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Mr. Vice-Chancellor Sir, I am grateful to God for giving me the opportunity to present this Inaugural lecture, the 190th in the university and the 3rd from the Department of Pharmaceutics.

What is Pharmaceutics?

Pharmaceutics is the discipline of Pharmacy which deals with all aspects of the conversion of a drug or new chemical entity into medicines. Medicines are delivery systems used to introduce drugs into the body in a safe, efficient, reproducible and convenient way (Aulton, 2002).

Pharmaceutics is diverse and includes the following subject areas:

*Physical Pharmaceutics* -This is the basic physical chemistry, which is necessary for the design and evaluation of drug delivery systems.

*Design and Formulation of Medicines* - Pure chemical substances with pharmacological properties that cannot be used directly by the patient in the raw form. Such chemicals have to be presented as formulated medicines or dosage forms such as tablets, capsules, suppositories, creams, ointments, aerosols, etc. Dosage form design therefore ensures predictable therapeutic response to the drug included in the formulation.

*Compounding and Pharmaceutical Technology* – These are the manufacture of medicines on a small and large scale, respectively.

*Pharmaceutical Microbiology* - This is involved with the control of micro-organisms in the manufacture of sterile and non-sterile pharmaceuticals and the search for, and development of agents/drugs for the control of pathogenic and spoilage micro-organisms.

Mr. Vice Chancellor Sir, my research work for over two and a half decades has been focused on two areas of Pharmaceutical Microbiology. These are the search for antimicrobial agents from plant sources and the assessment of the microbiological quality of medicinal products manufactured in Nigeria.

In this lecture titled "**Prospects of plant products as antimicrobial agents**" I will present examples of antimicrobial agents isolated from plants

studied in my research work and discuss the relevance of further research in this area considering their potential for future use.

## Introduction

From antiquity, nature has been a rich store of remedies for relief from various ailments affecting mankind. Plants, marine organisms and micro-organisms produce structurally diverse compounds, which are useful as drugs, lead structures or raw materials.

Plants have been used for thousands of years in traditional medicine. The earliest written records on Egyptian, Chinese, Indian, Greek and Roman traditional medicine have listed medicinal plants and prescriptions used in treating various ailments. In Africa, medicinal recipes from plants have been passed orally from generation to generation.

The discovery and development of antibiotics during the 1940s provided potent antimicrobial agents with high specificity for clinical use, which substantially decreased morbidity and mortality from bacterial infections. This led to a significant decline of interest in antimicrobial agents from other sources such as plants. Soon after its discovery however, antibiotics became less effective when used, as micro-organisms developed counter measures to deal with their lethal effects. The widespread use and misuse of antibiotics for human consumption and animal feed has led to the development of resistance in a variety of pathogenic bacteria. This has resulted in the emergence of multiple resistant strains, which are responsible for treatment failures worldwide. For example, Methicillin Resistant *Staphylococcus aureus* (MRSA) is one of the most difficult bacteria to eradicate in hospitals and to treat in patients. This is because they are resistant not only to β-lactam antibiotics, but to a wide range of antimicrobial agents such as macrolides, amino glycosides and fluroquinolones (Marple *et al*, 1989).

The mechanisms by which antibiotic resistance is developed can be divided into three main categories: the inactivation of the antibiotic by modification of its active moiety; the specific modification of the macromolecular target by mutation; and the prevention of antibiotics from reaching their targets



through decreased uptake or more commonly, active removal of antibiotic by efflux pumps (Walsh, 2000).

In the last decade, development of resistance to antibiotics by pathogenic organisms has become a serious problem necessitating the discovery of newer antimicrobial agents. This is important as the pipeline of new drugs is almost exhausted and the incentives to develop newer antimicrobial agents are weak. Pharmaceutical companies have reduced their investment in the development of new antibiotics because they are not as profitable as agents for treating other conditions such as heart disease, Alzheimer's or arthritis, which have long-term usage potentials.

These developments have stimulated the search for effective antimicrobial agents from plants and other sources, which can be used alone or in combination with other agents to control infectious diseases.

The following anonymous observation from the World Health Report on Infectious Diseases (2000) describes briefly how antimicrobial therapy has changed over the years!

Year 2000 B.C "Here, eat this root."
Year A.D. 1000 "That root is heathen. Here, say this prayer."
Year 1850 "That prayer is superstitious. Here, drink this potion."
Year 1920 "That potion is snake oil. Here, swallow this pill."
Year 1945 "That pill is ineffective. Here, take this penicillin."
Year 1955 "OOPS, bugs mutated. Here take this tetracycline."
Years 1960-1999 Thirty-nine more OOPS's.
"Here, take this more powerful antibiotic."
Year 2000 the bugs have won! "Here, eat this root."

## Contributions to the Search for Antimicrobial Agents from Plants

Antimicrobial agents are compounds capable of destroying or inhibiting micro-organisms even at low concentrations. They vary in their spectrum of activity and can be classified as antibacterial, antifungal or antiviral agents.

Plants contain many substances which are mainly secondary metabolites involved in defence against predators such as micro-organisms, insects and herbivores. Sometimes these metabolites give plants their odour, pigmentation and flavour and may serve to attract pollinators.

Numerous studies have been published on the antimicrobial activities of plant extracts and many of these studies have included plants used in ethno medicine. Some of the compounds responsible for the antimicrobial activity observed from the plants have been isolated by activity-guided fractionation and characterized using various spectroscopic methods. The antimicrobial agents from plants can, therefore, be classified into groups such as essential oils and terpenoids, tannins, phenols and alkaloids to name a few (Cowan, 1999).

Although I worked with chlorhexidine acetate, a synthetic antimicrobial agent, during the pursuit of an MSc degree at the University of Manchester, my research interest in antimicrobial agents from plants was developed during my PhD programme in the Department of Pharmaceutics of this university, when I worked on the antibacterial activity of the essential oil of *Cymbopogon citratus* (DC.) Stapf. (Lemon grass). I was privileged during this time to have Prof E. O. Ogunlana as my supervisor. Prof Ogunlana is an inspired teacher and mentor who has provided exemplary leadership in academic work, service to the Faculty, the University, the Pharmacy profession and the nation. He has positively influenced my life and those of other colleagues in the Faculty of Pharmacy. I therefore wish to seize this opportunity to thank him for the very good foundation he laid for all of us.

## Essential Oils and Terpenoids

*Cymbopogon citratus* (DC.) Stapf, commonly known as lemon grass, has been cultivated over many years for medicinal purposes. Information collected from Ghana, Liberia, Nigeria, Sierra Leone and Guinea Bissau indicate its use in a variety of traditional preparations.

Screening studies by various workers (Kokate and Varma, 1971; Chiori *et al*, 1977) using agar diffusion methods had shown the antibacterial effects of the essential oil from the leaves of *Cymbopogon citratus*. This stimulated



our interest to undertake a detailed investigation of the antibacterial activity of the essential oil of the plant known as lemon grass oil (LGO) (Onawunmi and Ogunlana, 1986).

The LGO was extracted by hydrodistillation of fresh leaves of lemon grass and the oil was dispersed in 20% DMSO, a solvent, which on its own, had no lethal effects on bacterial cells (Onawunmi, 1987a). In these studies, LGO showed rapid bactericidal activity against Gram-negative and Gram-positive bacteria with the latter being more susceptible. Subsequent post-doctoral work (Onawunmi, 1989a) determined the fungistatic and fungicidal effects of LGO against type cultures and clinical isolates of Candida spps, *Aspergillus fumigatus*, *Microsporum gypseum* and *Trichophyton mentagrophytes*. The most resistant organism was *A. fumigatus*, while *M. gypseum*, *T. mentagrophytes* and the Candida spps were highly susceptible.

The LGO was analysed for its composition and standardized by determining the relative percentage of the three main components of the oil using analytical gas chromatography. The main components of the oil were identified using chromatographic and spectrometric methods as α-citral, β citral and myrcene. While α-citral and β-citral individually elicited antibacterial activity against the test organisms, myrcene did not show any observable activity on its own. However, myrcene provided enhanced activities when mixed with either α- or β-citral (Onawunmi *et al*, 1984).

A number of compounds known to be minor components of LGO were also tested for their antibacterial and antifungal activity. While heptenone, dipentene and limonene showed no antibacterial activity, linalool, citronellal, citronellol and geraniol at 100% purity showed some activity against the test organisms but not against *Ps. aeruginosa*. Citronellol and citral also showed good activity against the test fungi, while dipentene and myrcene were inactive.

The antimicrobial activity of pure citral was subsequently studied to quantify the effects against various bacteria and fungi (Onawunmi, 1989b). Citral showed appreciable antibacterial and antifungal activities against the test organisms. The results were similar to those obtained with LGO and affirmed

the role of citral as the main antimicrobial component of LGO. These findings also showed that citral or LGO either singly or in combination with other agents could be useful as preservatives in addition to other uses in the pharmaceutical, food, soap and cosmetic industries.

It was also necessary to determine the mode of action of LGO, as this definitely would contribute to the development of knowledge in this area of natural products. In view of this, we focused our work on the mode of action of LGO by determining the effect on the cytoplasmic membrane and cell wall (Onawunmi and Ogunlana, 1985). Evidence of membrane damage was observed as LGO induced leakage of potassium ions, 260nm absorbing materials and protein from *E. coli* cells. In order to detect whether the cytoplasmic membrane was directly affected, *E. coli* cells with the outer membrane and peptidoglycan removed (spheroplasts), were exposed to LGO. Rapid lysis occurred up to the MIC, which confirmed the results obtained with the leakage studies.

Subsequent studies (Ogunlana *et al*, 1987) were undertaken to show conclusively the effect of LGO on the morphology of *E. coli* cells and peptidoglycan synthesis. Various morphological changes were observed using the electron microscope after exposing the cells to different concentrations of LGO for different times. The electron microscope studies were useful in confirming the mode of action of LGO, which demonstrated effects on the cytoplasmic membrane and peptidoglycan. The studies showed that LGO acted as a cell wall inhibitor and the morphological changes produced in the cells indicated the involvement of the penicillin binding proteins.

The antimicrobial activity of LGO was also assessed *in vivo* when incorporated as a preservative in Aqueous Cream B.P (Onawunmi, 1987b; 1989a). The creams containing various concentrations of LGO were challenged with selected Gram-positive and Gram-negative bacteria as well as yeasts and fungi. From the studies, a concentration of 0.5% LGO was found to effectively preserve Aqueous Cream B.P against bacterial and fungal contaminants. The results from these challenge tests have provided a basis for the effective utilization of LGO in the preservation of some topical formulations.



The effect of various hydrophilic bases (polyethylene glycol, o/w emulsion, tragacanth and methyl cellulose gels) on the antibacterial activity of LGO was determined in order to identify the most suitable base for the formulation of LGO (Onawunmi, 1994). The use of an inappropriate base could result in the production of a useless formulation with no observable antibacterial activity. The antibacterial activity of LGO was reduced by the presence of the bases. However, the polyethylene glycol base appeared suitable as it gave the best release of LGO and had the least effect on its antibacterial activity.

In practice, preservative combinations are used to expand the spectrum of activity and reduce toxicological hazards by the use of lower concentrations of component preservatives. The antibacterial activity of phenoxyethanol in combination with LGO *in vitro* was therefore investigated (Onawunmi, 1988). The combination increased the spectrum of activity of phenoxyethanol and reduced the effective concentrations of both compounds necessary for activity.

More recently, Bankole *et al* (2005) carried out experiments to determine the potential of using the powder and essential oil from the dried ground leaves of Lemon grass (*C. citratus*) to control storage deterioration and aflatoxin contamination of melon seeds. LGO at high doses (0.5% and 1%) completely inhibited aflatoxin production in shelled melon seeds inoculated with toxigenic *Aspergillus flavus*. The efficacy of the LGO in preserving the quality of melon seeds was statistically at par with that of fungicide treatment with iprodione.

Related work from other laboratories also showed that the essential oils isolated from some medicinal aromatic plants (*Aframomum danielli* and *Zingiber officinale)*, widely used in Sao Tome e Principe, showed broad-spectrum antimicrobial activity. Investigation of the antimicrobial activity of the major constituents showed that citral had the highest antibacterial and antifungal activity (Martins *et al*, 2001).

Similarly, citral, geraniol and perrialdehyde at 500µg/ml were reported to have completely killed pathogenic *E. coli* strains and *Salmonella typhimurium* (Kim *et al*, 1996). The authors therefore suggested that these

compounds could serve as potential antibacterial agents to inhibit pathogen growth in food. These findings on the appreciable antimicrobial activity of citral support our previous reports on citral.

The antimicrobial activity observed in our study of LGO encouraged us to examine another essential oil for activity.

***Boswellia dalzielli*** Hutch (Burseraceae) is the West African species of Frankincense producing plants. The official species are *B. carteri, B. frereana* and *B. serrata*. It is used in ethnomedicine to treat gastrointestinal disorders, septic sores, venereal diseases and rheumatism. *B. dalzielli* yields an oleo-gum resin, which is less known compared to the frankincense from the official species.

The essential oil obtained through hydrodistillation of *B. dalzielli* gum resin (Boswellia oil) was analyzed by GC-MS. Alpha pinene (21.1%) was the most abundant component along with other oxygenated monoterpenes (sabinyl acetate, p-cymene, cis-verbanol, isopinocamphene, pinocarvone, lyratyl acetate, *p*-cymene-8-ol, myrtenal, verbenone and myceol). The oil demonstrated significant antifungal, antibacterial and anti-inflammatory activities (Alemika *et al*, 2001).

The methanol and aqueous extracts of *B. dalzielli* stem bark showed antimicrobial activity and the active compounds were isolated using activity-directed fractionation. Incensole isolated from the ethyl acetate fraction showed moderate activity against Gram-positive bacteria using bioautographic methods although no activity was shown in the agar diffusion test (Alemika *et al*, 2004a). This experience in which the activity of incensole could not be reproduced using the agar diffusion method is instructive. Although incensole showed moderate activity, it did not explain all the activity observed in the crude methanolic extract indicating the presence of other constituents with antimicrobial activity. Protocatechuic and gallic acids, which were subsequently isolated from the phenolic sub fraction of *B. dalzielli* stem bark, exhibited appreciable activity against both Gram-positive and Gram-negative bacteria. A novel stilbene glycoside with moderate activity against Gram-positive bacteria was also isolated.



Other studies have since shown that some essential oils possess appreciable antimicrobial activity, which could be meaningfully exploited in the food, cosmetic and pharmaceutical industries (Dorman and Deans, 2000).

Several workers have also investigated combining these plant products with other antimicrobial agents to improve their efficacy (Giordani *et al*, 2006). Santolina oil exhibited synergistic effect with clotrimazole against *C. albicans*, while anethole, a constituent of clove oil, showed synergistic activity when combined with miconazole and amphotericin B. Similarly the essential oil of *Cinnamomum cassia* exhibited strong antifungal activity alone and potentiated the activity of amphotericin B, *in vitro*. This may show promise for the development of less toxic and more effective therapies for candidiasis.

In general, further developmental studies on the therapeutic applications of essential oils/ oil components alone or in combination with other agents should be undertaken to ensure full utilization of these active antimicrobial products from plants.

## Other Antimicrobial Agents from Plant Sources

The studies on LGO stimulated my interest in searching for more useful antimicrobial agents from plants. The Antimicrobial Plant Group (APG) supported by the International Program in the Chemical Sciences (IPICS) started off with the list of 48 plants screened by Ogunlana and Ramstad as reported in a research paper published in 1975. Other plants were also selected based on their ethnomedical uses for treating infections as this method had been reported to provide the best chance of isolating active compounds. Proper identification of the plant was essential and required close collaboration of the research group with a botanist. Several reports had identified plants incorrectly leading to contradictory reports on antimicrobial activity and antimicrobial components for the same plants. Bioactivity directed fractionation of the selected plant extracts was undertaken in collaboration with colleagues in the Faculty. The antimicrobial activity of the crude extracts and fractions were monitored using agar diffusion or bioautographic methods. A maximum concentration of 40 mg/

ml was tested for crude extracts, while fractions were tested using lower concentrations. The selection of a suitable solvent for the crude extracts and fractions was important, as the solvent used should not have any intrinsic antimicrobial activity. Preliminary studies led to the selection of 50% methanol in water as the solvent for our test protocol as it was shown not to possess any antimicrobial activity.

The test organisms used were type cultures of known resistance selected to represent a wide spectrum of micro-organisms. The test panel consisted of two Gram positive *(B. subtilis* and *Staph. aureus)* and two Gram-negative (*E. coli* and *Ps. aeruginosa*) organisms and Candida species to represent fungi. *Ps. aeruginosa* and Candida species were selected because of their known resistance to many antimicrobial agents. Therefore, plant extracts or compounds with activity against these organisms were considered interesting for further study. Pure compounds with antimicrobial activity were isolated and identified using various spectroscopic methods and the activity was quantified using the agar dilution or broth dilution methods to obtain the minimum inhibitory concentrations (MICs). Some examples of the antimicrobial agents isolated from plants screened in the last 10 years are now presented as further contribution to the search for antimicrobial agents from plants.

## Tannins

Tannins are inhibitory to fungi, yeasts and bacteria however very few studies have been carried out with purified tannins of known molecular structures (Scalbert, 1991).

The genus Acalypha (Family Euphorbiaceous) consists of about 570 species many of which are used in traditional medicine. The juice from the leaves of *Acalypha wilkesiana* is used to treat skin infections (Oliver, 1959) while a decoction of the leaves of *A. hispida* has been used in the treatment of wounds, ulcers, abscesses and leprosy (Schindler, 1939). *A. hispida* is also widely distributed in Asia where the leaves have been used as a remedy for thrush and boils while other Acalypha species have been used for the treatment of diarrhea and skin complaints (Perry, 1980). Reports from



several workers (Adesina *et al*, 1980; Alade and Irobi, 1993) have indicated antimicrobial activity of *A. wilkesiana* against various micro-organisms. In our investigation aimed at moving the studies ahead and specifically isolating the antimicrobial agents we undertook antimicrobial screening of 6 species and varieties of Acalypha comprising weeds and ornamental species. This initial screening confirmed the antibacterial activity of the 6 plants against various bacteria. *A. hispida* and a specific variety of *A. wilkesiana* known in horticulture as red Acalypha were however the only two which exhibited both antibacterial and antifungal activity. These two species were therefore selected for activity directed fractionation to isolate the antimicrobial agents in the two plants. The activity directed fractionation of *A. wilkesiana* and *A. hispida* leaves resulted in the isolation of gallic acid, corilagin and geraniin as the compounds responsible for the observed antimicrobial activity. The Gram-positive organisms were more susceptible than the Gram-negative organisms and *C. pseudotropicalis* (Adesina *et al*, 2000). While gallic acid is ubiquitous in plants and its antibacterial activity is known (Lamikanra *et al*, 1990) our study established the role of specific tannins such as corilagin and geraniin in the antimicrobial activity of Acalypha species.

In further pursuit of our objective of developing these active plant species into standard pharmaceutical products of therapeutic value we undertook, in collaboration with our colleagues in the Faculty of Clinical Sciences, an open non-comparative study to evaluate the efficacy and safety of *A. wilkesiana* ointment in superficial skin infections (Oyelami *et al*, 2003). The ointment was found to be very effective in the treatment of *Tinea pedis*, *Pityriasis vesicolor* and *Candida intertrigo*. It was therefore established that Acalypha ointment could be used for the treatment of these skin infections. The establishment of the active components in our laboratory removes the common obstacle in the use of crude extracts as the ointment can be standardized with respect to the now known active components.

Several tannins or related phenolic compounds have been reported to possess antiviral activity. For example, corilagin, previously isolated in our studies from Acalypha species, has been reported to inhibit HIV reverse transcriptase (Singh *et al*, 2005).

## Alkaloids

*Chrysophyllum albidum* G. Don-Holl. (Sapotaceae) is a tree with edible fruits, which grows widely in West Africa. The tree bark is used as a remedy for yellow fever and malaria while the leaves are used for the treatment of skin eruptions, diarrhoea and stomach ache. The cotyledons from the seeds are used in ointments for the treatment of vaginal and dermatological infections in Western Nigeria.

As part of our studies on antimicrobial agents from Nigerian medicinal plants, different parts of the tree were screened for antimicrobial activity. The leaves, stem, root and seed cotyledons all possessed antimicrobial activity against various organisms. The seed cotyledons were then selected for further studies as the methanolic extract was found to exhibit appreciable activity against some bacterial and Candida species. Activity directed chemical analysis of this extract led to the isolation and identification of eleagnine, tetra-hydro-2-methylharman and skatole. While eleagnine was the main antimicrobial constituent, tetrahydro-2-methylharman also showed some activity. Skatole on the other hand only showed limited activity against *E. coli* and *Staph. aureus* (Idowu *et al*, 2003).

The antimicrobial activity of the isolated compounds justifies the ethno medical use of the seed cotyledons in the treatment of vaginal and dermatological infections. Further studies are in progress to evaluate the antimicrobial activity in detail, assess the toxicity and study the mechanism of action of eleagnine.

Eleagnine is a closely related isomer of borreverine, an alkaloid isolated as the antimicrobial component (Maynart *et al*, 1980) of *Borreria verticillata,* a common tropical plant used in ethnomedicine for skin infections.

## Phenolics

*Steganotaenia araliacea* Hochst (Apiaceae/Umbelliferae) is used in East and West African ethno medicine for treating gastrointestinal disorders, peptic ulcers, and various diseases of microbial origin. Activity directed fractionation isolated protocatechuic acid as the main antimicrobial and antioxidant principle (Alemika *et al* 2004b).



A number of species of the genus Ficus have been used as ingredients in some traditional remedies. For example, the juice of *F. cycomorus* is used for some skin diseases while the bark, fruit and latex decoction of *F. vogeliana* and *F. capensis* are used in the treatment of gonorrhoeae, bronchitis, dysentery and for wound healing. The inference from these reported uses of the Ficus species is possession of antimicrobial properties. This prompted our investigation of the antibacterial activity of the fruits of *Ficus barteri*, a common Ficus species growing luxuriantly, with copious fruits, near the Faculty of Pharmacy building (Ogungbamila *et al*, 1997a).

Activity directed fractionation led to the isolation of piceatannol as the main antimicrobial compound. Other compounds isolated were trans-resveratrol, which had no antibacterial activity against any of the four test organisms and catechin, which had very feeble activity against *B. subtilis*. We also investigated the aerial parts of *Bryophyllum pinnatum* (Ogungbamila *et al*, 1997b) a plant used in many traditional recipes in West Africa for the treatment of ulcers, allergic inflammation and epilepsy. The main antibacterial constituent was found to be gallic acid, which accounted for about 0.014% of the fresh aerial part. Other minor constituents of the plant isolated were luteolin and epigallocatechin-3-O-syringate.

Sato *et al* (1996) found that gallic acid derivatives obtained from the extracts of *Terminalia chebula* were very effective against several bacterial species including methicillin sensitive *Staphylococcus aureus* (MSSA) and MRSA strains.

A recent review of antimicrobial agents from plants has also shown that phenolics are the main antimicrobial chemical agents isolated from plants (Rios and Recio, 2005).

## Further Screening

Currently, twenty weeds growing on the University campus, some of which are reported to be used ethnomedically for treating infections have been screened for antimicrobial activity. After ranking according to their activity, five candidate plants are currently being looked into in detail.

## Prospects for Future Use

In general, the findings from our studies on the activity of antimicrobial agents from plants in Nigeria were similar to those reported by other workers. These agents when evaluated *in vitro* for antimicrobial activity using current bioassays showed a low level of activity especially against Gram-negative organisms. The MIC values were much higher than those obtained with antibiotics, which had MIC values in the range, 0.01-10 μg/ml.

Thus, despite all the plants screened and antimicrobial agents isolated in the past decades, none of these plant agents have been as active and non-toxic as the penicillins and other antibiotics in use. The few antimicrobial agents from plants that have shown exceptional and broad-spectrum activity such as sanguinarine, an alkaloid derived from rhizomes of *Sanguinaria canadensis* L, are too toxic for systemic use. Sanguinarine was shown to possess anti-protozoal activity and in addition inhibited the growth of Gram-positive bacteria (including those resistant to penicillin) at 15.6 μg/ml, Gram-negative bacteria at 7.8-250 μg/ml and pathogenic fungi at 1.95-7.8 μg/ml (Vichkanova *et al*, 1969). Sanguinarine also showed an MIC of 10μg/ml against *Staph. aureus* and 39 μg/ml against *E. coli* (Stermitz *et al*, 1975). It has thus only been used in oral health products such as mouth rinses or toothpastes (Godowski, 1989).

Are we then to stop the search for antimicrobial agents from plants? Certainly not!

First of all, studies (Stermitz *et al*, 2000; Tegos *et al*, 2002) have shown that inhibiting or disabling multidrug resistance pumps (MDRs) by adding MDR inhibitors increased the penetration of several plant antimicrobial agents into bacterial cells. This resulted in striking increase in activity of these agents even against resistant Gram–negative organisms. For example, rhein, the main antimicrobial agent from rhubarb was only slightly effective against *E. coli*. The addition of two MDR inhibitors increased the activity significantly. The remarkable 2000-fold increase in activity demonstrated the potential of rhein as an effective antimicrobial agent against Gram-negative bacteria. Even *Ps. aeruginosa*, which is known to be resistant to almost all antimicrobial agents and antibiotics due to its outer membrane



and MDRs, was also rendered susceptible to rhein. Other plant antimicrobial agents such as plumbagin, gossypol and coumestrol all showed potentiated activity in combination with MDR inhibitors against many organisms. This study showed that plant antimicrobial agents are potentially as effective as antibiotics if they are delivered into the cell. Future studies may provide other novel or innovative ways of achieving this, hence the need to continue the search for antimicrobial agents from plants. While plant antimicrobial agents are not currently used as such in chemotherapy, these studies predict that the future development of combination therapy involving such plant-derived agents is indeed very promising.

Secondly, many studies have found that the efficacy of β-lactam antibiotics can be improved by combination with antimicrobial agents from plants such as baicalin, corilagin, and epigallocatechin gallate, to name a few.

The Chinese herb, *Scutellaria amoena* C.H. Wright, has been used in traditional Chinese medicine to treat a wide range of infectious diseases. Baicalin, a flavone isolated from the herb, showed moderate activity against MRSA and other strains resistant to other β-lactams (MIC 64 μg/ml). When the β-lactams such as ampicillin, amoxycillin, benzylpenicillin and methicillin were combined with baicalin at a concentration of 16 μg/ml their activity was potentiated. The study showed that baicalin had the potential to restore the effectiveness of β-lactam antibiotics against resistant strains of *Staph aureus* (Lui *et al*, 2000).

The leaves of *Arctostaphylos uva-ursi* and its extract are described in the Japanese *Pharmacopoeia*. The extract is known to contain antimicrobial agents and is used orally for the treatment of infectious diseases. Shimizu *et al* (2001) found that an extract of *A. uva-ursi* markedly reduced the MICs of β-lactam antibiotics against MRSA. The effective compound was isolated and identified as corilagin. Corilagin is a polyphenol, which we have also isolated from Acalypha species as one of its antimicrobial components (Adesina *et al*, 2000). While corilagin alone showed weak anti-MRSA activity (128 μg/ml) low concentrations of corilagin (16 μg/ml) markedly decreased the MIC of oxacillin and other β-lactams against MRSA strains used in the study. Corilagin also caused some reduction in the MICs of streptomycin and tetracycline against some strains of MRSA.

Carbapens are relatively new β-lactams that have a broad spectrum and strong activity against many pathogens but they do not show high levels of activity against MRSA. Combinations of carbapens and epigallocatechin gallate, a main constituent of the catechins extracted from tea (*Camellia sinensis*), exhibited potent synergy against twenty-four clinical isolates of MRSA (Hu *et al*, 2002). This synergy was similarly shown *in vitro* using an aqueous extract of tea.

These combinations of plant antimicrobials and antibiotics are worthy of further evaluation *in vivo* against MRSA infections. These agents may have potential for development as adjuncts to β-lactam treatments against resistant strains of bacteria such as MRSA.

Thirdly, we need to continue searching for more useful antimicrobial agents from plants and also investigate the mechanisms by which compounds can directly or indirectly serve as anti-infective agents. Some agents may not appear very active and current bioassays may not clearly show the usefulness of some isolated agents but new bioassays or test systems may reveal the usefulness of such compounds in future. Although women have used cranberry juice for years to prevent and treat urinary tract infections, researchers have now found that cranberry and blueberry juices competitively inhibit the adsorption of pathogenic *E. coli* to the urinary tract epithelial cells. The two components of cranberry juice, shown *in vitro* to inhibit adhesion, are fructose and proanthocyanidins. Other suggested mechanisms of action proposed for cranberry juice activity include its ability to acidify urine and the antiseptic effects of some of its contents, such as hippuric acid (Kontiokari *et al*, 2001).

Lee *et al* (2003) evaluated the antibacterial activities of various fruit and vegetable extracts on common potential pathogens including antibiotic resistant strains. All purple and red vegetable and fruit juices had antibacterial activity, while all green vegetable juices showed no antibacterial activity. Garlic juice and tea had significant activity against the test organisms. They suggested that tea and garlic have the potential for broader applications as antibacterial agents. Preliminary studies with grapefruit juice in our laboratory showed antibacterial activity *in vitro* against various organisms. This activity could be related to the observed effectiveness of grapefruit seeds in treating



urinary tract infections reported by colleagues in the Faculty of Clinical Sciences (Oyelami *et al*, 2005). Grape fruit oil extracted from the peel of grape fruit *(Citrus paradisi*) contains high concentrations of some of the grapefruit juice components. Abulrob *et al*, (2004) treated MRSA and MSSA strains with grapefruit oil components in combination with antibacterial agents to determine whether the components could modulate or potentiate bacterial sensitivity to the antibacterial agents. The preliminary data showed that a bergamottin epoxide and a coumarin derivative in the oil enhanced the susceptibility of the test MRSA strains to ethidium bromide and norfloxacin. These components are, therefore, potentially useful for the enhancement of the activity of therapeutic agents used for the treatment of bacterial infections.

Fourthly, the rapid spread of the human immunodeficiency virus (HIV) especially in Sub-Saharan Africa is an important reason for searching for antiviral agents from plant sources. A number of medicinal plants have been reported to have anti-HIV properties and activity directed fractionation of crude extracts have provided compounds with activity against HIV. Many of these agents have mechanisms of action complementary to those of existing antiviral drugs. Although no plant derived drug is currently in clinical use to treat AIDS, promising activity has been shown by some plant products alone and in combination with other antiviral agents. This suggests that plant products have the potential to be useful drugs for the treatment of HIV infection (Singh *et al*, 2005).

Tan and Vanitha (2004), reviewed seven Chinese herbs with immuno-modulatory and antimicrobial activity. Some of these herbaceous plants such as ginseng and ginger had direct inhibitory effect on microbes but they also contained compounds that selectively modulated cells of the immune system. Therefore, studying the influence of these compounds on immune cells as well as microbes can provide useful ideas for the development of potentially useful agents for the treatment of viral infections.

Finally, the search for antimicrobial agents from plants must continue since the majority of people in Nigeria depend on herbal medicines to meet their health needs. Studies *in vitro* and *in vivo* in clinical trials that provide a rationale for the use of such herbal products will be beneficial.

Variations in the levels of active ingredients in herbal medicines, which could result in therapeutic ineffectiveness, increased side/adverse effects or inducement of resistance can be avoided by standardization of the herbal preparations using the active components identified in the plant extract as indices. These plant products must also be used rationally. Put simply, rational drug use is using the right drug for the right indication, in the right dose and frequency, for the right duration and at the right cost. Antimicrobials, for example are used irrationally when inadequate doses are taken or when they are used for non-microbial diseases. Such irrational use has led to increased antimicrobial resistance by microorganisms.

## Microbiological Quality of Drug Products

All medicinal products including herbal preparations must comply with certain microbiological specifications in order to be used safely and effectively. Such specifications must not only be enforced during their registration by the regulatory body but compliance must be monitored subsequently, to ensure that the requirements have been sustained during manufacture and in the final products.

Quality assurance includes all the procedures necessary to ensure that a product meets specified quality. It includes formulation design and development, good manufacturing practice (GMP), quality control and post marketing surveillance. GMP is that part of quality assurance concerned with ensuring that medicines are manufactured to the required quality. For example, raw materials particularly those of natural origin must be of high microbial quality and the equipment and manufacturing environment must be suitable, properly cleaned and monitored. In addition, staff must be in good health and should have good knowledge about personal and production hygiene.

Medicinal products such as oral and topical preparations are generally not required to be sterile, however, it is desirable that they contain only low levels of microbial contamination and that no pathogens are present. This microbiological requirement is necessary because high levels of microbial contamination may result in spoilage and degradation of the product or constitute a health hazard to the user.



Medicines intended for oral administration should not contain more than $10^3$ aerobic bacteria or $10^2$ fungi per gram or ml of product. In addition, there should be absence of *E. coli*. However, higher levels may be permitted for products containing raw materials of natural origin (BP, 2003).

Periodic microbiological tests to determine total microbial counts and presence of pathogens in finished medicinal products can be used to confirm the efficiency of the GMP systems of the manufacturer.

A study to determine the microbial content of 43 samples of locally prepared herbal proprietary products was therefore undertaken to establish the safety or otherwise of the use of these preparations (Onawunmi and Lamikanra, 1987). No previous attempt had been made to study the level of microbial contamination of packed herbal preparations, which are readily available in Nigeria. 7 samples (16%) contained bacterial counts $>10^6$ orgs/ml, while 6 samples (14%) had fungal contaminants $>10^6$ orgs/ml. The contaminants were predominantly Gram-positive organisms and fungi, which were linked to the raw materials used, which are mainly of natural origin and the low pH values of the products. The presence of fungal contaminants in oral products is particularly undesirable in view of the ability of some fungal cells to produce mycotoxins. The use of these preparations can therefore be a source of infection, especially for children and pregnant women for whom they were intended. Although herbal medicines can be prepared for consumption over a very short time, proprietary packs, which can be stored over long periods, calls for the inclusion of preservatives. The choice of preservative in terms of effectiveness and compatibility with the product is not a trivial matter but is based on professional skills and product development studies. The presence of high levels of contamination in the preparations studied highlights the need to enforce GMP in the manufacture of such proprietary herbal preparations. The study also highlighted the need to document the risks from such products since a considerable number of Nigerians depend on these products.

A survey of the microbiological quality of some oral and topical pharmaceutical products manufactured in Nigeria was also undertaken to determine the extent and nature of microbial contamination. Of the 144 samples examined, 26 samples (18.1%) contained microbial counts greater

than $10^5$ orgs/ml. The highly contaminated samples were mainly aqueous products such as Magnesium trisilicate mixture (MTM) and Kaolin and morphine mixture. The main contaminants isolated from these contaminated products were Bacillus species, yeasts and Gram-negative bacteria. The type of organisms isolated suggested that the main sources of contamination were the raw materials used in the production, especially the water, and the manufacturing environment. While most of the organisms isolated from these samples are not usually considered to be pathogens, they were present in high numbers therefore rendering the product unfit for human use.

As a follow up on the previous survey, a further study was undertaken to assess the microbiological and chemical quality of six commercial samples of MTM (one of the problem products identified) and the raw materials used for its production (Bamgbade and Onawunmi, 2002). Three samples were not of satisfactory quality chemically, while four samples (including the three samples that were not chemically satisfactory) contained high microbial counts. In addition, some of the raw materials such as magnesium trisilicate powder were not satisfactory microbiologically. Preservatives (parabens and chloro-form) evaluated in MTM using challenge tests were not effective against *B. subtilis* indicating the need for strict adherence to GMP in the manufacture of this product (Bamgbade and Onawunmi, 2001).

The quality of the water used in the production of aqueous preparations is usually responsible for the quality of the final product. Water for pharmaceutical manufacturing has to be treated appropriately to meet specifications for the preparation of liquid dosage forms. This is an expensive venture in Nigeria, as manufacturers have to treat raw water on site to obtain potable water, which still has to be further treated to meet specifications for pharmaceutical manufacturing. It is therefore not surprising, that many small manufacturers find it difficult to cope with this expense and end up with compromised products.

In the course of my career, I have had opportunities to look at the quality of water used in pharmaceutical manufacturing and have found that running and maintaining the water treatment processes is a continuous exercise, which requires regular microbiological monitoring of the different treatment processes (filtration, distillation, deionisation and disinfection with UV). This



monitoring is necessary to determine when replacement of filters, UV lamps and regeneration or replacement of the deionizer resins are due as failure in any of these processes will result in contaminated water and production of contaminated aqueous products.

Recently, NAFDAC directed that two batches of Mist Kaolin manufactured in a Lagos-based pharmaceutical firm should be withdrawn from the market and destroyed because they were found in post-marketing surveillance to contain high microbial levels. The factory was subsequently closed down as preliminary checks by NAFDAC inspectors and the findings showed that the contamination was a result of the company's poor compliance with GMP. These findings justify the need for regular monitoring of manufacturing plants and elaborate post marketing surveillance by the regulatory agencies.

## Conclusion

Mr. Vice Chancellor Sir, the search for useful antimicrobial agents from plants has gained momentum in the past decade. Hundreds of compounds from plant sources have been shown to possess activity either alone or in combination with other agents against pathogenic and spoilage organisms *in vitro* and *in vivo* in a few cases. In this lecture, I have discussed the prospects of these plant products as antimicrobial agents either for chemotherapeutic or preservative purposes in medicines, cosmetics and food. Although substantial work have been reported on these agents, further studies are required to understand their mechanisms of action and establish their effectiveness *in vivo* by subjecting them to tests in animals and humans. There is a need to exploit the potential synergistic effects of plant-derived agents with known antibiotics/antimicrobial agents in chemotherapy.

In addition, toxicity tests and quality assurance are important for the safe and effective use of plant products. For example, in Belgium in 1990, due to inadequate quality control, one of the herbs used in a slimming preparation was incorrectly identified. As a result of this, the manufacturer utilized *Aristolochia fangchi*, which contains renal toxins. By 1994, there were 70 cases of renal fibrosis attributed to the slimming preparation, 30 of which resulted in terminal renal failure (Vanhaelen *et al*, 1994). More recently, the Medicines and Healthcare Products Regulatory Agency in Britain warned

consumers not to purchase two herbal products manufactured in Nigeria, which may contain the toxic herbal ingredients *Strophantus sarmentosus*, or Aristolochia species. Aristolochia species can cause kidney failure and cancer, while Strophantus species have a powerful action on the heart and could result in serious heart problems, including abnormal heart rate and heart failure. Indeed, NAFDAC, which "listed" the two herbal preparations, has now delisted them based on the reports. The Federal Government should always ensure through its regulatory agencies, that all drugs including herbal medicines are safe for use before 'listing' and/or registration. This is particularly important considering the increase in kidney problems in the country, which may not be unconnected with indiscriminate use of such products.

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Thank you for listening and God bless.

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