal preparations is based on the lack of a proof of their efficacy.

Validation of ethnomedicinal claims

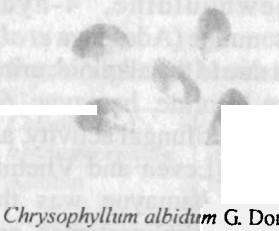
Early works at justifying traditional medicines in Ile-Ife include the proof that chewing sticks do kill oral microflora and therefore are effective in cleaning the mouth (El Said *et al*, 1971). The extract of *Senna podocarpa* Guill. et Perr.(Caesalpinaceae) pod (Asuwon ibile) used as purgative and worm expellant has been shown to be as good a laxative as commercial Senna (Elujoba and Iweibo, 1988). *Ocimum gratisimum* Linn. (Lamiaceae) (Efirin) oil calms overactive gut and thus cures diarrhoea. It demonstrates considerable inhibitory activity against strains of enteroaggregative *E. coli* irrespective of their antibiotic susceptibility spectrum (Orafidiya *et al*, 2000).

I, together with my colleagues and postgraduate students have also shown that the extracts of *Senna alata* Linn. (Caesalpinaceae) (Asunwon Oyinbo) (Caesalpinaceae) used as a purgative and for various skin diseases, such as ring worm and eczema, is antimicrobial and spasmolytic, thus justifying its use in stomach trouble. The flowers were actually more active than the leaves (Ogunti *et al*, 1991). The extract of *Newbouldia leavis* Seem (Bignoniaceae) (Akoko) root bark used in the treatment of stomach trouble, enlarged spleen, dysentery and earache is spasmolytic and a CNS depressant justifying its use in stomach troubles (Correira da Silva *et al*, 1965). *Chrysophyllum albidum* G. Don-Holl. (Sapotaceae) (Agbalum?) cotyledons, used to treat vaginal and dermatological infections, was found to be active against *Candida albicans and C. pseudotropicalis* confirming its efficacy against vaginal infections (Idowu *et al*, 2003). The stem of *Baphia nitida* Lodd. (Leguminosae) (Irosun), the leaves of *Pycnathus angolensis* Welw. (Myristicaceae) (Akomu), bulb of *Crinum jagus* (Thomps) Dandy (Amaryllidaceae) (Isu meri), leaves *Caliandra heamatocephala* Hask (Mimosaceae) and stem *Cissus quardrangularis* L. (Vitaceae) (Okun iyalode), used against various microbial infections are active against bacteria thereby justifying their use against skin infections (Omobuwajo *et al*, 1992; Adesanya *et al*, 1992; Nia *et al*, 1999; Adesanya *et al*, 1999). In addition C. *quandragularis* dehydrates the skin thus killing the top layer of the skin and the microbe, while *B. nitida* red pigment is used in local cosmetics.

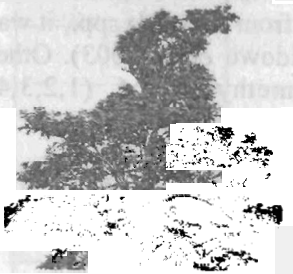
However, not all preparations are active on test models. For example it was found that *Adansonia digitata* L. (Bombacaceae) (*Ose*, Baobab tree) claimed to be effective in treating sickle cell anaemia patients was not active as an antisickling agent *in vitro* (Adesanya *et al*, 1988), but does

this prove that the plant is inactive? Is it right to look at direct effect only? What is the actual recipe used by the practitioner? Such results raise fundamental questions as to whether the test systems really relate to what is happening in the body. Traditional practitioners have always claimed that their treatment is for the whole body and not the symptoms alone. It is also rare to have preparations based on only one plant. The question then arises as to which of the components of the recipe is /are active? These questions raise the problems of looking at traditional medicines in the light of orthodox medicine. This exposes the inadequacy of the method of evaluating reports on single plants which is common in literature.

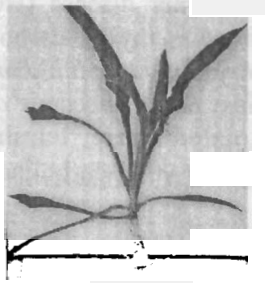
Senna alata Linn. (Caesalpinaceae) (Asunwon Oyinbo) (Caesalpinaceae)



Chrysophyllum albidum G. Don-Holl. (Sapotaceae) (Agbalumo)



Newbouldia leavis Seem (Bignoniaceae) (Akoko)

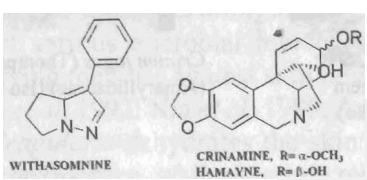


Crinum jagus (Thomps) Dandy (Amaryllidaceae) (Isu meri)

A search through literature showed that most local researchers end their work with only using crude soft extract of a single plant in justifying ethno-medicinal claims. The extracts were never separated and active compounds analyzed. The problem has to do principally with proper training in the field and lack of up to date items of equipment, a crisis not

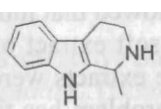
helped by the dearth of competent hands. However, it is pertinent to note that today, artemisinine and its derivatives are more popular and economic as an antimalarial than the extract of *Artemisia annua* Linn. (Compositae). So is reserpine from *Rauvolfia vomitoria*, quinine from *Cinchona succiruba* Linn. (Rubiaceae) and taxol from *Taxus brevifolia* Nutt. (Taxaceae) (McGuire *et al*, 1989).

At the Faculty of Pharmacy, we have tried to take many of such works to completion to really explain the observed activities. Recent works include the report that showed that the abortifaecient principle in *L. brevifolia* was due to the presence of saponins (Elujoba *et al*, 1990). I have shown that the spasmolytic activity of *Newbouldia leavis* is due to pyrazole alkaloid, withasominie. Other new alkaloids described in the plant are newbouldine, 4-hydroxy newbouldine and 4-hydroxywithasomnine (Adesanya *et al*, 1994). The antibacterial activity of *C. jagus* was due to the alkaloid, crinamine. Other alkaloids described in the plant are lycorine, hamayne, 6-hydroxycrinamine. None of the alkaloids had any antifungal activity, although other activities have been reported for them (Leven and Vlietnick, 1981). Recently, it was also demonstrated that hamayne was the alkaloid responsible for the inhibition of acetylcholinesterase enzyme indicating a role in the treatment of Alzheimer disease (Houghton *et al*, 2004). Eleaginine (1,2,3,4tetrahydro-1-methyl-β-carboline), was the main antimicrobial compound in *Chrysophyllum albidum*. Apart from *Candida* spp., it was also active against *Staphyloccocus aureus* (Idowu *et al*, 2003). Other compounds isolated were tetrahydro-2-methylharman (1,2,3,4-tetrahydro-1,2-dimethyl-β-carboline) and skatole (3-methylindole).



WITHASOMNINE

CRINAMINE, R= α-OCH_3
HAMAYNE, R= β-OH



ELEAGININE

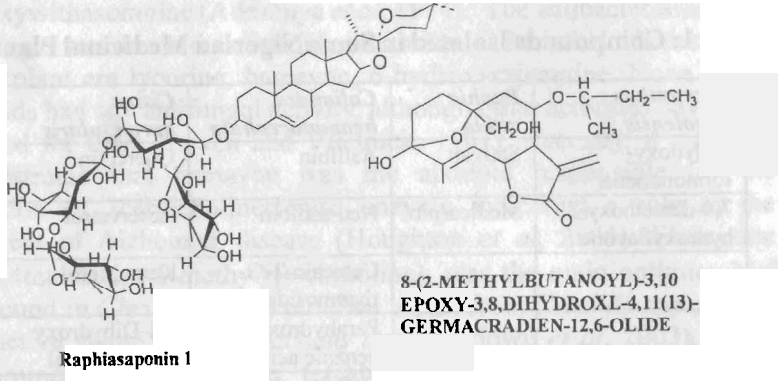
However, it was not all the time that new compounds were isolated or that the results directly explained the activity observed for the plant preparation. For example, we found that the active fractions of *Calliandra heamatocephala*, *Cissus quadrangularis*, *Baphia nitida* and *Pycnathus angolensis* contain mainly different kinds of flavonoids with low activities that were not comparable to those of the extracts (Table 1) (Omobuwajo *et al*, 1992; Nia *et al*, 1999; Adesanya *et al*, 1999). In some cases the activity might be due to high concentrations of known compounds. It was found that the active antimicrobial compound in *Dacroydes edulis* (G. Don) H. J. Lam (Burseraceae) (Elemi) was due to a high concentration of gallic acid in the leaves (Ajibesin, 2005), while the anti-gonorrhoeal compound in *Gladiolus* species (Baka) might be due to fructose in the bulb as has been reported for other plants.

Table 1: Compounds Isolated in Some Nigerian Medicinal Plants

Pycnanthus angolensis	*Baphia nitida*	*Caliandra heamatocephala*	*Cissus qurangularis*
2-Hydoxy-formononetin	Sativan	Astilbin	Quercetin
7,4-demethoxy-2'-hydroxyflavone	Medicarpin	Neo astilbin	Reservatrol
		Catechin-3-O-rhamnoside	Keampferol
		Parahydroxy benzoic acid	3.4-Dihydroxy benyl ethanol
		Caffeic acid	Piceatannol
		Protocatechuic acid	Palliadol
		Lupeol	Parthencissin A
		Betulinic acid	Quadrangularin A
			Quadrangularin B
			Quadrangularin C

Traditionally, many preparations are used for purposes other than medicine. These include cosmetics, dyeing, painting, fishing and carving. Justifying their use could also lead to the development of the preparations for better results. One of such is the use of the fruit of *Raphia hookeri* G. Mann et Wendland (Arecaceae) (Oguro) for stupefying fish in the Niger Delta region of Nigeria. The active compound was identified as a saponin and characterized as diosgenin-3β-O- α-L-rhamnopyranosyl-(1→3)-β-

D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside and named raphia-saponin 1 (Obuotor 2004). It gave LC_{50} of 0.82μg/ml in 24hr acute toxicity test using *Clarias gariepinus* (African catfish, Aro). It also gave HI_{50} of 6.64 μg/ml in a standard haemolytic test. A new sesquiterpene lactone, 8-(2-methylbutanoyl)-3,10-epoxy-3,8-dihydroxyl-4,11,(13)-germacradien-12,6-olide was isolated from antimalarial fraction of the aerial part of the plant *Tithonia diversifolia* (Hemsley) A. Gray (Asteraceae) (Elufioye *et al*, 2005). It is remarkable to note that these two compounds were characterized using mostly the services of the Central Science Laboratory of this University.



Raphiasaponin 1

8-(2-METHYLBUTANOYL)-3,10 EPOXY-3,8,DIHYDROXL-4,11(13)-GERMACRADIEN-12,6-OLIDE

Irrespective of the results from these studies the isolated compounds are important as leads in drug development and in standardizing the herbal preparations. They are also useful in monitoring the disposition of herb constituents in man and monitoring herb-orthodox drug interactions in the emerging field of herbal pharmacokinetics.

Safety studies

However, many plants in our environment are toxic and there is currently no comprehensive information on such plants to serve at least as a guide to users and by so doing assist in complying with the WHO directive on the use of traditional medicines. This directive encourages developing countries to supplement their health programme with traditional herbal preparations provided it is proven to be non-toxic (WHO 1985). The problem of toxicity is more relevant when one realizes that even now

mistakes are made with the collection of food plant and this usually leads to death of families. A common example is pounded yam. It is known that some species of yam are poisonous e.g *D. dumentorum* Pax. (Dioscoraceae) (*Esuru*) with the poison being discorine and dihydrodiscorine (Williaman *et al*, 1953). However, cross pollination and hybridization may lead to hybrids that are poisonous but are confused with the food plants. Another example is *Stachytapheta indica* (Verbanaceae) (Iru eku) which is used in Akwa Ibom for malaria but is toxic at high doses, while its co-generic specie, *S. jamaicensis* (Iru eku) is a sedative and non-toxic. These examples emphasize the need for care in collecting plants and the importance of taxonomy and proper identification of plants. Lack of proper identification, hybridization and miscollection of co-generic species are the major reasons why some plants reported to be active may appear not to be at times.

The current testing methods in literature rely almost solely on whole animal lethality using rats and mice mostly. There are fewer studies looking at the enzyme activities and organ damage to assess toxicity of the commonly used herbs. However, these tests raise more questions than they answer. For example, will these results be transferable to situations in man given that some of these preparations have been used by herbal practitioners for a long time. The fact that these plants have always been collected in the wild could lead to collection of non-toxic species or varieties due to intraspecies variations. In addition, what route of administration is relevant and how can long term toxicity be evaluated? In the absence of any alternative, the rat still seems the most relevant animal for a preliminary evaluation.

Senna podocarpa leaves are extensively used as a purgative and also as a guinea worm and sore healing remedy in Southern Nigeria. It has been extensively investigated as laxative and a replacement for *Senna acutifolia*, the official Senna of the British Pharmacopoeia (Elujoba and Iweibo, 1988). Repeated oral administration of both plants for four weeks to rats resulted in tissue degenerative changes in the liver and kidneys for both plants at doses between 1 and 5g/kg. Testicular effects were observed at the low doses (Adefemi *et al*, 1988). We have also looked at *Khaya ivorensis* A. Chev. (Meliaceae) (Oganwo) a common feature in antimalarial recipe prescribed by traditional medical practitioners. Earlier investigations had shown that the plant possesses some level of antiplasmodial activity. Oral administration of the ethanolic extract daily for two weeks followed by examination of the brain, spleen, heart, liver and kidneys for dismorphological features

showed that it has some level of tissue toxicity especially nephrotoxicity which was dose dependent. Morphological abnormalities were also found within the white matter of the cerebral cortex but these toxicities were reversible at low doses. However, the margin between therapeutic dose (100mg/kg) in rat and the toxic dose of 500-1000mg/kg makes it safe to be used (Agbedahunsi *et al*, 2004). We also investigated plants implicated as poisons in our survey of traditional medical doctors and ordinary people in Akwa Ibom State. Ethanolic extracts of seven plants were screened using rats and the LD_{50} estimated. The results showed variations in mode to toxicity. Only the extract of *Stachytapheta indica* was toxic by oral and intraperitoneal route. *Anchomanis difformis* (Bill) Engl. (Araceae) (Opego) and *Caladium bicolor* L. (Araceae) tubers were not toxic by either oral (OP) or intraperitoneal (IP) administration. *Teleferia occidentalis* Hook (Curcubitaceae) (Ugwu) root, *Coula edulis* Bail (Olacaceae) (Ekom) roots, *Euphorbia kamerounica* Pax. (Euphorbiaceae) (Oro agogo) leaves, *Pterocarpus milbreadii* L. (Fabaceae) (White camwood, Mkpa) root, and *Blighia sapinda* K. Konig (Sapindaceae) (Isin) leaves were toxic to various extents through the intraperitoneal route. Although the fruit and seeds of *B. sapinda* are known to be poisonous, this is the first report of the toxicity of its leaves by IP route (Ajibesin *et al*, 2002). *C. bicolor* is known to contain a high concentration of calcium oxalate crystals that cause the mouth to itch on consumption. This result showed that the route of administration and mode of preparation are important in demonstrating toxicity of plants.

STUDIES BASED ON INFORMATION FROM OTHER SOURCES

Although ethnomedicine provides a ready source of materials to work with, there are other methods of selecting plants. These include a systematic evaluation of plants in a certain defined area, exploitation of biochemical pathways or simple serendipity.

Antisickling studies

Sickle cell disease is prevalent in Africa South of the Sahara and it has been estimated that about 45% of Nigerians carry the gene. Offspring with the SS gene become sicklers and bear the brunt of this incurable disease. Although modern management of the disease is well established, it is correct to assume that traditional herbalists have been

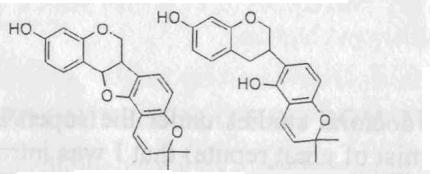
managing it for some generation. The story of the accidental discovery of the root of 'Fagara' *Zanthoxylum xanthoxyloides* (Lam) Waterm. (Rutaceae) (Orin ata) for the management of sickle cell patient by Professors Sofowora and Isaacs-Sodeye and others in this University is also well known. The extract of Fagara reversed already sickled cell red blood cells to normal *in vitro*. The active constituents were later identified as phenolic acids. This discovery led to the search of co-generic species for plants with higher activity. A biological screen of species growing in Nigeria showed that *Z. gilletti* (De Wild) Waterm. (Syn; *F. macrophylla*) is significantly more active than *Z. xanthoxyloides*. The other less active species are *Z. rigidifolium, Z. leprieurii* and *Z. lemairai* (Adesanya and Sofowora, 1983).

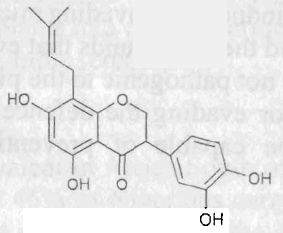
Microscopic examination of the reversed red cells during antisickling assays showed that such cells actually did not return to normal biconcave cells as confirmed by other workers but to a round shape raising the question of viability of such cells in the microvasculature as the extract had been shown to be non-toxic in different toxicity models (Headings *et al*, 1979; Isaacs-Sodeye *et al*, 1975). This suggested that these membrane acting agents might affect the enzymes in the red blood cell. Our studies showed that the treated red cells possess active enzymes particularly glucose 6-phosphate and 6-phosphogluconate dehydrogenases and these enzymes did not leak from the cells (Osoba *et al*, 1989). However, the membrane acting agents in the extract may affect the membrane cation permeability and Ca^{2+} mobilization in the membrane (Honig *et al*, 1978). The actual role of these compounds in the loss of biconcave shape is yet to be explained.

Phytoalexins

It was during my doctoral studies under the supervision of Dr. M. F. Roberts (a biochemist of great repute) that I was introduced to the idea that plants have a chemical defence system called phytoalexins. These are small chemicals induced by invading microbes leading to synthesis of mRNA, enzymes and the compounds that eventually ward off the attack of microbes that are not pathogenic to the plant as pathogenic ones have their own system for evading the defence. The theory was that such compounds may be capable of preventing the growth of human pathogens.

In our work, these compounds were successfully induced in a few beans species (Family, Leguminosae, order; Phaseolinae) *Phaseolus vulgaris*, *P. lunatus*, *P. mungo*, *P. coccineus*, and *P. aureus*. Comparative thin layer chromatography and bioautography of the healthy and diseased seedlings led to the isolation and structural elucidation of several isoflavonoids as the phytoalexins in these plants (Table 2). The major and most common phytoalexins were phaseollin and kievitone. They were shown to accumulate over time only in the diseased plants (O'Neill *et al*, 1983; Adesanya *et al*, 1984; 1985a; 1985b; O'Neill *et al*, 1986). Microscopic examination revealed the gross effects of active isoflavonoids on the development of hyphae of the fungus *Cladosporium cucmerinum*, as disorganized growth patterns involving swollen hyphal tips, cellular granulation, and the production of septal plugs (Smith, 1978; Adesanya *et al*., 1986). Results of comparative antifungal studies, using *Aspergillus niger* and *Cladosporium cucumerinum*, of all the 26 compounds isolated with good yield, their chemical derivatives together with demethylmedicarpin, medicarpin, and isomedicarpin isolated from *Trifolium repens* (Table 2) showed that all the compounds, except phaseollinisoflavan, were not particularly active especially against *A. niger* (causative agent of some type of aspergillosis in man) indicating that they are not likely to be useful as antifungal agent in man. Other investigations have shown that phaseollinisoflavan is also very active against zoopathogens (Smith, 1982).





The fact that the process involved genetic induction led to the use of these compounds as chemotaxonomic markers in other plant families. The distinction between *Vigna* and *Phaseolus* species has long been a matter of taxonomic interest. However, most of the compounds, including those with isoflavan structures that occur in the genera Phaseolus, Vigna, Lablab, and Erythrina, are of little chemotaxonomic importance. Our work taken with other investigations clearly showed that the ability to produce methoxylated derivatives, once thought to be restricted to Vigna, is far more widespread. Only phaseollinisoflavan found in the typical *Phaseolus* species, *P. vulgaris* and *P. coccineus*, may be of chemotaxonomic importance. It is interesting to note that *P. lunatus* produced no cyclized derivative of its many prenylated compounds and has the novel 8-hydroxylation pathway. These results support earlier observations that *P. lunatus* is different from other *Phaseolus* species in its protein characteristics (Kloz, 1962; Kloz and Klozova, 1974; Ingham 1990).

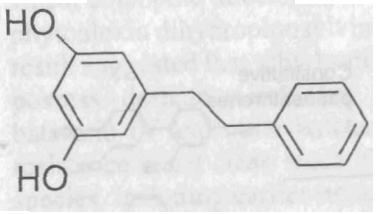
These studies were later extended to a few species of yams (*Dioscorea* species) grown in Nigeria with the aim of evaluating their potential in prolonging dormancy, disease resistance, crop protection, and chemotaxonomy in the various sections. These tubers deteriorate rapidly on storage due to microbial infection, with an estimated loss of over half of the yearly harvests in Nigeria (Degras, 1993). Thus, concerted efforts are being made to find ways of increasing their shelf life (dormancy period) and also prevent microbial susceptibility.

The peel of *D. rotundata* (White yam, isu funfun) has been shown by previous workers to contain the constitutive antifungal phenanthrenes, batatasin I and hircinol (Coxon *et al*., 1982), and we showed that the inducible compounds in diseased *D. rotundata*, *D. alata* (water yam, isu ewura), *D. dumentorum* (Esuru), *D. bulbifera* (Isu awun), and *D. mangenotiana* (Elephant yam, dagi daji, Ege Esusu)) are dihydrostilbenes (Table 3) with dihydropinosylvin being the most common and abundant in some species and absent in others (Table 3) (Fagboun *et al*, 1987; Adesanya *et al*, 1989; Cline *et al*, 1989; Kaganda and Adesanya, 1990) (Table 3). Other compounds isolated are batatasin III, batasin IV, demethylbatatasin IV, dihydroresveratrol, 3, 5 dimethoxydihyrostilbene with three of them being reported in literature for the first time. We also showed that dihydropinosylvin and batatasin IV are significantly more toxic (<25 µg/ml, respectively) to *Aspergillus fumigatus* than demethylbatatasin IV (>100 µg/ml), and dihydroresveratrol was not active (Adesanya *et al*, 1989). This suggested

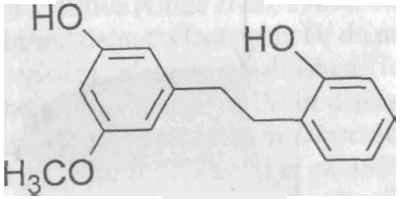
Table 2: Inhibition of Spore Germination in *Cladosporium cucumerinum* and *Aspergillus niger* by isolated Isoflavonoids

Compound		Minimum inhibitory concentration (μg ml^{-1})	
		C. cucumerinum	A. niger
Isoflavones			
i.	Daidzen	50-75	50-75
ii.	Genistein	50-75	50-75
iii.	Isoprunetin	50-75	25-50
iv.	2'- Hydroxydaidzein	25-50	50-75
v.	2'- Hydroxygenistein	50-75	25-50
vi.	2'- Methoxygenistein	50-75	25-50
vii.	2'- Hydroxyisoprunetin	25-50	50-75
viii.	2,3- Dehydrokievitone	25-50	25-50
ix.	2,3- Dehydrokievitol	>100	>100
x.	Phaseoluteone	10-25	10-25
Isoflavnones			
xi.	Dalbergioidin	75-100	50-75
xii.	Isoferreirin	10-25	10-25
xiii.	5-Deoxykievitone	25-50	50-75
xiv.	Kievitone	10-25	25-50
xv.	Kievitone. Hydrate	>100	>100
xvi.	2',4',5',7-Tetramethyl-	>100	>100
xvii.	2',4',5-	25-50	25-50
xviii.	Kievitol	>100	>100
xix.	3'(γ,γ-dimethylallyl)-	25-50	25-50
xx.	Cyclokievitone	50-75	50-75
xxi.	2',4',5-	>100	>100
xxii.	1'',2''-	25-50	50-75
Pterocarpans			
xxiii.	Demethylmedicarpin	25-50	50-75
xxiv.	Isomedicarpin	10-25	50-75
xxv.	Medicarpin	10-25	25-50
xxvi.	Phaseollidin	10-25	40-50
xxvii.	Phaseollin	25-50	50-60
Isoflavans			
xxviii.	Demethylvestitol	25-50	50-75
xxix.	Phaseollinisoflavan	10-25	50-75
Coumestan			
xxx.	Aureol	25-50	50-75

their possible use as antifungal in man. Similar compounds were also isolated in *D. batatas* (Japanese yam) by researchers in Japan and they also showed that induced phenarthrenes and dihydrostilbenes are generally more effective against fungi than bacteria, and the dihydrostilbenes more active than the phenanathrenes (Takasugi *et al*, 1987).



DIHYDROPINOSYLVIN



BATATASIN IV

Thus it appears that the phenanthrenes might be part of the first line of chemical defence in the yam tuber and when this is breached by microbes (Figure 1); the hypersensitivity reaction in the infected cells leads to induction and accumulation of dihydrostilbenes in the surrounding next layer of cells. These compounds assist in stopping the invading microbe if it is non-pathogenic to yam (Figure 1). The lack of activity of demethylbatatasin IV suggests that it could be a product of fungal/plant detoxification process.

It is interesting to note that while all the other species contain an appreciable amount of dihydropinosylvin after induction, *D. magenotiana* contains no appreciable amount of this phytoalexin and rots considerable faster than other species suggesting that the level of induced compounds in the plant might actually bear a correlation with susceptibility. Chemical investigations in breeding experiments have also linked resistance to anthracnose disease in *D. alata* to the level of phenolics in various yam cultivars (Alozie *et al.*, 1987) indicating a possible role for inducible compounds in the prediction of species and cultivars resistant to infection.

Figure 1. Part of the chemical defence of yams

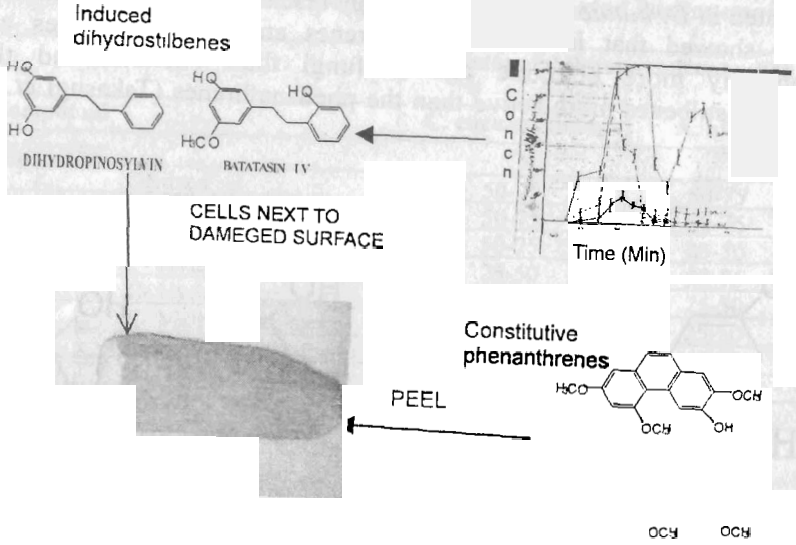


Table 3: Occurrence of bibenzyl Phytoalexins in some Nigerian *Dioscorea* species

Species	Dihydro-pinosylvin	Demethyl-batatasin IV	Batatasin IV	Dihydro-resveratrol	4,2'-dihydroxy-3,5-dimethoxybibenzyl
D. rotundata	+++++	++	++		
D. alata	++++	+++++	++		
D. mangenotiana	+				++
D. dumentorum	+++			+++++	
D. bulbifera	++++	+++++			

Key: + shows level of abundance

DORMANCY STUDIES

Constitutive compounds responsible for dormancy in the bulbils of *D. batatas* have been isolated and characterized as the phenanthrene batatasin I and the dihydrostilbenes (bibenzyls) batatasin II, III, IV and V (Hashimoto *et al*., 1972; Hashimoto and Tajima, 1978, Cline *et al*, 1989). The levels of these compounds vary significantly in different species (Ireland *et al*, 1981). Batatatin IV and batatasin III also inhibited lettuce and *Sorghum bicolor* seed germination, hypocotyls elongation, and wheat coleoptile development (Hasimoto and Tajima, 1978). The major phytoalexin dihydropinosylvin was not as active (Cline *et al*., 1989). The result suggested that dihydropinosylvin and demethylbatatasin IV do not possess dormancy-inducing characteristics already established for batatasin IV and other batatasins. The role of batatasin IV in disease resistance is not clear, since it accumulates to a low level in *Dioscorea* species, including earlier investigated *D. batatas* (Takasugi *et al*, 1987; Cline *et al*, 1989), (Figure 1) and has also been reported to occur naturally in *D. batatas* by some other workers (Hashimoto *et al*, 1972).

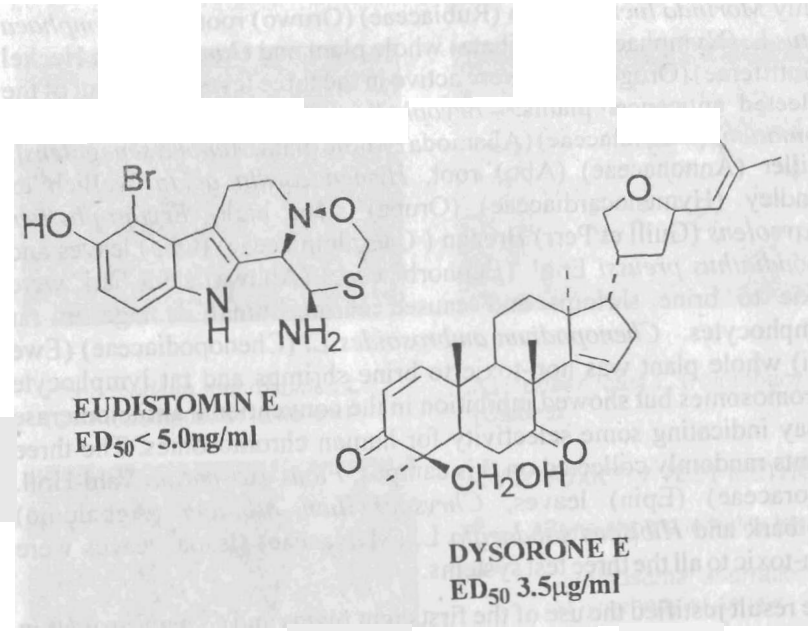
Literature search revealed that dihydropinosylvin and dihyroresveratrol were derivatives of stilbenes, pinosylvin and trans-resveratrol, respectively. The stilbenes are proven antimicrobial compounds existing in diseased *Pinus* and *Arachis* species, respectively (Ingham, 1976; Schoppner and Kindl, 1979). We are aware of the various activities associated with stilbenes in literature which include oestrogenic and anticancer activities. (George *et al*, 1988; Juneja *et al*, 1985). The yields of these induced compounds are also always too low for evaluation on other biological models.

An attempt to increase the effectiveness of dihyropinosylvin with my colleagues Prof. Ogundaini and late Prof F. O. Ogungbamila led to the design and synthesis of 6 dihydrostilbenes based on this major phytoalexin. These products together with their stilbene and alcohol derivatives were tested on lethality to brine shrimp and inhibition to seed germination. None of the compounds synthesized was as active as dihydropinosylvin (Sogbaike *et al*, 2002). I must say and we would have liked to continue the work by designing and synthesizing other analogues but it had to be terminated due to lack of adequate laboratory for some of the chemical reactions involved in this work.

TOXICITY AND ANTICANCER STUDIES

I was introduced to cytotoxicity and anticancer studies during my postdoctoral visits to the Institut de Chimie des Substances Naturelles (ICSN), CNRS, Gif-sur-Yvete, France. There, I was challenged with isolating the cytotoxic constituents of materials collected as a result of systematic biological survey of the fauna and flora of New Caledonia in the pacific and Vietnam. One of such plants was *Dysoxylum roseum* C. DC. (Meliaceae). *D. roseum* is used as fish poison and for alleviating aches and pains while members of the family have been shown to be antibacterial, CNS depressant, antiinflammatory, immunomodulatory and cardioactive. Earlier works on co-generic species had shown the presence of alkaloids and these were presumed to be the most likely active constituents. However, to our surprise the active turned out to be apotitucallane triterpenes, dysorones A, B, C, D and E with dyrosone E exhibiting moderate toxicity ED_{50} 3.5 µg/ml against human buccal carcinoma KB cell lines *in vitro* (Adesanya *et al*, 1991). Similar activity directed fractionation of the marine tunicate *Eudistoma album* F. Monniot. (Polycitorodae) led to isolation of brominated -carboline alkaloids, Eudistomin E, which is already known for its potent antiviral activity and novel eudistalbin A. Both were active at ED_{50} <5.0ng and 3.2 g/ml respectively while the third eudistalbin B is a new inactive natural product (Adesanya *et al* 1992). Several hederagenin saponins together with novel farnesyl glycoside, (1-O-α-L-rhamnopyranosyl-(1-6)-α-L-rhamnosyl-(1-2)-α-L-arabino-pyranosyl-(1-3)-β-glucopyranosyl-alltrans-farnes-1-ol), were isolated from *Lepisanthes rubiginosa* (Roxb.) Leenh. (Sapindaceae) collected from Vietnam. The non-specific toxicity of this plant *in vivo* in mice bearing P339 murine leukemia and human lung A549 tumour cells *in vitro* was not retained by any of the ten isolated compounds (Adesanya *et al*, 1999).

Two important lessons were learnt from this work. The first is that when working with natural products, it is better to have an open mind as the fixation on alkaloids by my hosts irrespective of the fact that an apotrucallane triterpene, dysobinin, with significant CNS depressant action was isolated from *D. ferum* led to a waste of resources and time in trying to repeat all known procedures for isolating alkaloids. The second was with eudistalbin B, which defied separation on advanced separation systems like HPLC, but was eventually purified by the old simple preparative thin layer chromatography.



It however did not take me long to realize that such isotope labelled biological assays using tissue cultured cancer cell lines are not going to be easy in Nigeria given the lack of suitable items of equipment and high cost of materials. In search of a locally viable option for screening plants for cytotoxicity, I teamed up with Prof. I. Awopetu of the Department of Zoology and Dr. F. A. Fakoya of the Department of Cell biology and Anatomy to develop an *in vivo* model for evaluating chromosomal aberrations in rats' lymphocytes as an indication of cytotoxicity and anticancer potential of plants. Currently, 9 plants common in recipes used in the management of cancer in SouthWestern Nigeria have been investigated for toxicity to brine shrimps and induction of chromosomal aberrations in rat lymphocytes. Our initial results presented at Conference in Canada led to several inquiries about our work and requests for collaboration. My PhD student, Mrs. A. Sowemimo, was then invited the Ontario Cancer Institute, University of Toronto, Canada to extend our screen to the inhibition of telomerase enzymes in human immortal cell lines (Cancer cells). This enzyme is known to accumulate preferentially in cancer cells and lead to continuous division of such cells.

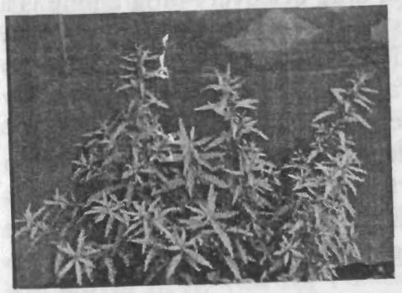
Only *Morinda lucida* Benth (Rubiaceae) (Oruwo) root bark. *Nymphaea lotus* L. (Nymphaceae) (Osibata) whole plant and *Garcinia kola* Heckel (Guttiferae) (Orogbo) root were active in the three test systems out of the selected anticancer plants. *Bryophyllum calycinum* Salisb (syn; *B. pinnatum*) (Crassulaceae) (Abamoda) whole plant, *Annona senegalensis* Miller (Annonaceae) (Abo) root, *Hymenocardia acida* Wallich ex Lindley (Hymenocardiaceae) (Orupa) stem bark, *Erythrophyllum suaveolens* (Guill et Perr) Brenan (Caesalpiniaceae) (Obo) leaves and *Spondiathus preussi* Engl. (Euphorbiaceae) (Abuwa) stem bark were toxic to brine shrimps and caused chromosomal damage in rat lymphocytes. *Chenopodium ambrosoides* L. (Chenopodiaceae) (Ewe imi) whole plant was non-toxic to brine shrimps and rat lymphocyte chromosomes but showed inhibition in the conventional antitelomerase assay indicating some selectivity for human chromosomes. The three plants randomly collected on this campus, *Ficus exasperata* Vahl-Holl. (Moraceae) (Epin) leaves, *Chrysophyllum albidum* (Agbalumo) rootbark and *Hibiscus sabdariffa* L. (Malvaceae) (Isapa) leaves were non-toxic to all the three test systems.

The result justified the use of the first eight plants and *C. ambrosoides* in the management of cancer in South West Nigeria although they appear to be nonselective and their mode of action may be different. All these plants, except *C. ambrosoides,* are also mutagenic and cytotoxic. *G. kola, M. lucida, C. ambrosoides* and *N. lotus* are the ones most likely to have anticancer properties since they act through the telomerase system. *C. ambrosoides* was capable of telomerase inhibition without exhibiting mutagenic activity *in vivo* which bodes well for the potential selectivity of the plant.

The student is now on a visit to another laboratory in Germany, to continue the work.

CONCLUSION

In my trip through the forest, I have found and presented information on over 30 plants. In the process, I have also isolated and described over seventy five compounds in literature with many of them new natural products. The path was smoothened by funds from many sources particularly scholarship and fellowships from organizations that include the Federal Government of Nigeria; Obafemi Awolowo University, Ile-Ife; AU/STRC, Lagos; ICSN/CNRS, Gif-sur-Yvette,



Chenopodium ambrosoides L. (Chenopodiaceae) (Ewe imi)

Nymphaea lotus L. (Nymphaceae) (Osibata)

Morinda lucida Benth (Rubiaceae) (Oruwo)

CYTOTOXICITY TEST METHODS

- Brine shrimp lethality test
- Chromosomal aberrations in rat lymphocytes *in vivo*
- Inhibition of Telomerase enzyme in human immortal cells *in vitro*

France; French Embassy, Lagos. Research grants were also obtained from the University Research Committee, International Foundation of Science (IFS), the International Programme in Chemical Science (IPICS), Sweden and Wellcome Trust, Nigeria. These works have also produced three PhDs (Drs O. R. Omobuwajo, R. Nia and E. Obuotor) and five MSc graduates of this University.

I hope by this lecture that I have shown that one has to have an open mind when working with nature. While looking for new medicines and justifying medicinal properties of plants, the results obtained could have potential uses in other fields such as botany, microbiology and agriculture. Knowing structures of active compounds is also important in

development and synthesis of other potentially useful compounds. I have also shown that the key to a successful drug discovery programme is a viable biological assay system. This is perhaps the greatest problem facing our chemists today. Collaboration across many disciplines is thus desirable and provision of adequate facilities is crucial. It is certainly more useful to Africans to know that our medicines are active and non-toxic but it is more rewarding to isolate the active principle and develop drugs from them. However, it is pertinent to note that this process is tedious and tortuous. It is also becoming more and more expensive with newer technologies and Nigeria is gradually losing the capacity to compete in this field.

It is hoped that the Nigerian Institute of Pharmaceutical Research and Development (NIPRD), in Abuja will rise to the challenge and offer creditable and certifiable services in providing standard bioassay methods that researchers especially chemist can use to monitor their plant extracts, fractions and synthetic products. They have to scan journals for new compounds being synthesized or isolated particularly from African plants and invite the researchers to submit such for testing as is being done by National Institutes of Health (NIH) in America. They can thus monitor progress in the development of drugs in specific areas and at a stage invite the researcher for discussions on results found interesting in their possession.

It is true we have the plants in the tropics for now but the rate at which the useful ones are being investigated, transported and grown in Europe is alarming. With advances in technology, tissue culture and separation sciences, it will not be long before Africa has little to offer in this field. Thus, it is pertinent to investigate and protect our germplasm now.

Mr. Vice-Chancellor Sir, distinguished guests, permit to end this lecture by recalling my question about the Europeans, the goat, the rope and us. Did the Europeans give us the goat without releasing the reins? The answer is as stated in one of the concluding paragraphs of the book by D. O Fagunwa and I quote "The key to this world is in the hands of no man". The reality is that by our lack of an adventurous spirit and curiosity, we (as Africans) are the ones re-proffering (surrendering) the rein to the Europeans.

And so, fare you well, the scientist returns to his laboratory.

Thank you.

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