ANTIVIRAL AND ANTIBACTERIAL SCREENING OF SOME NIGERIAN MEDICINAL PLANT EXTRACTS

Thesis submitted for the degree of

Doctor of Philosophy

at the University of Leicester

BY

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September 2000

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DECLARATION

I hereby declare that this dissertation was written by me and it is a record of my own work. It has not been presented before in any previous application for a Doctor of Philosophy degree. References made to published literature have been duly acknowledged.

DEDICATION

To Mum and Dad

ACKNOWLEDGEMENT

I would like to express my gratitude to the Almighty God for being my sufficiency in all things. His mercy, protection, leading, wisdom, love and grace made it possible for this project to be carried out. Great is His faithfulness!

My thanks and gratitude goes to my supervisor, Prof. Steven H. Myint for his guidance, understanding, criticisms, correction and encouragement in seeing to the success of this work. I also acknowledge the assistance of Prof. Bill Grant, who stepped in when Prof. Myint left and took the time to go through the thesis, page by page, making valuable corrections and suggestions.

My special thanks and appreciation to Dr Primrose Freestone, without whom this project will not have been completed, for giving her time and assistance during the determination of the active products in the extracts. To my colleagues in Lab 125 in the persons of Mrs. Stephanie Euden, Andrew Kurdzeil, Nigel Cook, Sarah Strugnell, Joseph Lu, Manar Moneef, Tony Maggs, Simon Kilvington, Gary Pinch, I say thanks for all your help, encouragement and for making my stay at Leicester a memorable one.

I acknowledge the support of my wife and son during the course of this study especially for their prayers, patience and understanding. To my brother, Dr J.K.Audu, I say thanks for all the support and encouragement rendered.

I acknowledge with thanks the help of the traditional healers, in the persons of Mallam Ibrahim, Baba Tula, Alhaji Maikano, and Mallam Sabo, for letting me into the secret of their art and showing me most of the plants used in this

study. I thank the Botany Department, Abubakar Tafawa Balewa University, Bauchi, Nigeria, for confirming the identification of the plants collected for this study. I acknowledge with thanks also, the assistance of Demo Kalla, Yayok Micah, Haruna Nma, Poulina Daniel, Jemima Karibo and Sabo Abubakar for their help in collecting, grinding and packing the plant samples. I also appreciate the help rendered by friends including Mr. And Mrs. Samaila Gadzama, Mr. And Mrs. Kiriya Karibo, Dr and Mrs. Adawa, Pastor Tunde Ishiaka, Mr. And Mrs. Emmanuel Oladipo, Dr (Rev) and Mrs. Rueben Danladi, Rev Paul Basset and all those too numerous to mention.

Finally I want to thank the World Bank Graduate Scholarship Program for providing most of the funding to undertake this project.

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ABSTRACT

Plants from Northern Nigeria with a history of use in both human and veterinary traditional medicine have been investigated *in vitro*, for antiviral and antibacterial activity and their cytotoxicity against human cells in culture determined. Aqueous extracts were tested against poliovirus, astrovirus, human and Equine herpes simplex virus and canine and bovine parvovirus, using the microtitre plate inhibition test. The hole-plate diffusion method was used for the antibacterial testing against a range of Gram positive and Gram negative bacteria.

Most of the extracts were non toxic to cells in culture and had activity against more than one virus at a dose rate of between 100 and 400 •g/100••. The most effective of the plant extracts were those of *Bauhania thonningi*, *Anacardium occidentale*, *Boswelia dalzeilii*, *Dichrostachys glomerata* and *Sterculia setigera*, which were found to be active against all the viruses tested. In addition, most of the extracts were active against those Gram positive bacteria tested. Two of the plants, *Anogeissus schimperi* and *Anacardium occidentale*, had good antibacterial activity against the Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa.*

Attempts were made to isolate and identify the active components in these extracts with activity against both viruses and bacteria using the gel filtration,

ion-exchange chromatography and Thin layer chromatography. Activities were associated with fractions identified as flavonoids, alkaloids, terpenoids and polyphenols and were found to, either individually or in combination, have antiviral action.

A field trial of the active plant extracts were carried out using cattle with clinical diarrhoea and dysentery and comparing recovery patterns with similar administering extracts to cattle with clinically diagnosed diarrhoea animals treated with a broad spectrum gut-acting antibiotic, neomycin-sulphate. No significant difference (**P=0.778**) was seen between the extract and the antibiotic in efficacy of treatment. This study supports claims of the successful usage of these plant extracts by the traditional healers in treating both human and animal infectious diseases.

CHAPTER ONE: INTRODUCTION I

1.0 GENERAL INTRODUCTION

1.1 TRADITIONAL HERBAL REMEDIES

More than 200,000 out of the more than 300,000 plant species identified to date can be found in the tropical countries in Africa and elsewhere (Sofowora, 1982). A large number of such plants, termed medicinal plants, are used on the African continent for the treatment of different diseases and complaints (Sofowora, 1982). The term 'medicinal plants' refers to any plant which, in one or more of its organs, contain substances that can be use for therapeutic purposes or which are precursors for the synthesis of useful drugs (Bep Oliver-Bever, 1986, Sofowora, 1982). Ethnobotany provides a source of information about these plants that are of great value for any scientist concerned with their investigation. Not only does this material represent a reservoir of pharmacologically active substances, but there is also the potential to chemically transform the products into more effective analogues (Vanden Berghe & Vlietinck, 1991).

One of the earliest records of the use of herbal medicine was that of Chaulmoogra oil from a species of *Hydnocarpus gaertn* which was known to be effective in the treatment of Leprosy (Le Strange, 1977). Also evidences of the use of extract of Opium poppy (*Papaver somniferus*) and Castor oil seed (*Ricinus communis*) were found from ancient Egyptian tombs as far back as 1500BC (Sofowora, 1982). Hippocrates, who is commonly referred to as the father of modern medicine, also used herbs as his medicines and some of the plants described by him included Opium poppy, Mint, Sage, Rosemary and Verbena (Le Strange, 1977).

Hence, plants are the foundation of the present allopathic health care system. Traditional medical systems are, however, holistic in nature, treating the entire being rather than only the disease (Kleinman, 1978, Sofowora, 1982).

In Africa, more than 80% of the population go to traditional doctors for treatment. In rural sub-Saharan Africa alone, the ratio of patients to a traditional doctor is 100 to 1,000:1 as compared to 10,000 to 100,000:1 for an orthodox doctor. Hence more people in this sub-region of Africa see traditional doctors (Ogunyemi, 1979; OAU/STRC, 1979). These use plants as an important source of medicine. Even though modern medicine is based on pure chemical substances (marketed as drugs), some plants are still used as pure substances in the manufacture of drugs (e.g. *Digitalis purpurea* for the production of Digitoxin). Sometimes they are altered to produce drugs (e.g. Diosgenin from various species of Yam for the manufacture of Progesterone) (Ayensu, 1978, Sofowora, 1982). These have led to increased interest in medicinal plants by the developed countries and drug companies (Vanden Berghe *et al.,*1985, 1986, 1991). For example, the herbal medicine market in the European Union countries is said now to be worth about US$7 billion a year (Wilkinson, 1998). In 1990 alone, Europe, Japan, and the US spent US$3.3 billion on over-the-counter herbal medicines (Vanden Berghe *et al.,* 1986).

The wide availability of chemically useful antibiotics and semi-synthetic analogues notwithstanding, a continuing search for new anti-infective agents remains indispensable (Vlietinck & Vanden Berghe, 1991). Most of the major anti-infective agents have indeed considerable drawbacks in terms of limited spectrum or serious side effects (Vlietinck & Vanden Berghe, 1991). Moreover, the combination of the genetic versatility of microbes and widespread over use of anti-infective agents has led to

increasing clinical resistance of previously sensitive infections (Rwangobo *et al.,* 1988, Vanden Berghe *et al.,* 1986). The same arguments plead in favour of the development of new anti-viral drugs, especially since the *pharmacopoeia antiviral armamentarium* is small and limited to the treatment of one or only a few specific viruses (Vlietinck and Vanden Berghe, 1991).

One of the possible strategies for finding new anti-infective agents could involve the search for compounds with chemotherapeutic activities supplementary to, and structures widely different from, those in current use (Mitcher & Balfour, 1985).

A number of reports concerning the anti-viral and antibacterial constituents of African medicinal plants have appeared (Watt & Breyer-Brandwijk, 1962; Sofowora, 1993; Bep Oliver-Bever, 1986; Vanden Berghe *et al.*, 1986), but the studies have not been extensive and thousands of plants still need to be investigated (Ayensu, 1978, Sofowora, 1982). Taking into account the enormous number (Sandberg and Bruhn, 1979) and the amazing structural diversity of the currently available antimicrobially and antivirally active plant constituents (Hudson, 1990), one might hope that promising systemic and/or locally acting anti-infective agents might be discovered in the plant kingdom (Vlietinck, 1987).

No botanist can tell with certainly how many species of plants exist in the world's flora today; however, estimates vary from 250,000 to 500,000 or even more (Akerele, 1993). In traditional societies, where human life enjoys an intimate relationship with the ambient vegetation, man has experimented with many of these species, usually putting them, whenever possible, into the mouth (Akpata, 1997). Most were innocuous, serving no purpose; a few, he found, nourished him, some were distasteful or even

toxic and made him ill or killed him. Occasionally, they seemed to relieve symptoms of sickness and a small number of them with narcotics or hallucinogenic effects transported him temporarily from this mundane existence to unreal realms which he could not understand (Etkin and Ross, 1982, Schwontkowski, 1993). From this experimentation- which must have been in process since man has been on the planet- he has built up an astonishingly complete knowledge of the properties of plants (Sofowora, 1982). Much of this knowledge, passed on orally from generation to generation in unlettered societies, is still extant in many parts of the world (Dunlop, 1975, Harley, 1941, Impsato, 1977, Schwontkowski, 1993, Sofowora, 1982).

There has been a resurgence of interest in ethnomedicine, ethnobotany and ethnopharmacology (Wilkinson, 1998). It has resulted in intensification of field studies amongst still viable primitive cultures on the one hand, and a culling of data hidden in the vast and diffuse literature of the past, on the other hand (Ayensu, 1978). Stimulation for this intensification has come not only from intellectual curiosity but also from the realization that the plant kingdom represents a vast emporium of untapped medical potentialities (Ayensu, 1978, Balley, 1937, Capassa, 1985, Elisabetskey, 1991, Elisabetskey and Morales, 1988). It is primarily in tropical parts of the world where ethnobotanical investigations are yielding the richest harvest (Fabry et al., 1998). There are several reasons: the wealth of the flora is usually far greater than in temperate zones and still traditional societies with histories of plant use for medicinal purposes abound in these areas (Ainslie, 1937, Beuscher *et al.*, 1994, Dennis, 1988, Fabry *et al.,* 1998, King and Tempesta, 1994).

Studies in medicinal plants in tropical areas of Southeast Asia have recently taken a surge forward but perhaps the Asiatic region where most

attention has been paid to traditional medicine is in India (Babbar *et al*, 1970, 1979, Chopra *et al.,* 1956). Following national independence, Indian science placed strong emphasis on chemical and pharmacological investigations of plants employed for thousands of years in Ayurvedic and other native systems of medicine (Chandrasena, 1935). One of the results, of course, has been the discovery of the properties of *Rauvolfia serpentina,* leading to new medicines that have revolutionized modern western medical practices (Hudson, 1990). The present increase of ethnobotanical field research in India is encouraged and attests to the vitality of this surge (Farnsworth and Soejarto, 1991, Taylor *et al.,* 1996). It is well known too, that intensive study of many of the ancient folk remedies still used in present-day China is well underway (WHO, 1986, Li *et al.*, 1988).

Africa on the other hand, presents a rather disheartening contrast. An enormous and extensively varied continent, with several unique floras, it appears, perhaps, to have been the cradle of the human race (Ayensu, 1978). It has been inhabited by thousands of locally distinct tribes of many diverse cultures. It can boast of very early civilization, such as Egypt and Ethiopia, which had extensive written history (Sofowora, 1982). There are still strong and viable cultures that unlike those of many other parts of the world resist the encroachment of outside influence (Brokensha *et al.,* 1980). Yet, what we know of the ethnomedicine and ethnopharmacology of vast areas of Africa is pathetically little. Some locally important and extremely valuable reports have been published (Watt and Brandwijk, 1962; Balley, 1937; Sofowora, 1982, Ayensu, 1978). They tantalize us with the immeasurable amount of folk knowledge, still hidden away in limbo in danger of perishing.

1.2 TRADITIONAL MEDICAL AND VETERINARY MEDICAL PRACTICE IN AFRICA INCLUDING NIGERIA

Those who practiced traditional medicine are referred to as traditional healers or traditional medical practitioners. They can be described as people who are recognized by the community in which they live as competent to provide health care by using vegetables, animal and mineral substances as medicines together with certain other methods (Etkin and Ross, 1982, Sofowora, 1982). These methods are based on social, cultural and religious backgrounds as well as on the knowledge, attitudes and beliefs that are prevalent in the community regarding physical, mental and social well-being and the causes of disease and disability (Sofowora, 1982).

The term traditional medicine or folk medicine refers to the knowledge of the mode of treatment or traditional beliefs, which is common to a group of rural people. It need not involve a specific medical system, but relates rather to use by tradition within a group or tribe of people, the population of which may or may not be defined (Sofowora, 1982).

Although it is not known exactly when the first men practiced herbalism in Nigeria, a number of theories have been advanced by scholars and traditional medicine practitioners alike, to explain the acquisition of this knowledge by early man (Akpata, 1979). One such theory is that early man deliberately selected specific plant materials for the treatment of his ailments since man had the ability to rationalize rather than to rely on instinct, as do animals (Sofowora, 1982). This choice was certainly not based on the knowledge of the plant constituents. Some anthropologists' state that early man lived in fear and that in order to allay this, he indulged in mystical and religious rituals. Thus, it could well be those religious

thoughts and its collection influenced the initial selection of plant material for medicinal purposes and a magic ritual accompanied administration. Some plants are still used in the rituals of traditional religion in Nigeria and many other parts of Africa today.

It has also been proposed that knowledge of medicinal plants was gained by accident, although this theory has been refuted by a number of traditional medical practitioners who claim that information on such was communicated to their ancestors in various ways (Akpata, 1979; Lambo, 1979). However, early man could have gained some scientific knowledge by watching the effects produced by various plants when eaten by domestic animals. Even today some herbalists try out new remedies on domestic animals, especially when testing for toxicity and on themselves or their relations (Schilhorn Van Veen, 1996). Such tests prove to the patient that the preparation is harmless and sometimes also confirm that the dosage prescribed is justifiable (Sofowora, 1982). Such information on African and indeed Nigerian medicinal and toxic plants has been passed orally from generation to generation and even today there are herbal cures which have not been written down (Ainslie, 1937, Balley, 1937, Etkin, 1981, Imperato, 1977).

According to some traditional practitioners (Akpata, 1979; Ogunyemi, 1979) another possibility is that knowledge of traditional cures came from wizards and witches. It is believed that some witches, whether living or dead, attend village markets in strange forms: as goats, sheep or birds. If their presence in this disguise is detected by someone very shrewd or gifted, such as a traditional practitioner, the practitioner is promised some useful herbal cures in return for not exposing the witch. The same reward would be offered if a real-life witch was caught in the process of performing an evil act (Sofowora, 1982).

Hunters have been reported as the original custodians of some effective traditional herbal recipes. Such knowledge could have been acquired when, for example, a hunter shot an elephant. If the elephant ran away, chewed leaves from a specific plant and did not die, it is believed the hunter noted the plant as a possible antidote for wounds or for relieving pain. Traditional practitioners also claim that when in a trance, it is possible to be taught the properties of plants by the spirit of an ancestor who practiced herbalism (Makhubu, 1978; Elewude, 1980).

1.2.1 Status of traditional medicine in Africa

In Africa region, traditional medicine has become a part of the people's culture even though this form of medicine is not as well organized as, for example, in India and China (Lin and Panzer, 1994). Practitioners include herbalists, bone setters, village midwives or traditional birth attendants, traditional psychiatrists, spiritual healers and other specialist (Akpata, 1979, Sofowora, 1982). Many countries in Africa now have a division, department or task force on traditional medicine, usually attached to their ministries of Health. Similar bodies also exist at state or local government level. Research in African traditional medicine and pharmacopoeia had been spearheaded by the Organization Of African Unity through its Scientific, Technical and Research Commission (OAU/STRC) (Sofowora, 1982).

Most African countries now have at least one research group investigating medicinal plants and the OAU/STRC continues to finance research on African medicinal plants in many countries including Angola, Egypt, Senegal, Ghana, Mali, Nigeria, Guinea, Uganda, Tanzania and Madagascar (OAU/STRC. 1979). It is noteworthy that a number of Research Institutes on traditional medicine in Africa now has herbalists on

their staff. Research has developed from screening of medicinal plants for bioactive agents to the development of drugs and dosage forms for natural products of merit (WHO, 1986).

1.2.2 Status of traditional veterinary medicine in Africa

Since the domestication of animals some 10,000 years ago, stock raisers and handlers have naturally been concerned about livestock health (Bierea, 1955). The oldest known veterinary texts originate from Egypt, India and China (Lin and Panzer, 1994). The latter nation is particularly renowned for its early elaboration and use of acupuncture in animal healthcare, which is still based on the same principles and essentially the same techniques and equipment today (Baïrachi Levy, 1991). Until the early 1900s most veterinary practices could be considered "traditional" in the sense that they derived from long experience and underwent little fundamental change in many of their tools and techniques. For their *materia medica,* historically both human and animal medicine has relied heavily on plant materials. Indeed, most of today's major pharmaceutical companies started, a century ago, by selling plant extracts (Mez-Mengold, 1971), and approximately a quarter of all prescriptions drugs currently sold in the western world still use active ingredients derived from plants (Cox and Balick, 1994).

It has become evident that, as in high-tech human medicine, conventional veterinary medicine and formal-sector resources alone are inadequate for meeting the basic animal health care needs many of the world's stock raisers in any sustainable way (Haan and Bekure, 1991, Schillhorn Van Veen and de Haan, 1995). Thus, in the mid-1970s scientists and developers began to look increasingly to local healthcare knowledge, and

practitioners for fresh ideas that were more 'practical, low tech and cost effective' (FAO, 1991).

In both first and third world countries, the search for alternatives has triggered the re-evaluation and appreciation of "ethnoscience"; local or indigenous knowledge and methods of cropping, stock raising, healing, managing natural resources and so forth (Brokensha *et al.,* 1980, McCorkle, 1995, Warren, 1991_a, 1991_b, Warren *et al.,* 1995). This sort of empirical agro-ecological and medical know-how has been elaborated down through generations; and it continues to be developed or modified by local groups as their ecological, economic, social, demographic and other circumstance change. Often, it is unique to a given culture or society and historically well adapted to its biophysical and human ecologies. Usually it is transmitted orally rather than in writing. And it forms the basis for much of local-level decision- making and action in many facets of daily life (McCorkle, 1995).

Ethnoveterinary Medicine constitutes one branch of ethnoscience. Its research, development and extension have emerged as a fertile field for the generation or regeneration and transfer of appropriate and sustainable veterinary alternatives to people everywhere, but especially to third world stock raisers like Nigeria (Lans and Brown, 1998a,b., McCorkle, 1995).

1.2.3 Traditional methods of animal disease prevention and control in the African Savannah

Stock raising has formed a vital part of the livelihood and culture of many African peoples especially those of the Savannah zone of sub-Saharan Africa. Livestock are also a mainstream of the economies of today's Savannah countries like Nigeria (Kudi *et al.,* 1998). Given the importance of stock raising to both household and national economies, African

herders long-standing concern with the well being of their animals is understandable. Livestock disease, in particular plays a perhaps more prominent role in Africa than elsewhere, the five most important diseases (ruminant trypanasomiasis, contagious bovine pleuropneumonia or CBPP, rinderpest, east coast fever or ECF and heart water) occur only rarely on other continents (Schilhorn Van Veen and Haans, 1995). Thus, Africans are confronted with more and unique livestock health problems. This help to explain herders considerable attention to disease prevention and control and the lengths to which they have been known to go to protect their animals (Ajayi, 1990).

Prevention and control of livestock disease have long been a critical concern in Africa, as testified in early Egyptian papyri (schwabe, 1978), numerous biblical references and later in the works of thirteenth-century Arab scholars such as Yaqut, Ibn Said and Abu Zacaria (Cuoq, 1975). Indeed, in the early middle ages, Arabia was a world centre of veterinary and other medical knowledge (Schwabe and Kuojok, 1981). With the spread of Islam some of this knowledge made its way into Africa, where stock raisers adopted, adapted, refined and combined it with local (and later European) knowledge and practice in their efforts to combat against the many diseases afflicting their herds (El-Kheir and Salih, 1980).

In the African Savannah area, the majority of traditional practices in this regard can be classed under two broad types (Ford, 1971), ecological and physiological. The former is commonly used when disease pressure is intermittent, localized and to some extent avoidable. The primary objective of the ecological approach is to forestall exposure to fatal diseases, such as tsetse-transmitted trypasomiasis, CBPP, rinderpest, anthrax and blackleg. A classic example of an ecological method of disease prevention is moving herds so as to avoid contact with sources of contagion

(Schilhorn Van Veen, 1996). In contrast to avoid such exogenous strategies, the physiological approach seeks to prevent or control disease by modifying endogenous processes. A good example is vaccination.

African stock raisers also employ strategies that represent something of a mix between ecological and physiological approaches such as magical and religious procedures that defy classification under either rubric (McCorkle, 1995). Nevertheless, the ecological physiological distinction provides a useful framework for organizing an overview of traditional African methods of disease prevention and control (Allan, 1965).

1.2.3.1Specific Ethnoveterinary medicine in Nigeria

During recent years, stock raising has taken great strides toward meeting Nigeria's increased agricultural needs. One of the major factors responsible for such progress has been improved health coverage for farm animals. Across the past decade, veterinary services provided by government, co-operatives and other agencies have been greatly strengthened, and veterinary hospitals all over the country are now staffed by trained veterinarians. Nevertheless, many areas still lack adequate access to modern veterinary services (OAU/STRC, 1979).

Fortunately, the past decade has also seen a great upsurge of interest in research on indigenous herbal drugs in Nigeria. Major biomedical laboratories and medical organizations, plus a few veterinary institutions are now screening numerous ethnobotanicals for pharmacological effects (Etkin, 1981Ford, 1971). The positive results of such investigations, however, strongly suggest that many ethnoveterinary botanicals may be equally useful, since many of the same items are employed for both human beings and animals (Haans and Bekure, 1991).

The validity of many traditional Nigerian remedies, including many of the treatments for microbial infections, parasitic diseases, gastrointestinal problems and reproductive dysfunction, has already been substantiated by scientific research. And it appears that most traditional Nigerian herbal remedies for domestic animals are safe at the prescribed dosages (Lambo, 1979). Moreover, some of these remedies are even capable of treating diseases for which modern medicine has not yet found a satisfactory or lasting solution. There is great need to unravel the mysteries of the indigenous pharmacopoeia and unearth its hidden treasures through scientific study. If research in human ethnomedicine is any guide, ethnoveterinary pharmacology has much to offer in the way of potent inexpensive, accessible and safe drugs to treat and prevent animal health problems (McCorkle, 1998).

Ethnoveterinary medicine in northern Nigeria

The Livestock Owners of Northern Nigeria are predominantly of the Fulani tribe. Originally they were pastoralists; they had no access to land and were forced to adopt a nomadic lifestyle. At present the Fulani are becoming more and more integrated into rural and urban communities (Schilhorn Van Veen, 1996). All indigenous knowledge and practices regarding cattle and other ruminants in Northern Nigeria can therefore be found among the Fulani. They have specific knowledge of the epidemiology and gross pathology of the diseases and ailments that affect cattle, sheep, goats and horses. This information is relatively easy for outsiders to obtain (Schilhorn Van Veen, 1996).

1. Contagious Diseases

Contagious diseases appear to be well understood by the Fulani. Judging by the actions they take, they must know about animal-to-animal transmission. When a contagious disease strikes, the Fulani separate their sick animals from the healthy ones. They warn neighbouring herders and make arrangement to use separate rangeland and watering places. Anytime an outbreak of rinderpest or contagious bovine pleuropneumonia (CBPP) occurred, the Fulani would not graze their cattle in the affected areas for two months. Pastures infected with endemic diseases such as blackwater and anthrax were not used for grazing for two years. Places where animals had died from these diseases would be covered with thorn bushes in order to prevent healthy cattle from grazing.

2. Local vaccination practices

The Fulani's response to foot-and-mouth disease (FMD) illustrates how indigenous knowledge sometimes outstrips contemporary Western science. Fulani sometimes move their cattle upwind of infected herds to prevent the disease from spreading, and sometimes they move them downwind to expose the animals to FMD, knowing that a mild case of the disease will not be fatal and will confer immunity. Nigerian cattle-owners know that fluid in the tongue blisters of animals infected with FMD is infectious to other animals. To control the spread of the disease, they collect this fluid, dip a tree thorn in it, and scratch the tongue epithelium of apparently healthy animals in order to vaccinate them. Vaccination against CBPP is standard procedure for the Fulani. They slice lung tissue from a diseased animal and implant it under the skin on the foreheads of their cattle, sealing the incisions with mud.

3. Chosen Tracks

Nigerian Fulani appreciate the role of insects in the spread of disease. Trypanosomiasis is linked to tsetse fly bite, whereas ticks are known to transmit other blood diseases. Common preventive measures among the Fulani include applying effective home-made fly repellents, lighting smudge fires to drive insects away and avoiding infested grazing areas and shade trees. The Fulani have special ways of controlling ticks. They feed host animals salty plants so that ticks fall off; they pick off ticks and burn them; and they burn off infested rangeland.

1.3 CONTROL OF VIRAL INFECTION

Control of virus infections, like other kind of infection control can be effected either as a prophylactic measure or therapeutically, in order to control and alleviate a virus infection which has already established (Beladi *et al.,* 1977, Bockstahler *et al.,* 1984, Brody *et al.,* 1994, Declereq, 1995).

Unlike most bacterial, fungal and parasitic infections, however, viruses are not autonomous organisms and therefore require living cells in which to replicate. Consequently, most of the steps in their replication involve normal cellular metabolic pathways, and this makes it difficult to design treatments to attack the virus directly, or its replication, without accompanying adverse effects on the cells (Brody *et al,* 1994). Fortunately, many viruses have unique features in their structure or in their replication cycles, and these constitute potential targets (Hirsch and Kaplan, 1990, Hudson, 1990). Some of the successful antiviral chemotherapy to date has been achieved with the herpes viruses because certain key viral enzymes have distinctive affinities for several nucleotide analogs (Blochstahler *et al.,* 1984, Declereq, 1995, Golstein and Swain, 1965, Hirsch and Kaplan, 1990, Kaul *et al.,* 1985).

1.3.1 Limitation of the use of vaccines

Two general features are considered to be responsible for most of the limitations in the use of vaccines (Norby, 1983). Firstly, there is the problem of genetic variation; there is no reason to think that viruses will eventually stabilize; consequently vaccines of all kinds will continually have to be modified to accommodate "antigenic drift" and the occasional emergence of recombinant forms (Hudson, 1990). Also, although some

viruses may occasionally be eradicated from human populations, as suggested by the apparent success against small pox, many other viruses probably await their turn to invade. Example of such is the exotic viruses, which are usually innocuous in their natural hosts and only gain recognition when humans stumble upon them accidentally. A similar trend may also occur amongst non human populations especially when domesticated animals are introduced into new environment or when wild animals are displaced (Hudson, 1990).

The second feature is the property of viruses to persist in wild populations, i.e., carriers or reservoirs. Smallpox could be eradicated because it was confined to humans. In contrast, most of the devastating animal viruses usually persist in wild populations that are difficult to control (Hudson, 1990). Thus, although many clinicians are optimistic that mass vaccination against measles will result in virtual disappearance of this virus (Mitchelle and Balfour, 1985), an analogous program would fail, for example, in the case of rabies, unless the problem of wild reservoirs could be solved.

1.3.2 Chemotherapy

For many years virologists have sought chemical means of combating virus infections (Brody *et al,* 1994). In spite of the intensive search for suitable synthetic antiviral chemicals, relatively little success has ensured. Although there is no doubt that many lives have been saved and numerous potentially crippling diseases have been averted through the use of judicious chemotherapy (Freestone, 1985, Larder and Darby, 1984, Mitchell and Balfour, 1985).

One of the principal reasons for these successes is the fact that, while the virus itself may not be cleared from the body, the alleviation of virus-induced immunosuppresion and histopathological effects are

commendable i.e., goals which can lessen the risk of secondary microbial infections and disease (Hudson, 1990). Even relatively innocuous infections, such as herpes simplex genital ulcers in humans are deemed worthy of appropriate chemotherapy because of their chronic discomfiture and risk of transmission (Hirsch and Kaplan, 1990, Hudson, 1990, Norby, 1983).

Since viruses are obligate intracellular parasites, antiviral agents must be capable of selectively inhibiting viral functions without damaging the host. Many drugs are available in the market today and their therapeutic strategies as shown in Table 1, are known to have problems of either being too toxic or because of viral latency (Brody *et al*, 1994).

In view of this, it seems reasonable to continue the search for more potent and more specific antivirals. The chances of success in the future are significantly greater. This is due to the fact that now we appreciate the value of a rationalistic approach to the problem based upon more intimate knowledge of virus replication and its relationship to cellular metabolism (Dunlop, 1975, King and Tempesta, 1994, Rozhon *et al.*, 1994, Vanden Berghe *et al.*, 1991).

Table 1: Chemotherapeutic Targets of Known Antiviral Drugs.

DRUG	TARGET	EXAMPLE OF VIRUS
Amantadine (Symmetrical)	Block Virus uncoating	Influenza
Methisazone (Marboran)	Block Synthesis of Viral Protein	Poxviruses
Guanidine (HBB)	Inhibit Viral Replication	Picornaviruses
Phosphonoacetic acid	Inhibit virus-induced DNA polymerase	Herpes Simplex Virus
Idoxuridine	Inhibit thymidine kinase	Herpes Simplex Virus
Vidarabine	Inhibit DNA polymerase	Varicella-zoster, Cytomegalovirus
Acyclovir	Inhibit virus-coded DNA polymerase	EBV and HSV
Virazole (Ribavirin)	Synthetic nucleoside against DNA and RNA (protein inhibitor)	Most DNA viruses
Dactinomycin	Inhibit DNA-dependent RNA synthesis	Most DNA viruses
Interferon	Induce production of cellular enzymes, blocking viral reproduction by inhibiting transition of viral mRNA.	Most DNA and RNA viruses

1.3.3 Photochemotherapy

Photochemotherapy has been referred to, traditionally, as the application of light sensitive dyes, such as neutral red and proflavine, to the treatment of topical herpes simplex infections Hudson, 1990). This form of therapy, otherwise referred to as photodynamic therapy, was used in many cases to treat cold sores, genital sore and keratitis, with some success (Bockstahler *et al.,* 1984; Pavan-Langston, 1984). The principle of the treatment depends upon the ability of the dye to intercalate between neighbouring nucleotides in the DNA (or RNA), or to bind electrostatically to the nucleotide bases. Upon exposure to light of the appropriate wavelength, usually in the visible or near-UV range, damage to DNA results in the form of single or double- strand breaks. This process takes place in both viral and cellular DNA, so that viral replication is inhibited (Piette *et al,* 1986).

In addition to the obvious potential for side effects resulting from damage to cell components the spectre of treatment-induced oncogenesis has been raised (Piette *et al.,* 1986). Herpes viruses possess cell-transforming capacity, especially when their ability to replicate and to kill cells has been inactivated by various means. For these reasons photodynamic dye therapy waned in interest and was no longer considered as a safe method of treating virus infection (Bockstahler *et al.,* 1984; Piette *et al.,* 1986).

Recently there has been renewed interest in phototherapy because of the realization that there are numerous naturally occurring plant substances, many of which have antimicrobial activity, which are active in the presence of light (Hudson, 1990). Some of these compounds are already familiar to clinical practice e.g. the psoralens used for the treatment of psoriasis. Many of them constitute the "active principle" of plants, which

have had a history of successful treatment of skin afflictions, including microbial infections (Spikes, 1975; Towers, 1980, 1984; Poulton and Ashwoodsmith, 1983; Towers and Hudson, 1987).

There are two limitations to this method of antiviral therapy. First the question of light penetration arises in connection with large animals, although visible radiation, especially at the red end of the spectrum, exhibits a surprisingly high degree of penetration in tissues, its use is still only limited to the external several millimetres of exposed skin (Bockstahler *et al.,* 1984). A solution to this problem would be the use of optical fibres that can be designed to reach almost any tissue in the body. The other limitation is the timing of the treatment.

1.3.4 Monoclonal antibody conjugate and similar approaches

Chemotherapy against virus infection would be made more attractive if it were possible to selectively target specific chemicals produce by the infected cells; thereby killing them while sparing the neighbouring uninfected cell, which expresses viral antigens on the cell surface (Hudson, 1990). The monoclonal antibody component of a chemical conjugate would selectively bind to appropriate viral antigens and subsequently become internalized, whereupon the attached chemical could then cause its damage (Edwards, 1983, Magee and Ristow, 1983).

In practice, more than half of the virus encountered have membranes and code for one or more viral proteins which are inserted into the cell membrane during replication (Oserff *et al.,* 1987). The insertion tends to occur at discrete sites of the membrane, known as "patches" which consist of clusters of antigen easily recognized by antiviral antibodies.

The rationale for this approach was first developed for the chemotherapy of experimental cancer (Edwards, 1983). The earlier chemicals used included some potentially toxic substances, which were apparently without effect on the host cells or animals by virtue of their irreversible linkage to the antibody molecule. Consequently only those cells expressing tumour antigens complementary to the antibody were destroyed (Magee and Ristow, 1983, Oseroff *et al.*, 1987, Mew *et al.,* 1983).

The infected cells only express surface viral antigens at a certain time in the replication cycle and therefore are probably only accessible to the conjugate for a limited period of time (Hudson, 1990). A tremendous advantage of this kind of treatment, compared with conventional chemotherapy is the possibility of destroying persistently infected cells. As long as the cells harbouring a latent viral genome or chronic virus infection express surface antigens, which they sometimes do, then they are amenable to attack (Hudson, 1990).

1.4 THE DEVELOPMENT OF ANTIVIRAL AGENTS FROM PLANTS

The acquisition of new agents with chemotherapeutic value in the fight against viruses and cancer is obviously a medical problem of high importance to modern man. Plant has been particularly valuable in the empirical search for new drugs since there is a tremendous historical legacy of folklore uses of plant preparations in medicine (Akerele, 1993, Dalziel, 1948, Harley, 1941, Kerharo, 1974). This has been exploited in the discovery of many classes of drugs where a plant product was the initial lead that led to development of broad classes of compounds. Some examples are cocaine which led to hundreds of local anaesthetics, morphine which led to all narcotic analgesics, and quinine which led to a large group of antimalarials (Lewis and Elvin-Lewis, 1977, Vlietinck, 1987). It is clear that many interesting and useful classes of biologically active chemicals have their origin in the plant kingdom (Hudson, 1990).

Several other key points: the chemical structures of active plant products tend to be complex and would therefore be very unlikely to be synthesized a priori in the search for new agents. The stereochemistry of plant products and natural products in general is complex; and where large series of related compounds have been prepared and tested the natural isomers tend to have the most activity (Chandrasena, 1935, Cruz, 1995). This leads to the hypothesis that compounds produced biologically tend to have biological activity. This is borne out, for if one screens a large group of randomly selected natural products versus an equivalent group of randomly selected synthetic compounds for almost any kind of biological activity, the natural products will show a higher percentage of actives (King and Tempesta, 1994, Ohigashi *et al.,* 1991).

The above facts alone would be enough to justify screening plants for antiviral activity, but there is yet another crucial consideration which militates for such screening: the long and voluminous history of the use of plants in folklore for the treatment of infectious diseases and related conditions (Freestone, 1985).

1.4.1 Selection, collection and identification of medicinal plants

1.4.1.1 Selection and collection of medicinal plants

The approaches which can be used to select plants for developments as medicines can be distilled into three models; selection based on folklore, selection based on botanical or chemical groups and random selection (Sofowora, 1982, Hudson, 1990). Each of these models has advantages and disadvantages that need to be considered.

Selection Based on Folklore

A major question is "how accurate are accounts of folklore medicine?" Considered in the context of usage over many generations it would seem that folklore has a reinforcement system built in so that plant drugs which are ineffective would be dropped from use over time. While those which are curative would be used increasingly (Akpata, 1979), resulting in a high percentage of useful drugs being found in plants used by particular culture which has had many generations for trial and error to occur. It is also clear that there is an excellent pre-screen for activity, since if the treatment succeeds and the patient lives, the knowledge tends to be passed onto future generations (Sofowora, 1982).

There are several major problems in the use of folklore as a tool to select plants for screening. First, since these folklore reports originate for a large part in primitive cultures and folklore medicine is administered by persons

who are believed to have supernatural powers (Witch Doctors, Shamans, Medicine Men etc.). Many of the reputed effects may be due to psychological factors or mind over matter considerations, which might be described as faith healing (Imperato, 1977). A second problem relates to the lack of botanical identification of the plants used in folk medicine. Another factor that complicates using folklore plants is that many cultures use extremely complex mixtures of plants. It is quite common in oriental medicine to use 30-60 plants in one preparation and this greatly complicates isolation of a pure active material (Akpata, 1979). Cost is a factor also in that since each sample needs to be searched out and checked with the ethnobotanical literature, the sample procurement costs are high but this may be balanced by the saving in screening costs if one gets more active plants (Hudson, 1990). Another deficiency of plant is that a limited number of plants exist with folklore history, so the number screened may be but a very small fraction of the total available and this would result in good leads being missed (Capassa, 1985).

Selection based on botanical relationship

It is logical to assume that some plant families will have higher yields of antiinfective agents than others, since there is a relationship between the morphological characteristics of a plant and its biochemical characteristics in terms of the secondary metabolites which it produces (Farnsworth and Kaas, 1981). Chemical relationships between particular types of secondary metabolites have been used to classify plants (chemotaxonomy), and it is common knowledge to anyone doing natural product research that botanically related plants tend to have similar kinds of chemicals present (Hudson, 1990). Therefore, once some background is available on what botanical groups yield active compounds, one can

select the group as priority candidates for further collection and screening in order to find more active materials (Ahmed *et al.*, 1996).

The positive aspect of isolation of analogues and better screening efficiency has to be against the negative points of repetitive isolation of known compounds and the fact that by restricting the variety of plants screened novel leads will be missed (Malone, 1983).

Random selection

Novel compounds may be found anywhere in the plant kingdom and to exclude particular groups of plants from screening decreases the chances of finding materials if interest at least in the initial phases of screening. Once large number of particular groups have been screened it may subsequently become possible to exclude various classes of plants from further consideration (Rios *et al.*, 1988).

The random screening method is the best for purposes of encountering novelty and also has the advantage that a ready supply of materials to screen is available and the cost to procure randomly collected samples is not high (Souza Brito, 1996).

1.4.2 Screening of higher plants for antiviral activity

The concept of antiviral drugs has been accepted slowly partly because the toxicity of many of the earlier antiviral agents. In contrast to the development of antibiotics, attempts to develop antiviral drugs have met a variety of problems. Being strictly dependent on cellular metabolic processes, viruses possess only limited intrinsic enzyme systems and building blocks that may serve as specific targets for a drug. An effective antiviral drug should not only display considerable specificity in its antiviral action but should also irreversibly block viral synthesis in order to stop cell

suicide due to the viral infection and restore normal cell synthesis (Vanden Berghe *et al.,* 1986). In addition to this inhibition, the antiviral agent must have a broad spectrum of activity, favourable pharmacodynamic properties and not be immunosuppressive. In the ideal situation the antiviral drug checks the infection while the immune system prepares to destroy the last virus particle (Babbar *et al.*, 1979, Shannon and Schabel, 1980).

There is also a need for new substances with extracellular virucidal activity, since many of the existing disinfectants and antiseptics fail to kill all known pathogenic viruses after 5-minute exposure time at room temperature (Springthorpe *et al.,* 1986, Sidwell, 1986).

1.4.2.1 Test organism and host cells

The viruses to be selected for initial evaluation of plant extracts are obviously of major importance. They must be chosen to represent different group of viruses according to their morphology and various multiplication mechanisms and a range of virus diseases where chemical control would be useful (Grunert, 1979). *In vitro* methods are more appropriate since they allow simultaneous screening of a battery of viruses. *In vivo* screening of extracts against a broad array of viruses, in contrast, is not only very expensive but also extremely laborious.

In vitro antiviral bioassays utilise thinly confluent monolayers of tissue culture cells with sufficient susceptibility to the infecting viruses that a visibly cytopathogenic effect (CPE) i.e. rounding up, shrinking or detaching cells from the monolayer, can be produced and readily observed microscopically within a week. A monolayer of cells consist of animal or human cells grown in culture medium (Van Hoof *et al.*, 1984; Vanden Berghe *et al.,* 1986).

1.4.2.2 Antiviral testing

In most previous cases the antiviral evaluation of crude extract has employed only a single protocol, designed to detect virucidal or prophylactic effects, i.e., those that were found to protect cells from a subsequent virus infection and those that interfered with virus replication in cultured cells. In only a few instances has a specific antiviral ingredient been characterized as a result of such studies (Hudson, 1990).

It should be noted that only in relatively few cases have particular species of plants been examined in more than one study. Even when this has been done inadvertently, there is no basis for a suitable comparison (Silva *et al.,* 1998), since the different studies used different test protocols, different parts of the plant, different types of test virus, or different methods of extraction. So that one cannot state whether or not the same ingredients could be implicated (Vanden Berghe and Vlietinck, 1991, Semple *et al.,* 1998). In any case, phytochemists have long known that there are also geographical and seasonal changes in contents of specific chemicals. Consequently we should not be surprised if two studies, or two tests conducted at different times, give apparently conflicting results for a given species, and in fact in one fairly comprehensive study this was shown to be the case (Souza Brito, 1993).

Preinfection treatment

Babbar *et al.,* (1970) have reported the screening of more than 2000 plant extracts for their ability to protect cultured cells or chick embryos against Newcastle virus and vaccinia virus. In the initial study they examined 620 extracts, although the methods of extraction were not described. Among these, 23 had antiviral activity of some kind, of which 9 were virucidal and 14 inhibited virus replication. Many of the plants clearly contained

interesting and effective antiviral compounds that work by mechanisms that might resemble interferon, but their properties did not allow them to be defined as classical interferon.

The method involves incubating the cell line with the plant extract and washing off the extract before infecting with the chosen virus (Abou-Karan and Shier, 1990). The idea here is for the extract to penetrate the cells and prevent the virus from infecting the cells. Plants found to have this properties would be useful for prophylaxis (Hudon, 1990).

Virucidal action

In the study of Macrae *et al.,* (1988), 34 species in the family *Euphobiaceae* were examined for virucidal activities. The reason for examining this family is the recognition that these plants are very popular in the pharmacopoeia of indigenous South Americans for the treatment of various skin infectious diseases as well as cancer. Various parts of the plant were extracted with methanol and these extracts were partitioned into aqueous and organic fractions (Macrae *et al.,* 1988), the expectation being that different classes of phytochemicals would separate into one of the fractions, e.g., phenolic compounds in the aqueous fractions. Two test protocols were used. In one the virus was mixed with the extract for 2h at 37°C, then the mixture was added to cultured mouse cells for 2h to allow adsorption of virus to the cells. The mixture was then removed and replaced with a standard overlay to allow development of plaques (Abou-Karam and Shier, 1990). This protocol would allow the expression of direct virucidal activity and any interference with virus adsorption or penetration. In the other protocol, the extract was present only in the agarose overlay following normal virus infection of the cells. (Amoros *et al.*, 1992a)

Aqueous extracts of most plants are likely to contain many compounds and tannins, some of which are virucidal. In a series of studies Konowalchuk and Speir (1976) found virucidal activity, as measured by a decrease in poliovirus type 1 pfu (plague forming unit), in a remarkably wide variety of fruit extracts and juices, including wines. They attributed these activities to the common presence of tannins and other phenolic compounds, although in no instance was an active ingredient identified.

Post infection treatment

This method of test involved the treatment of cells with the virus, allowing the virus to adsorbed. After adsorption, the excess virus was removed and plant extract in liquid medium was added to the cells and incubated. Farnsworth and colleagues (1985, 1991) screened a total of 600 plants (mostly of North American Origin), using this method, for antiviral activity. The extracts were essentially defatted ethanol extracts of dried and powdered plant material. Although experimental details are scant, it is probable that an inhibition in virus replication was the main target, although the data can only really be regarded as qualitative (Farnworth and Soejarto, 1991).

In another study, Chang and Yeung, (1988), evaluated aqueous extracts of 27 traditional Chinese herbs for activity against HIV-1. Their test protocol consisted of incubating together the extract (non-toxic concentration), cells and virus, followed by cultivation for four days and assay for percentage of viral antigen-producing cells. This test protocol would cover virucidal effects as well as activity interfering with any stage in the replication cycle (Cardellina *et al.*, 1993, Ono *et al.*, 1990).

1.5 CHOICE AND USE OF PLANT MATERIAL

1.5.1 Introduction

As far as the search for useful phytochemicals is concerned, plant resources are unlimited at present; but those resources are dwindling fast, thanks to progress and the onward march of civilization (Davidson *et al.*, 1996). In fact, little has been done, so far, to exploit the plant world for antiviral compounds. Although a significant number of studies have used known purified plant chemicals (Hudson, 1990, Beladi *et al.,* 1977), very few screening programs have been initiated on crude plant materials. This is in spite of the centuries of tradition, in most of the world's cultures, of controlling microbial infections by means of plant materials, in spite of the recognition by many organic chemists that mother nature is the worlds most creative and efficient chemist (Borris, 1996).

1.5.2 Plant extracts and purified compounds

The investigation of plant as antivirals is faced at the outset with a choice of starting with either a plant considered to be a source of useful materials, or a characterized phytochemical which for some reason is considered worthwhile to evaluate (Chessin *et al.*, 1995). The advantage of purified compound is that a virologist can examine it immediately without the need for help from phytochemists or botanists.

In the case of plant material the first step is correct botanical identification of the plant, which is usually done at the time of collection or shortly thereafter (Cannel, 1998). The material then has to be chopped, homogenized, macerated, or whatever, to give something that can be extracted. Extraction will then follow a fairly standard regimen worked out on the basis of experience. This regimen, like the details of the antiviral

testing methodology, is largely individualistic although certain general principles are commonly found (Silva *et al.*, 1998). Usually, the plant is extracted sequentially by organic and organic/aqueous mixtures in such a manner as to preserve the integrity of the phytochemicals as much as possible. The organic phases can usually be diluted eventually into cell culture medium, if necessary via dimethylsulfoxide; but obviously one has to be careful with crude extracts since the active ingredients may be quite dilute already. The use of concentrated extracts introduces the possibility of cytotoxicity caused by other unrelated substances or solvents.

Identification and final purification of a bioactive compound and final structure and verification of its activity is generally a job for a chemist (Vanden Berghe *et al.*, 1996).

1.5.3 Evaluation of crude extract- pitfalls and benefits

The analysis of plant extracts at any stage of the purification is fraught with several dangers. One of these is the obvious possibility that the solvent itself, such as petroleum ether, chloroform or acetone, could produce toxicity to the test virus or to the cell culture (Hudson, 1990).

A second problem is the possibility of antagonism or masking of the antiviral by some other component of the extract. This may come from the plant material itself, which could contain a compound that antagonizes the antiviral activity or bind to it, rendering it reversibly or irreversibly inactive (Hudson, 1990). Alternatively, components of the virus containing-medium or the cell culture medium, especially serum components, could operate in a similar manner to mask the real antiviral activity. Since extracts should in any case be tested at several dilutions, then those results would probably indicate the presence of complicating factors such as these (Cannel, 1998).

Antiviral activity could also disappear during the course of fractionation and repeated extraction, and this could be due to masking, sequestering by another compound, dilution, or loss of protective substances, or real inactivation (Clark, 1996). Chemical modification or hydrolysis may cause the later. For example, in the case of natural glycosides that possess antiviral activity, if a sugar residue is inadvertently removed by hydrolysis, then the aglycone moiety may no longer be active. Essential side chains also could easily be lost during purification (Hudson, 1990). If one has an idea of the type of compound being sought, then judicious modifications in the technique could be made accordingly. There are now fairly well established techniques for purifying the major classes of phytochemicals.

There is also the possibility of synergism between two or more compounds that together could provide useful antiviral activity. If such synergism were separated during purification, then the antiviral activity could be lost (Amoros *et al*, 1992b). Synergism could happen both at the level of the animal model or in the real disease situation. This may explain the success of many medicinal plant extracts, which could be therapeutically useful for several apparently unrelated syndromes by virtue of the synergistic effects of two or more components that complement each other *in vivo*. In fact a combination of extract may be even more potent for the same reason (Sofowora, 1982). It is worth highlighting this situation because if no impressive antiviral compound can be identified in certain traditional medicinal preparations used for suspected viral syndromes; these does not necessary negate the claim made by the advocates of the preparation. It may reflect the fact that the successful use of the preparation was due to other factors that might augment a weak antiviral activity (Hudson, 1990).

Another point to bear in mind in the testing of plant extracts, especially when medicinal plants are used is that tradition has laid down a foundation of materials that can be used therapeutically. It has also specified the mode of use i.e. the extraction method, the part of the plant to be used, the appropriate season for gathering materials and the details of administration (Martin, 1995). Therefore at the outset of a screening program involving medicinal plants, it is wise to pay attention to the "folkloric" use of the plant as well as its precise identification.

1.6 AIMS OF THIS STUDY

1.6.1. Justification

The history of herbal medicine is as old as human history. In the continent of Africa, the application of herbs for internal and external uses has always been a major factor in the practice of medicine. The treatment of wound with concoctions prepared from leaves, bark and roots is a daily occurrence in an African community. Because of the astringent or disinfectant properties of certain plant parts, such applications have been highly successful for generations. The alkaloids in plant families such as the nightshade, the poppy and the pea, have been well known for healing qualities to the herbalists over the centuries (Daziel, 1948, Eisenberg *et al.,* 1993). Modern man recognizes the familiar plant derivatives from these families as alleviants in strychnine, quinine, nicotine, cocaine and morphine.

The West African flora is known to contain a multiplicity of drug plants and alkaloidal poisons (Frieburghaus *et al.,* 1996, Sofowora, 1982). There are other plants, which contain important derivatives that are not native to West Africa, but are currently grown there for food. Example, bromelein, an enzyme isolated from the stumps of pineapples after harvest, is an anti-inflammatory pharmaceutical used in the treatment of sprains and contusions (Sofowora, 1982). The importance of plant derived pharmaceuticals and the size of the world market for such drugs require that research in this field should be strongly encouraged and financially supported by the government and private pharmaceutical industries of West Africa (King and Tempesta, 1994).

Research on medicinal plants should be intensified at two levels; Firstly, botanical gardens and research institutes should be encouraged to collect, classify and grow the known medicinal plants to assure the authencity of the materials being used by local herbalists. Secondly, biochemist and pharmacologists should be encouraged to analyzed the chemical composition of a number of plants that are reported in this publication to ascertain and extract the active principles of the species that seem to have promise in the formulation of new effective drugs. Such studies will help eliminate those ingredients of plant that are of little medicinal value and encourage the promotion of those species with potential. The overall effect of any scientific study will help to variously establish and confirm the credibility of the use of herbals as an effective source of both traditional and modern medicine.

1.6.2 Specific aims

a) To screen some medicinal plants form Nigeria, used in traditional medicine, for antiviral and antibacterial activity.

b) To isolate and characterize the active principle(s) from such plants.

c) To evaluate some of the plants in a field trial of cattle with clinical diarrhoea of unknown aetiology in Nigeria.

CHAPTER TWO: INTRODUCTION II

2.0 MEDICINAL PLANTS

2.1 ETHNOBOTANY OF MEDICINAL PLANT SELECTED FROM NIGERIA:

2.1.1 *Anacardium occidentale* L.

Family: *Anacardiaceae*

Common Name: Cashew, Cashu, Canju

Part Use in Traditional Medicine: Leaves, Bark and Nut/Seed.

Cashew is a multi-purpose tree common in many parts of the tropics. It is found growing wild on the drier sandy soils. It grows up to 15m in height and has a thick and tortuous trunk and branches so winding that they frequently reach the ground. The cashew tree produces many resources and products and the family is made up of 60 genera and nearly 600 species. The bark and leaf of the tree are used medicinally; the nut has international appeal and market value as a food. Even the shell around the nut is used medicinally and has industrial applications in the plastic and resin industries for its phenol content.

It was discovered by Europeans and first recorded in 1578, and from there taken to India, then to East Africa and the rest of the world. In the 16th century Brazil, cashew fruits and their juices were taken by Europeans to treat fever, to sweeten breath, and to conserve stomach activity (Smith *et al.,* 1992). The Tikuna tribe in Northwest Amazonia considers the fruit

juice to be medicinal against influenza and brew a tea of leaves and bark for diarrhoea (Schultes, 1990). The Wayapi tribes in Guyana use the bark tea for a diarrhoea and /or colic remedy for infants (Grenand *et al.,* 1987). In Brazil, a bark tea is used as a douche for vaginal secretion or as an astringent to stop bleeding after a tooth extraction (Cruz, 1995). The fruit juice and bark tea are a very common diarrhoea remedy throughout the Amazon today, used by Curanderos and local people alike (Scultes, 1990). In Nigeria the leaves and bark are use for treating diarrhoea, Fever, Dysentery and diabetes (Irvine, 1961). The leaves and or the bark are also used in Brazil for eczema, psoriasis, scrofula, dyspepsia, genital problems and venereal diseases, impotency, bronchitis, cough, intestinal colic, leishmaniasis and syphilic related skin diseases (Cruz, 1995). North America practitioners use cashew for diabetes, cough and bronchitis, tonsillitis, intestinal colic, diarrhoea and as a general tonic (Cruz, 1995. Schwontkowski, 1993).

The bark and leaves of cashew are a rich source of tannins, a group of phytochemicals with physiological activities. These tannins have demonstrated an anti-inflammatory effect (Mota *et al.,* 1985). Another group of phytochemicals named anacardiac acids are found in cashew with the highest concentration found in nutshells. Several clinical studies has shown that these chemicals exhibit tyrosinase inhibitory activity, have molluscacidal properties and are cytotoxic to certain cancer cells (Jurberg *et al.,* 1995, Lauren *et al.*, 1987, Mendes *et al.,* 1990, de Souza *et al.,* 1992, Kubo *et al.*, 1994). Its anti-microbial properties (Cowan, 1999, Himejima and Kubo, 1992) were documented in a 1982 clinical study (Laureus *et al.,* 1982) and its effectiveness against leishmanial ulcers were documented in two clinical studies (França *et al.,* 1993, 1996). The family are best known for their phenols and phenolic acids- anacardor and

anacardiac acid, terpenes, triterpenes, polyphenols and tannins (Chaltopadhya & Khare, 1969).

2.1.2 *Boswelia dalzielli* L.

Family: *Burseraceae*

Common Name: Hano, Ararabi, Hararrabi, Basamu.

Part Used in Traditional Medicine: Bark and Gum Resin.

It is a very common tree in the high Savannah, extending as far north as 13^0 latitude where the soil is suitably rich and well watered. The leaf, flowers, fruit or bark are all distinctive. When young or middle aged the stem is erect and the limbs ascend steeply forming a high crown, foliated down to a low point. Older trees exhibit a short, massive bole from which the heavy limbs spread out forming huge crowns with drooping extremities (Dalziel, 1948).

The bark is most conspicuous, pale brown, with large papery pieces peeling off and at times hanging in shreds from the stem. The slash is reddish-brown and a scented gum resin exudes, partly drying into nodules, almost white in colour, readily crumbled (lely, 1925).

The leaves are 30-45 cm long, pinnate with some 7-9 pairs of long, slender, pointed, deeply toothed leaflets. These increase in size towards the top end of the leaf, the basal pair often being very small and distinct in shape. The terminal pair is frequently partly united into one sessile, in colour light green and shining with the venation raised on both surfaces (Ayensu, 1978).

The flowers appear from January to April, and are large bunches of racemes at the tips of the large blunt twigs. The racemes are from 14-20 cm long and bear numerous white flowers on long stalks. The flowers disc is red and the flower scented. The fruits are 3-angled capsules, elongated pear-shaped, with prominent bulges opposite the seeds. The capsule split into three and small seeds are released, each with a sharp spike at the top end (Ayensu, 1978, Etkin, 1981). The plant is being use as an anti-inflammatory agent and also against diarrhoea in Nigeria (Sofowora, 1982)

The gum resin, being scented, is used for burning in houses, or fumigating cloths. It is also used as ingredient in some herbal concoctions. The leaf contain Betulinic acid, which is use as ant-carcinomic, antimalarial, antiviral and as a prostaglandin-synthesis-inhibitor (Duke, 1992a,b., Dunstan *et al.*, 1998, Huang *et al.,* 1995). Alkaloids and tannins have been isolated from the stem bark (Baoua *et al.*, 1976, Duweijua *et al.,* 1993).

2.1.3 *Sterculia setigera* (Del.)

Family: *Sterculiaceae*

Common Name: Kukkugi.

Part Used in Traditional Medicine: Leaf, Bark, Seed/Fruit.

This is a common tree in northern Nigeria, inhabiting granite country and may be found in quantity on hills or valleys amongst rocks (Dalziel, 1948). It does not occur in the extreme north on laterite formations. There is rarely any length of bole, the stem branching low down and the large, crooked limbs spreading wide apart to form a very open crown of irregular form. It can be distinguished at once by its purple bark. Amongst rocks the

roots are above ground for several feet, clasping the boulders or creeping between the crevices. Roots flanges are common. The branches are so soft that they, or even the whole of a fair-sized tree may be swayed to and fro by the hand, without difficulty (Hussani *et al.,* 1991).

The bark is purple and quite smooth except for occasional large grey scales, which leaves yellow patches of very distinctive appearance. A gum exudes from the crimson slash and a watery sap flows at certain seasons. It is used for the treatment of tapeworm and whitlow in Ghana (Irvine, 1961, Dalziel, 1948), boils, fever, diarrhoea, vermifuge and chronic syphilis in Cameroon and Nigeria (Dalziel, 1948, Ayensu, 1978).

The leaves are about 10-12 cm long and 7-10 cm broad. They are light green and downy on both sides, the nerves prominent on the under surface. There is a stalk about 3 inches long. The leaves are soft in texture and when macerated, used in the treatment of boils, diarrhoea, fever, snake-bite and gonorrhoea in Nigeria, Ivory coast and Burkina Faso (Irvine, 1961, Kerharo and Bouquet, 1950, Ayensu, 1978).

The fruits are pods about 7-10 cm long and 5 cm in diameter. There are 4 or 5 together radiating from a twig end and they are the readiest means of identification. They are rounded in the middle and pointed at the end with a groove on the under side along which the pods splits. They are covered with greenish hairs, in a manner similar to the fruits of the Baobab tree. The seeds, about a dozen, are purplish with a horny, yellow aril at the base and they are attached to both edges of the pod, sitting on small bosses, which are covered with short stiff brownish hairs that penetrate the skin of the fingers. The fruit and seed are used for the treatment of digestive problems in Sierra Leone and Guinea (Irvine, 1961, Dalziel, 1948, Ayensu, 1978).

Chemicals isolated include acetic acid (Gum) and butalin (bark), which are used as antibacterials (Duke, 1992a, Jeffery and Baxter, 1983), fungicide and pesticides (Duke, 1992b), anti-infalmmatory (Bisset, 19940, and antiviral and anticarcinogenic (Seki *et al.*, 1990)

2.1.4 *Anogeissus leicarpus* (Gull. & Per.) (A. schimperi)

Family: *Combretaceae*

Common Name: Marke

Part Used in Traditional Medicine: Leaf, Bark.

This is the most evenly and widely distributed of all trees; extending from the southern rain belt to the extreme north of Nigeria. The trees have a height of 21 m with girths of 2 m. The species is rarely distinguished at a distance by its feathery, birch-like foliage and drooping branches, which at first acutely ascending, bend over and droop in graceful curves.

The bark varies according to habitat. A dark gum exudes from the bark, which is a very inferior adhesive, as it is cloudy when mad up into solution. The slash is pale yellow with thin dark lines. The bark is phytochemically said to contain tannin, mucilage and gum (Dalziel, 1948, Githens, 1949, Oliver, 1960, Lewis and Elvin-Lewis, 1977). In Nigeria, it is used as a cure for worms in both humans and animals. Also the bark is chewed for coughs (Lewis and Elvin-Lewis, 1977) and as a chewing stick to clear and prevent against oral infections (Akpata and Akinrimsi, 1977, Odebiyi and Sofowora, 1997, Norton and Addy, 1989).

The leaves are on long, slender, drooping twigs and are oval, slightly darker than below with a silky pubecens which is most marked in the

young foliage. They tend to assume one plane. The leaves are used for the treatment of diarrhoea, wounds and dysentery in northern Nigeria.

Other uses included the wood, which is burnt entire, and fine, white ash used for fixing dyes. The leaves, mixed with salt, make a yellow dye. The sapling and branches produce forked poles for building. Phytochemically, courmarins were isolated from the stem bark (Fadulu, 1975, Ndiyi and Okwute, 1988)

2.1.5 *Bauhinia reticulata* (Schum.) (or B. thonnogi)

Family: *Leguminosae*

Common Name: Kalgo, Camel's Foot, Cow's hoof.

A small to medium-sized tree or shrub from 5-9 m in height with large divided leaves resembling Camel's or Cow's hoof. It occurs over large areas of Nigeria as pure growth, particularly as secondary growth in farmed land, where it is a great pest to the farmers who desire to continue sowing. It produces large drooping white flowers and a brown seedpod, which looks like the mimosa seedpod.

The bark is a dull, dark grey, sometimes with a rust-red tinge, deeply fissured and ridged with hard, brittle bark of some thickness, which fall in large, ragged sections. The slash is bright crimson, turning brown on exposure and shows the fibrous nature of the bark. The leaves are bifoliate, the depth to which the leaf is divided varying a great deal and the angle being sharp or rounded. The texture is tough (Ayensu, 1978).

In Brazilian herbal medicine, it is described as hypoglycaemic, depurative and diuretic. It is considered a good blood cleaner and a leaf decoction is

used internally and externally for elephantiasis and snake-bite as well as other skin conditions (de Almeida, 1993, Cruz, 1995). It is widely used in South America by diabetics to help balance blood sugar levels as well as help with other symptoms produced by diabetes like polyuria, renal disorders and other urinary problems (Bernardes, 1984, Herboper, 1997).

Hypoglycaemic activity was first reported in 1929 clinical study and was followed by another study in 1931 (Juliane, 1929, 1931). Another study was funded in 1945 to try and determine the active constituents responsible for its activity (Costa, 1945). North American practitioners and herbalists are now using it for diabetes; hyperglycaemia and polyuria as well (Schwontkowski, 1993, Easterling, 1993).

In Nigeria, it is use for treating wounds and some febrile conditions. Phytochemical analyses showed that the leaves contain tannins (Githens, 1947,Watt and Breyer-Brandwijk, 1962, Lewis and Elvin-Lewis, 1977). Also contain Betulinic acid, which is an anti-infalmmatory, antibacterial, antiviral and antimalarial agent (Duke, 1992a,b)

2.1.6 *Guiera senegalensis* (J.F.Gmelin)

Family: *Combretaceae*

Common Name: Sabara.

A shrub or very occasionally, a small tree, which covers wide areas of otherwise barren land in the bush Savannah. It also covers the bare rocky slopes and summits of the flat-topped hills. In appearance it is a bushy plant with several stems, dusty grey-green in colour measuring 1-1.5 m in height. It is most conspicuous in fruit. The stem is subject to a gall caused by a grub whose brown excretions fill the cavity in the gall.

The bark is grey and that of the young stems and branches is brown and covered with loose, brown fibres running vertically. The leaves are a dusty grey-green, but a fresh green when new, and are rather dry and leathery in composition (Kerharo, 1974).

The shrub is burnt round cattle, sheep and goats camps as fly repellent. It is also a remedy for colds in such herds. The leaves concocted with water are a medicine for internal complaints, a preventive of leprosy and applied externally, a cure for skin irritations (Etkin and Ross, 1982, Kerharo, 1974). They are also drunk by women after childbirth. The leaves are often added to food to prevent indigestion.

Leaves contain traces of harmane alkaloids, flavonoids, tannins and a bitter principle elastine (Le Grand, 1989). 2,4,5-Tri-O-galloyquinic acid isolated from the leaves inhibited HIV replication and viral reverse transcriptase (Mahmood, 1993). Antibacterial effect of leaf extracts has also been demonstrated (Laurens *et al.,* 1985, Bassene *et al.,* 1995, Bosisio *et al.,* 1997 Kudi *et al.,* 1999). The anti microbial activity of tannins is also well documented (Branter and Grein, 1994, Haslam, 1996, Scalbert, 1991).

2.1.7 *Ziziphus spina-christi* (Willd)

Family: *Rhamnaceae*

Common Name: Kurna

This species is generally found in and around towns where it attains a height of over 12 m with girths of over 2 m. Bright green foliage and tangled crown, commonly spherical in young trees, are readily recognized. Large trees have a great thicket of long, slender twigs that intertwine and emerge here and there from the crown to a distance of several feet, after

the manner of the bramble. It is a good shade tree and this and its edible fruits are the reason for it being planted and preserved in towns (Lely, 1925).

The bark is grey and deeply scoured with long fissures and ragged ridged scales that fall in large sections 15 cm long. The slash is cerise. The leaves though they are arranged spirally around the twigs, tend to assume one plane. The upper surface is bright green with a bluish bloom and the venation roughens the underside (Lely, 1925).

On account of its strength and supposed immunity from white ants, the wood is used for the rafters of plat-roofed mud houses. The fruit is eaten fresh and useful in treating diarrhoea and dysenteric conditions. The leaves, besides providing fodder for goats and cattle, are crushed and applied to cure skin diseases. It is also use for the treatment of worm infestation, fever and sexually transmitted diseases (Sofowora, 1982). Phytochemically known to contain Betulinic acid in the stem bark, which is an antimalarial, anti-inflammatory and antiviral agent (Fujioka *et al*, 1994, Dunstan *et al.*, 1998, Huang *et al.*, 1995)

2.1.8 *Cassia goratensis* L.

Family: *Leguminosea*

Common Name: Rumfu.

The *Cassia* plant family has 300-600 species of trees, shrubs, vines and herbs. Many species have been used medicinally and these tropical plants have a rich history in natural medicine.

This is a small and common tree whose only value, apart from medicinal use by the natives, is ornamental. They are found in clumps of small trees

about 10ft high. They are also singly growing to a height of 6 and even 9 m. In its young state it is apt to be confused with *Cassia occidentalis* and *Cassia tora*, which latter are a shrubby herb with similar flowers and leaves.

The leaves are pinnate, 25-30 cm long with about eight pairs of oval leaflets which are bright green and smooth on the upper surface; grey, with the venation raised on the under surface. They are soft in texture and very often blotched or spotted.

The pods and leaves boiled in water are used for washing and purification after childbirth. The same infusion is taken internally as a cure for fever. It is also use as purgatives and laxatives (Elujoba *et al.*, 1989, Hussain *et al.,* 1991).

Leaves of *Cassia* species are known to be used in the treatment of leprosy, diarrhoea, cough, swellings, stomachache and pneumonia (Sofowora, 1982). Phytochemically the genus contains chrysarobin (an anti-parasitic principle official in European pharmacopoeias), tannins, anthraquinones and sugars (Morton, 1977, Dalziel, 1948, Irvine, 1961, Elujoba *et al.,* 1989, Cruz, 1995, Feng *et al.*, 1962).

2.1.9 *Detarium senegalense* (Gmel.)

Family: *Leguminosae*

Common Name: Taura.

This is found in northern Nigeria as a small tree from 4-6 m high with a few erect branches forming a small flat-topped crown. Often, however, quite large trees can be seen, even in the driest country and further south large specimens up to 12 m high with girths over 1.5 m are not

uncommon. In the southern provinces, the same tree will grow to a height of 24 m with a 4 m girth. The larger portion of the height is almost always the crown, which is spreading and dense, giving good shade. The tree is readily distinguished by its bluish bark and round compressed fruits. The small trees show enlarged tips to the branches from which the leaves spring. In places it is common as to form almost pure forest over small areas (Elujoba *et al.,* 1989).

The bark is bluish-grey, with large polygonal scales. On young trees there are yellowish patches where the scales have fallen and the bark of the smaller branches is ochrous and powdery. The slash is pale crimson. The leaves are pinnate, 16 cm long with 15-25 cm alternate or opposite elliptical leaflets some 3 inches long and 4 cm broad. Those nearer the top are more oval, those at the base rounder. The tip has a slight cleft. The leaf stalks are very short and stout and covered with dusty brown hairs. The surface is waxy and the texture is rather leathery (Dalziel, 1948, Kerharo, 1974).

The tree flesh is use, by the natives, in the manufacture of sweetmeat "madi". The leaves are used in the treatment of diarrhoea, fever and bacterial venereal diseases (Dalziel, 1948).

2.1.10 *Khaya senegalensis* (A. Juss.)

Family: *Meliaceae*

Common Name: Madachi, Mahogany.

This common and well-known dry zone mahogany occurs more or less plentifully all over northern Nigeria and has a distribution from the rain forest to the French boundary. Its habitat is the banks of streams but its soil and water requirement are comparatively modest and any little valley

or moist hallow is sufficient. Normally it is a small tree some 15-18 m high with a girth of 2 m, but 21-24 m and 3 m girth are common. Usually not more than 6 m of its height is clean bole, but no rules can be laid down, as the stem may or may not divide into 2 to 3 large limbs which ascend vertically and form the characteristic open wide-spreading crown. In gregarious clumps the tree may have as many as 5 or 6 of these false stems and form an enormous crown. The bole and main limbs show a wavy habit, especially noticeable in small tree. The base of the bole is often much swollen by the repeated bark chipping for the bitter tonic (Lely, 1925).

The bark is dark grey and covered with small thin scales. A red sap exudes from the bright crimson slash. The leaves are pinnate with 4-6 pairs of leaflets. The leaves of young trees will bear as many as 10 leaflets. The leaf is bright and shiny when young but darkens and dulls and the greyish under surface is typical (Kerharo, 1974).

The wood is used for furniture, canoes and mortars. The bark as a bitter tonic after boiling with water and powdered as a cure of sore backs of horses. The leaves are gathered for camel and cattle fodder. The seed, usually dried, fried, beaten up and boiled to extract the oil, are used for anointing the body, by pagans and also use as dressing in wound management (Sofowora, 1982).

Phytochemically, catechnic tannins, gum resins, bitter principle and calicedrin have been isolated and demonstrated as antibiotic (Dalziel, 1937, Githens, 1949, Lewis and Elvin-Lewis, 1977, Oliver, 1960, Watt and Breyer-Brandwijk, 1962).

2.1.11 *Butyrospermum parkii* L.

Family: *Sapotaceace*

Common Name: Kadanya, Kadai, Shea butter tree.

This is one of the commonest species of the Savannah forest and has a wide distribution. An average tree is about 9 m high with a girth of 1-2 m, though large specimens over 12 m high with a girth of 3 m is by no means infrequent. A short, stout bole and large, spreading limbs, gnarled and crooked, form a widely spreading crown of considerable density, which, from the drooping habit of the lower branches, reaches almost to the ground in many specimens. It is the type species of the tree Savannah and in some parts of the country form a large percentage of the forests.

The bark is dark grey, sometimes almost black, sometimes, particularly in the case of trees growing in barren situations, almost white. It has deep, vertical fissures and prominent, square scales of great thickness. The rough scaling extends to the quite small branches. A milky sap exudes from the crimson slash and from the leaf stalks and twigs, when broken.

The leaves are strap-like, of an average length of 23 cm and a width of 5 cm. The springing of the leaves, from the end 5cm of the twigs, form a shape similar to a rosette. The oil is extracted and butter made by the natives by a preliminary boiling in water, followed by repeated pounding and stirring in cold water, the oil which rises to the surface being skimmed off and placed to harden in calabashes (Ayensu, 1978). It is eaten as such, burnt as an illuminant and used as a base for certain medicines for the treatment of diarrhoea, fever and as wound dressing in Nigeria (Ayensu, 1978, Lely, 1925).

2.1.12 *Psidium guajava*

Family: *Myrtaceae*

Common Name: Guava, Goiba, Gouwa.

Guava fruits often grow well beyond the size of tennis balls on well-branched trees or shrubs reaching up to 20 meters in height. Guava fruit today is considered minor in terms of world trade but is nevertheless widely grown in the tropics, enriching the diet of millions of people in the tropics of the world. It is a common shade tree or shrub in door yard gardens providing shade while the guava fruit are eaten fresh and made into drinks, ice cream and preserves. The fruit contain numerous seeds that can produce a mature fruit-bearing plant within four years (Morales *et al.*, 1994).

Centuries ago, Europeans adventurers, traders and missionaries in the Amazon basin took the much enjoyed and tasty fruits to Africa, Asia, India and the pacific tropical regions where it is now cultivated throughout the tropical regions of the world (Kerharo, 1974).

The leaves and bark of the tree have a long history of use for medicinal purposes and are still employed today. A tea made from the leaves and /or bark have been used by many tribes for diarrhoea and dysentery and other tribes employ it for stomach upsets, vertigo and to regular menstrual periods (Hawkes, 1983). In traditional medicine today, guava is still employed as a natural medicine (Duke and Vasquez, 1994, Grenand *et al.,* 1987, Forero, 1980). Guava leaves still remain in the Dutch pharmacopoeia and the leaves are still used as a diarrhoea treatment in Latin America, Central and West Africa, and Southeast Asia. This long history of use has led modern day researchers to study guava leaf

extracts and its uses as an effective treatment for gastrointestinal disorders has been validated in over eight clinical studies (Smith et al., 1992). Guava fruit has also been studied and has demonstrated hypoglycaemic properties (Morales *et al.,* 1994, Lazoya *et al.*, 1994, Caceres *et al.,* 1993)

Ethnobotanical uses include its use against cold, dysentery, catarrh, fever, jaundice, diarrhoea, scabies, swelling, vermifuge, wound, epilepsy and respiratory conditions (Roman-Ramos *et al.,* 1995, Cheng *et al.,* 1983). Phytochemically, substances isolated include amino acids, ascorbic acid, sugars, vitamin B6 (Lozaya *et al.,* 1994).

2.1.13 *Dichrostachys glomerata* (Chiev.)

Family: *Mimosaceae*

Common Names: Dundu, Kurkur, Burli, Kufana.

It is a small to medium tree 4-5 m in height with branches starting right at the base of the tree. Leaves alternates and are bipennate, 8-10 pairs par stock with a pair of thorns at the base of each stock. There are 15-25 pairs of leaves 5-6 by 1-2 mm in size. The stem is 6-8 cm long with long peduncles (Kerharo, 1974).

Their common habitat is mostly in the Savannah region of West Africa. It is commonly use in traditional medicine as a diuretic and treatment for rheumatism and haemorrhages (Kerharo and Adam, 1974). The back is taken orally as an anti-emetic. It is also known to be use for the treatment of toothache, dental carriers and gingivitis. Externally, the leave is used as

a wound dressing, massaged into intercostale spaces to relieve pains due to rheumatism and swellings. It is also known to be used for mental conditions (Kerharo, 1974).

Phytochemically, it was found to contain amino acids, alkaloids, saponin, tannin, quinones, glycosides, steroids and terpenes (Duke, 1992a,b., Dunstan *et al.*, 1998, Fujioka *et al*, 1994, Huang *et al*, 1995).

2.2 NAPRALERT PROFILE FOR PLANTS WITH ANTIBACTERIAL AND ANTIVIRAL ACTIVITY

NAPRALERT is an acronym for **NA**tural **PR**oduct **ALERT**. It is dynamic database that is updated periodically and has been copyrighted from 1975 to date by the board of trustees of the University of Illinois. The data in NAPRALERT represents a synthesis of information from more than 150,000 scientific journal articles, books, abstracts and patents, collected systematically from global literature. Information covered concerns mainly ethnomedical data on plants, biological effects reported for extracts of living organisms and occurrence of secondary chemical metabolites in living organisms.

The profile shown below was for the 4 plants that were studied in detail. Profile for the other plants were not obtained form NAPRALERT because of the cost ($750/plant species).

2.2.1 *Anacadium occidentale*

The following compounds have been isolated from different parts of the plant from different countries and are presented in Table 2.

Table 2: Profile of compounds isolated from different parts of *Anacardium occidentale.*

PLANT PART	GROUP OF COMPOUNDS	COMPOUNDS ISOLATED	COUNTRY OF ISOLATION	REFERENCE
FRUIT	a. Benzenoid	Acetophenone, Benzaldehyde, Salicyclicacid, Gallic acid	Venezuela, Brazil, Nigeria, Indonesia, USA.	Ogunlana & Ramstad, 1975, Desai et al., 1975, Keshinro, 1985, Rahman et al., 1978, Chhabra et al., 1987, Kubo et al., 1986, Wall et al., 1996, Sardjono 1976, Murthy et al., 1982, Laurens and Paris, 1977, Arya et al., 1989,
	b. Flavonoid	Hypsoside	India	
	c. Vitamin	Ascobic acid	Brazil	
	d. Alkanal	Butan-1-al, B-Mythyl	USA, Madagascar	
	e. Monoterpene	Phellandrene	Venezuela, Brazil, India	
	f. Sesquiterpene	Cryopyllene, Selinene, Alicyclic	Tanzania, India	
	g. Lipid	Octanoic acid, Ethyl esters.	USA	
	h. Tannin	Tannin	India	
	i. Steroid	Campesterol	Venezuela	
LEAF	a. Flavonoid	Agathisflavone, Apigenin, Kaempferol, Myricetin, Quercetin, Robustaflavone, Siltosterol.	Venezuela, India, Madagascar, Indonesia	
	b. Benzenoid	Benzoic acid, Gentisic acid	India	

SEED	a. Triterpene	Amyrin, Cycloartanol, Squalene	Brazil, India	Parro, 1971, Varghese et al., 1971, Subramanian & Nair, 1969, Maia et al., 1969, Gedan et al., 1972, Tyman et al., 1992, Beri, 1970, Malini & Vanithakumari 1990,Felcman & Braganca, 1988, Mota et al., 1985, Gill & Akinwumi, 1986, Dhar et al., 1968, Barros et al., 1970, Tyman & Jacobs, 1971.
	b. Benzenoid	Anarcadic acid, Anacardol, Arachidic acid, Cardanol, Resorcinol	Brazil, Nigeria, Tanzania, USA, India, Indonesia, Mozambique, Kenya, Sri Lanka.	
	c. Lipid	Capric acid, Linoleic acid, Myristic acid, Gadoleic acid, Myristoleic acid, Oleic acid, Lauric acid, Hexadecadienoic acid, Palmitic acid, Palmitileic acid, Stearic acid	Venezuela, Brazil, USA, India	
	d. Alkane	Docasan-1-ol, Elaidic alcohol	India	
	e. Flavonoid	Leucocyanidin	India	

STEM	f. Inorganic Minerals	Sodium, Magnesium, Phosphorus, Sulphur, Chlorine, Potassium, Calcium	USA
	g. Protied	Protein	Venezuela
	h. Steroid	Stigmasterol	USA
	a. Flavonoid	Afzelechin, Catechin	Venezuela, India
	b. Steroid	Campesterol, Cholesterol, Sitosterol	Venezuela, India
	c. Inorganic Meneral	Chromium	India
	d. Benzenoid	Digallic acid	Venezuela
	e. Tannin	Tannin	India

2.2.2 *Sterculia setigera;*

The following compounds have been isolated from different parts of the plant from different countries and are presented in Table 3.

Table 3: Profile of compounds isolated from the Bark of *Sterculia setigera.*

PLANT PART	GROUP OF COMPOUNDS	COMPOUNDS ISOLATED	COUNTRY OF ISOLATION	REFERENCE
STEM	Alkaloids Sterols Terpenes	N/A N/A N/A	Sudan Senegal	Elsheikh et al., 1990, Almagboul et al., 1988, Kerharo, 1963, El-Kheir & Salih, 1980.
LEAF	N/A	N/A	N/A	N/A
ROOT	N/A	N/A	N/A	N/A

N/A = Not applicable

2.2.3 *Anogeissus schimperi;*

The following compounds have been isolated from different parts of the plant from different countries and are presented in Table 4.

Table 4: Profile of compounds isolated from the Bark of *Anogeissus schimperi*

PLANT PART	GROUP OF COMPOUNDS	COMPOUNDS ISOLATED	COUNTRY OF ISOLATION	REFERENCE
STEMBARK	Courmarin	Ellagic acid, 3-3'-Di-0-Methyl	Nigeria	Nduji & Okwute, 1988
		Flavellagic acid, 3-3'-4'-Tri-0-Methyl	Nigeria	Fadulu, 1975.
LEAF	N/A	N/A	N/A	N/A
FRUIT	N/A	N/A	N/A	N/A
ROOT	N/A	N/A	N/A	N/A

N/A = No Information from Napralert.

2.2.4 *Boswelia dalzeilii;*

The following compounds have been isolated from different parts of the plant from different countries and are presented in Table 5.

Table 5: Profile of compounds isolated from the Bark of *Boswelia dalzeilii*

PLANT PART	GROUP OF COMPOUNDS	COMPOUNDS ISOLATED	COUNTRY OF ISOLATION	REFERENCE
STEMBARK	Alkaloid	N/A	Niger	Baoua et al., 1976.
	Tannins	N/A	Cameroon	Duwiejua et al., 1993
LEAF	N/A	N/A	N/A	Baoua et al., 1976.

N/A = No Information from Napralert.

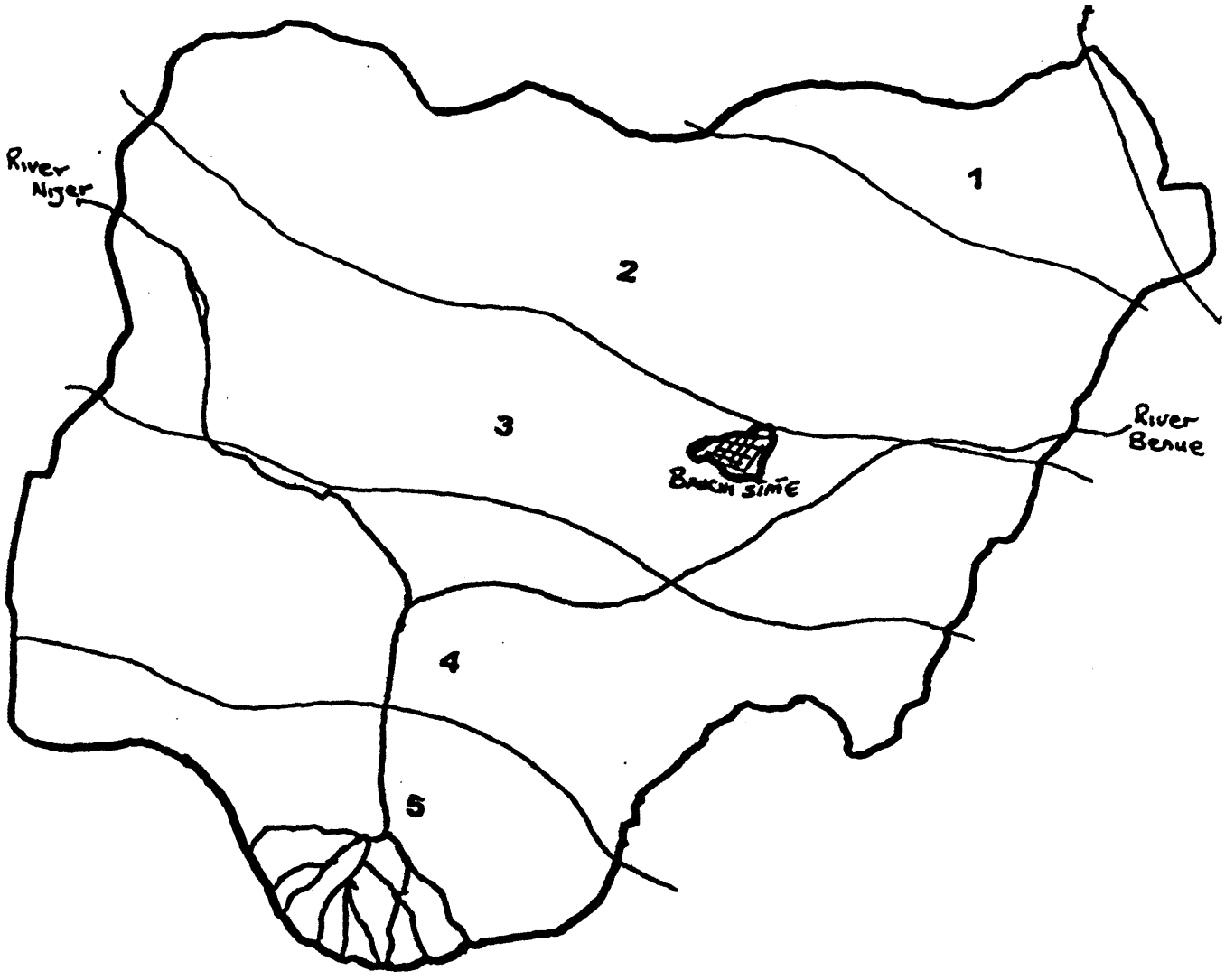
CHAPTER THREE: MATERIALS AND METHODS

3.1 THE STUDY AREA

Bauchi Local Government Area lies between latitudes 10°N and10° 30"N and at an altitude of 690.2m above sea level (Bauchi State Urban and Regional Planning, 1992). Bauchi State has an average annual rainfall of 1091.4mm. The peak of the rain occurs in August with the highest relative humidity of 66.5% and the lowest of 16.5% in February. The mean maximum temperature of 37.5°C occurs in April and the minimum 13.7°C in January (Meteorological Station, Bauchi Aerodrome, 1993-1995).

Bauchi State is located within the Northern Guinea Savannah vegetational belt of Nigeria. The area is known to compose mainly of grassland with inersparsed trees (Fig. 1).

Figure 1: Map of Nigeria: Vegetational Zones



KEY:

SAHEL SAVANNAH	1
SUDAN SAVANNAH	2
GUINEA SAVANNAH	3
RAIN FOREST	4
MANGROVE SWAMP	5

3:2 COLLECTION OF PLANT SAMPLES

Collection of the medicinal plants was based on their use in ethnomedicine and ethnoveterinary medicine. Plants use for the treatment of diarrhoea and feverish conditions were selected with the help of 4 traditional medical practitioners. Plant samples were collected fresh from the Savannah forest of Northern Nigeria and brought to the Botany Department of Abubakar Tafawa-Balewa University, Bauchi, Nigeria for voucher specimen and confirmation of identification. All samples collected were oven dried slowly at 30^{o}C until dried. The dried plant material was then grounded using an electric grinder and placed in dry polythene bags in an aerated environment at room temperature and transported to the United Kingdom for analysis.

3:3 EXTRACTION OF CRUDE EXTRACT

Various methods have been developed for the extraction of crude extract from plants. The major problem in extraction is the presence of interfering compounds like lipids, pigments, plasticizers and grease. Methods have been developed to remove these from crude extracts. Two methods of extraction were used in this study.

3:3:1 Water extraction

In this type of extraction, 10 g of each of the plant materials was mixed with 200-ml sterile distilled water and left overnight. The mixture was then filtered and the residue discarded. The filtrate was evaporated *in vacuo* at 30^{o}C and freeze-dried. The freezed dried residue was kept at 4°C before use. When required the residue were then suspended in sterile distilled water (1g in 28ml), filter sterilized and used for antibacterial and antiviral testing. Unused solutions were discarded.

3:3:2 Solvent extraction

This is widely use during early purification or prior to final purification by chromatography, crystallization or precipitation. Solvent extraction provides the ease of liquid handling, the potential for high-throughput operation and the potential for adaptation to continuos operation. The important criteria for solvent selection include price, toxicological constraint, availability, solute selectivity, recovery difficulties, physical properties and operational hazards. Extraction methods used in this study were those that prevent compound decomposition, side reaction or rearrangement during extraction process.

3:3:2:1 General procedure

Dried (10g) of plant material was extracted with 200ml of 80% ethanol and filtered. The 80% ethanol filtrate was evaporated *in vacuo* (at 30^{o}C) and partitioned in n-Hexane : 90% Ethanol (9:1). The N-Hexane phase was discarded and the 90% ethanol phase residue resuspended in sterile distilled water and evaporated *in vacuo* and freeze-dried (Silva *et al.*, 1998).

The crude extract was triturated (1g) with 180ul distilled water. This was later mixed with 28ml of tissue culture medium (with 2% foetal calf serum), pH adjusted to 7.4 with 0.1N NaOH or 0.1N HCL. The extract was finally filtered sterilized through a 0.2µm filter under aseptic conditions before antiviral and antibacterial testing.

Extraction of Anacardium occidentale L.

Twenty gram of dried ground stem bark of the plant was weighed into a 500-ml schott bottle and 200ml of 80% ethanol added. It was allowed to stand for 24 hours and then filtered using 0.2 *µm* Aquadisc filters (Sortex®). The filtrate was then evaporated in vacuo (at 30°C) and partitioned in n-Hexane: 90% Ethanol (9:1). The n-Hexane phase was discarded and the 90% Ethanol phase residue resuspended in 30ml of sterile distilled water and evaporated in vacuo. About 50ml of sterile distilled water was again added and the mixture freeze-dried. About 0.8-1g of extracted material was recovered and stored in a cool dry place until required for use (Cannel, 1998).

Extraction of Boswelia dalzeilii L.

Twenty gram of dried ground stem bark of the plant was weighed into a 500-ml schott bottle and 200ml of 80% ethanol added. It was allowed to

stand for 24 hours and then filtered using 0.2 μm Aquadisc filters (Sortex®). The filtrate was then evaporated in vacuo (at 30°C) and partitioned in n-Hexane: 90% Ethanol (9:1). The n-Hexane phase was discarded and the 90% Ethanol phase residue resuspended in 30ml of sterile distilled water and evaporated in vacuo. About 50ml of sterile distilled water was again added and the mixture freeze-dried. About 2-3g of extracted material was recovered and stored in a cool dry place until required for use (Cannel, 1998).

Extraction of Sterculia setigera (Del.)

Twenty gram of dried ground stem bark of the plant was weighed into a 500-ml schott bottle and 200ml of 80% ethanol added. It was allowed to stand for 24 hours and then filtered using 0.2 μm Aquadisc filters (Sortex®). The filtrate was then evaporated in vacuo (at 30°C) and partitioned in n-Hexane: 90% Ethanol (9:1). The n-Hexane phase was discarded and the 90% Ethanol phase residue resuspended in 30ml of sterile distilled water and evaporated in vacuo. About 50ml of sterile distilled water was again added and the mixture freeze-dried. About 0.5-0.7g of extracted material was recovered and stored in a cool dry place until required for use (Cannel, 1998).

Extraction of Anogeissus Leicarpus (A. schimperi) (Gull. & Per.)

Twenty gram of dried ground leaf of the plant was weighed into a 500-ml schott bottle and 200ml of 80% ethanol added. It was allowed to stand for 24 hours and then filtered using 0.2 μm Aquadisc filters (Sortex®). The filtrate was then evaporated in vacuo (at 30°C) and partitioned in n-Hexane: 90% Ethanol (9:1). The n-Hexane phase was discarded and the 90% Ethanol phase residue resuspended in 30ml of sterile distilled water and evaporated in vacuo. About 50ml of sterile distilled water was again

added and the mixture freeze-dried. About 0.2-0.5g of extracted material was recovered and stored in a cool dry place until required for use (Cannel, 1998).

Extraction of Bauhinia reticulata (B. thonningii) (Schum.)

Twenty gram of dried ground leaf of the plant was weighed into a 500-ml schott bottle and 200ml of 80% ethanol added. It was allowed to stand for 24 hours and then filtered using 0.2 μm Aquadisc filters (Sortex®). The filtrate was then evaporated in vacuo (at 30°C) and partitioned in n-Hexane: 90% Ethanol (9:1). The n-Hexane phase was discarded and the 90% Ethanol phase residue resuspended in 30ml of sterile distilled water and evaporated in vacuo. About 50ml of sterile distilled water was again added and the mixture freeze-dried. About 0.1-0.3g of extracted material was recovered and stored in a cool dry place until required for use (Cannel, 1998).

Extraction of Guiera senegalensis (J.F.Gmelin)

Twenty gram of dried ground leaf of the plant was weighed into a 500-ml schott bottle and 200ml of 80% ethanol added. It was allowed to stand for 24 hours and then filtered using 0.2 μm Aquadisc filters (Sortex®). The filtrate was then evaporated in vacuo (at 30°C) and partitioned in n-Hexane: 90% Ethanol (9:1). The n-Hexane phase was discarded and the 90% Ethanol phase residue resuspended in 30ml of sterile distilled water and evaporated in vacuo. About 50ml of sterile distilled water was again added and the mixture freeze-dried. About 0.3-0.5g of extracted material was recovered and stored in a cool dry place until required for use (Cannel, 1998).

Extraction of Ziziphus-spina-christi (Willd).

Twenty gram of dried ground leaf of the plant was weighed into a 500-ml schott bottle and 200ml of 80% ethanol added. It was allowed to stand for 24 hours and then filtered using 0.2 μm Aquadisc filters (Sortex®). The filtrate was then evaporated in vacuo (at 30°C) and partitioned in n-Hexane: 90% Ethanol (9:1). The n-Hexane phase was discarded and the 90% Ethanol phase residue resuspended in 30ml of sterile distilled water and evaporated in vacuo. About 50ml of sterile distilled water was again added and the mixture freeze-dried. About 0.4-0.6g of extracted material was recovered and stored in a cool dry place until required for use (Cannel, 1998).

Extraction of Cassia goratensis L.

Twenty gram of dried ground leaf of the plant was weighed into a 500-ml schott bottle and 200ml of 80% ethanol added. It was allowed to stand for 24 hours and then filtered using 0.2 μm Aquadisc filters (Sortex®). The filtrate was then evaporated in vacuo (at 30°C) and partitioned in n-Hexane: 90% Ethanol (9:1). The n-Hexane phase was discarded and the 90% Ethanol phase residue resuspended in 30ml of sterile distilled water and evaporated in vacuo. About 50ml of sterile distilled water was again added and the mixture freeze-dried. About 0.5-1g of extracted material was recovered and stored in a cool dry place until required for use (Cannel, 1998).

Extraction of Detarium senegalensis (Gmel.).

Twenty gram of dried ground leaf of the plant was weighed into a 500-ml schott bottle and 200ml of 80% ethanol added. It was allowed to stand for 24 hours and then filtered using 0.2 μm Aquadisc filters (Sortex®). The

filtrate was then evaporated in vacuo (at 30°C) and partitioned in n-Hexane: 90% Ethanol (9:1). The n-Hexane phase was discarded and the 90% Ethanol phase residue resuspended in 30ml of sterile distilled water and evaporated in vacuo. About 50ml of sterile distilled water was again added and the mixture freeze-dried. About 0.3-0.7g of extracted material was recovered and stored in a cool dry place until required for use (Cannel, 1998).

Extraction of Khaya senegalensis (A. Juss.)

Twenty gram of dried ground stem bark of the plant was weighed into a 500-ml schott bottle and 200ml of 80% ethanol added. It was allowed to stand for 24 hours and then filtered using 0.2 μm Aquadisc filters (Sortex®). The filtrate was then evaporated in vacuo (at 30°C) and partitioned in n-Hexane: 90% Ethanol (9:1). The n-Hexane phase was discarded and the 90% Ethanol phase residue resuspended in 30ml of sterile distilled water and evaporated in vacuo. About 50ml of sterile distilled water was again added and the mixture freeze-dried. About 1.5 - 2g of extracted material was recovered and stored in a cool dry place until required for use (Cannel, 1998).

Extraction of Butyrospermum parkii L.

Twenty gram of dried ground stem bark of the plant was weighed into a 500-ml schott bottle and 200ml of 80% ethanol added. It was allowed to stand for 24 hours and then filtered using 0.2 μm Aquadisc filters (Sortex®). The filtrate was then evaporated in vacuo (at 30°C) and partitioned in n-Hexane: 90% Ethanol (9:1). The n-Hexane phase was discarded and the 90% Ethanol phase residue resuspended in 30ml of sterile distilled water and evaporated in vacuo. About 50ml of sterile distilled water was again added and the mixture freeze-dried. About 0.8-

1.5g of extracted material was recovered and stored in a cool dry place until required for use (Cannel, 1998).

Extraction of Psidium guajava

Twenty gram of dried ground stem bark of the plant was weighed into a 500-ml schott bottle and 200ml of 80% ethanol added. It was allowed to stand for 24 hours and then filtered using 0.2 μm Aquadisc filters (Sortex®). The filtrate was then evaporated in vacuo (at 30°C) and partitioned in n-Hexane: 90% Ethanol (9:1). The n-Hexane phase was discarded and the 90% Ethanol phase residue resuspended in 30ml of sterile distilled water and evaporated in vacuo. About 50ml of sterile distilled water was again added and the mixture freeze-dried. About 0.8-1g of extracted material was recovered and stored in a cool dry place until required for use (Cannel, 1998).

Extraction of Dichrostachys glomerata (Chiev.)

Twenty gram of dried ground leaf of the plant was weighed into a 500-ml schott bottle and 200ml of 80% ethanol added. It was allowed to stand for 24 hours and then filtered using 0.2 μm Aquadisc filters (Sortex®). The filtrate was then evaporated in vacuo (at 30°C) and partitioned in n-Hexane: 90% Ethanol (9:1). The n-Hexane phase was discarded and the 90% Ethanol phase residue resuspended in 30ml of sterile distilled water and evaporated in vacuo. About 50ml of sterile distilled water was again added and the mixture freeze-dried. About 0.3-0.5g of extracted material was recovered and stored in a cool dry place until required for use (Cannel, 1998).

3:3:2:2 Extraction of Detannified Extract

Ten (10g) of plant material was extracted with methanol and filtered. The filtrate was concentrated *in vacuo* and 10-20% distilled water added. The solution was defatted with n-Hexane and the n-Hexane phase discarded. Aqueous Methanol layer was evaporated to remove Methanol and then partitioned against chloroform. The tannin precipitated and was removed leaving the chloroform portion. The chloroform portion was washed with 1% aqueous NaCl and dried over anhydrous Na_2SO_4 to give a detannified chloroform extract (Silva *et al.*, 1998).

3:3:2:3 Extraction of Crude Saponin

The plant material (10 g) was defatted with 200-400 ml of n-Hexane and the n-Hexane portion, which may contain fats, waxes and chlorophyll, discarded. The residue was then extracted with ethanol and filtered. The filtrate was evaporated *in vacuo* and the residue resuspended in water saturated with n-Butanol. The n-butanol phase was mixed with few millilitre of diethyl ether and crude saponins were precipitated. The liquid portion was discarded and the precipitate air-dried and stored at 4°C until ready for use. The extract was diluted in the cell culture medium (1g in 28ml) immediately before use (Cannel, 1998, Vanden Berghe *et al.*, 1996).

3:3:2:4 Extraction of Alkaloid

Plant material was defatted with n-Hexane and extracted with methanol, filtered and concentrated in vacuo. The concentrated methalonic crude extract was treated with 1% aqueous HCl and partitioned against diethyl ether. The diethyl ether phase was discarded and the aqueous-acid phase was further alkalinized with NH_4OH to control pH and then

partitioned against chloroform. The chloroform portion contained 1^{o}, 2^{o} and 3^{o} alkaloids while the aqueous extract contained quaternary alkaloids (Cannel, 1998). They were evaporated in vacuo, freeze dried and kept at $4^{o}C$ until ready for use. The extract was dissolved in cell culture medium (1g in 28ml) immediately before use (Vanden Berghe *et al.*, 1996).

3:4 PROCEDURE FOR REMOVING LIPIDS AND PIGMENTS

3:4:1 Removing lipids from plant samples

The plant material was percolated with n-hexane. The n-hexane portion was discarded and the residue was then extracted as described earlier.

3:4:2 Removing pigment from plant sample

The aqueous extract was partitioned in n-hexane and the n-hexane portion discarded. Then it was partitioned against chloroform and the chloroform portion discarded. The aqueous portion was filtered through celite^{R}(Fisher®).

3:5 ANTIVIRAL AND ANTIBACTERIAL TESTING

3:5:1 Antiviral testing

3:5:1:1 Cell type and medium used

Two (2) types of cell line were used in this study and were a human colonic cancer cell line (HT-29) and Merlin-Darby canine kidney cell line (MDCK). Human colonic cancer cells are adenocarcinoma cells with 130 passages. These support the growth of many types of viruses especially those with the gut as their predilection site.

Suitable medium for their growth consist of medium 199 (Gibco-modified Earle's salt) with sodium bicarbonate and L-glutamate. To a 500ml of the medium, 10% heat inactivated foetal bovine serum, 10ml of procaine (5000 units)/streptomycin (5000ug/ml) and 5ml fungizone were added before use as a growth medium. The medium was stored at 4^{o}C and wormed to 37^{o}C before use.

The Melin-Darby canine kidney cell line consists of epithelial cells with 260 passages. They require a different growth medium from that of HT-29. The medium is Eagle's Minimum Essential Medium (EMEM- Gibco) with 2mM glutamine. To a 500ml bottle of the medium, 1% of non-essential amino acid and 10% of foetal bovine serum were added, stored at 4^{o}C and warmed to 37^{o}C before use.

3:5:1:2 Cell culture

One (1ml) of frozen cells was taken out of the liquid nitrogen cylinder and thawed rapidly. It was then poured into a capped test-tube and 9ml of

warm medium added. The mixture was centrifuged at 100 g for 5 minutes twice each time discarding the supernatant. The final sediment was then suspended in 10ml of growth medium in a small tissue culture flask and was incubated at 37°C (with 5% CO_2) for between 4-7 days until confluent.

For further propagation, medium in a confluent flask was discarded and the flask washed with 10ml Phosphate Buffered Saline (PBS). One milliliter(1ml) of trypsin in versene (0.05%) trypsin) was poured onto the confluent cells and rocked round until the trypsin covered the entire cell line. The flask was left for about 5 minutes and the cells removed by hitting the flask with the heel of the hand. Then 9ml of medium were added and aspirated several times to break cell clumps. The cell suspension ("seed") was divided into 2 new flasks, made up to the appropriate volume with more growth medium and returned to the incubator at 37°C with 5% CO_2.

3:5:1:3: Virus preparation

Three milliliter of infected HT-29 culture stock with complete cytopathologenic effect (CPE) was pipetted into a flask of confluent HT-29 cells. The flask was incubated at 37°C and observed for complete CPE (4-7days). All infected tissue cultures with complete CPE were pooled into a 500ml Schott bottle and kept at -70°C until viral concentration was needed.

The pool of infected tissue culture stock was freeze-thawed 3 times and 3ml chloroform per litre and solid NaCl to 1M was added. The mixture was shaken for 15 minutes at 37°C until the salt was dissolved and centrifuged for 30 minutes at 600 g in the MSE centrifuge. The supernatant was decanted into a sterile bottle and 105g per litre

polyethylene glycol 6000 (PEG 6000) was added and dissolved at room temperature and left overnight at 4°C. It was centrifuged again for 30 minutes at 600 g and the supernatant discarded. The pellets were resuspended by transferring about 5ml PBS from tube to tube. To the pooled suspension an equal volume of chloroform:iso-amyl alcohol (24:1) was added and vortexed for 30 seconds. The mixture was again centrifuged for 30 minutes at 600 g. The top layer was removed into a fresh tube and aliquoted into 1ml Eppendorfs and stored at -70°C.

3:5:1:4 Enumeration of tissue culture infective dose 50 ($TCID_{50}$)

The media from confluent flasks of HT-29 cell line was discarded and each flask washed with 10ml of sterile PBS. The flasks were then trypsinized with 1ml trypsin/versene solution and allowed to stand for 3-5 minutes. Each flask was tapped gently to dislodge the cells from the flask. Dislodged cells were resuspended in 9ml-growth medium and cell suspension placed onto Neubauer chamber at the edge of the coverslip until monolayer was formed. Numbers of cells in 6 large squares were counted using a microscope at **5 x 20** magnification. The mean number of cells per square (**X**) was taken and calculated thus;

$$\mathbf{X} \times \mathbf{25} \times \mathbf{10^4} = n^{o} \text{ cells/ml}$$

For 1 well, 250 ul of liquid is required at 1×10^5 cells/well

∴ Actual nº of cells per 250 ul = **X/4** = **Y**

Dilution factor = $\mathbf{Y/1 \times 10^5}$

So, to dilute the cells,

Dilution factor - **1** = Volume of growth medium required per ml of suspension.

Then, 250 ul of the corrected seed of cells (**1 x 10^5** cells per well) were added to each well in a 96 well titre plate and incubated at 37°C (with 5% CO_2) until confluent (Normally 4 to 5 days).

Viral dilutions were made from neat to 10^{-8}. Each well was inoculated with 100ul of the required dilution (using 10 wells per dilution) .The microtitre plates were sealed with parafilm seal and incubated for a further 4 days at 37°C.

Staining the titre plates

Excess medium was decanted and formal-saline added into a plastic container containing the titre plates covering all their wells. It was allowed to stand for 30 to 60 minutes then was drained off. Crystal violet stain (Sigma®), 1% in 70% methanol, was added to the plastic container covering all the titre plate wells and left for 15 to 30 minutes. Excess stain was poured away and container flooded, to the side of the titre plate, with water until all free stain was washed away. Microtitre plates were inverted onto absorbent surface until dry (Burleson *et al.*, 1992).

Enumerating the titre plates

The wells were examined for zones of clearing. When in doubt whether a cleared zone was due to infection or to deteriorated tissue, the plates were examined under the microscope. The cells around the zone appeared abnormal if it was a plaque.

The $TCID_{50}$ was the dilution of the test suspension that produced a cytopathologenic effect (CPE) in 50% of the wells and represented the dose of virus used in the test. This was done by the method of Reed and Muench (Vanden Berghe et al., 1986).

3:5:1:5 Antiviral testing of extracts

The viruses selected for testing are shown in Table 6.

Table 6: Viral Battery Used.

Astrovirus	RNA	SS	Non Enveloped
Poliovirus	RNA	SS	Non Enveloped
H. Simplex. Virus 1	DNA	DS	Enveloped
Equine H. Simplex virus 1	DNA	DS	Enveloped
Canine Parvovirus	DNA	SS	Enveloped
Equine Parvovirus	DNA	SS	Enveloped

Propagation of viruses

All viruses (except the parvoviruses) were grown on HT-29 cell line. Parvoviruses were grown on MDCK cell line. All viruses were concentrated and stored at -80^{o}C until ready to use. Titres of 10^{-5} of each of the viruses were used for all tests.

Antiviral testing of extract

The antiviral testing was done as described by Vanden Berghe et al., (1986) and involves the use of 96 well microtitre plates. The plate was divided into 6 columns, A-F (Figure 2). One hundred µl of each of the following was added to each well.

Three types of test were conducted.

a. Preinfection test

In this test, the cells in the microtitre plates were incubated with the extract for about an hour (37°C with 2% CO_2). Excess extract was decanted and the virus added as described above (Hudson, 1990).

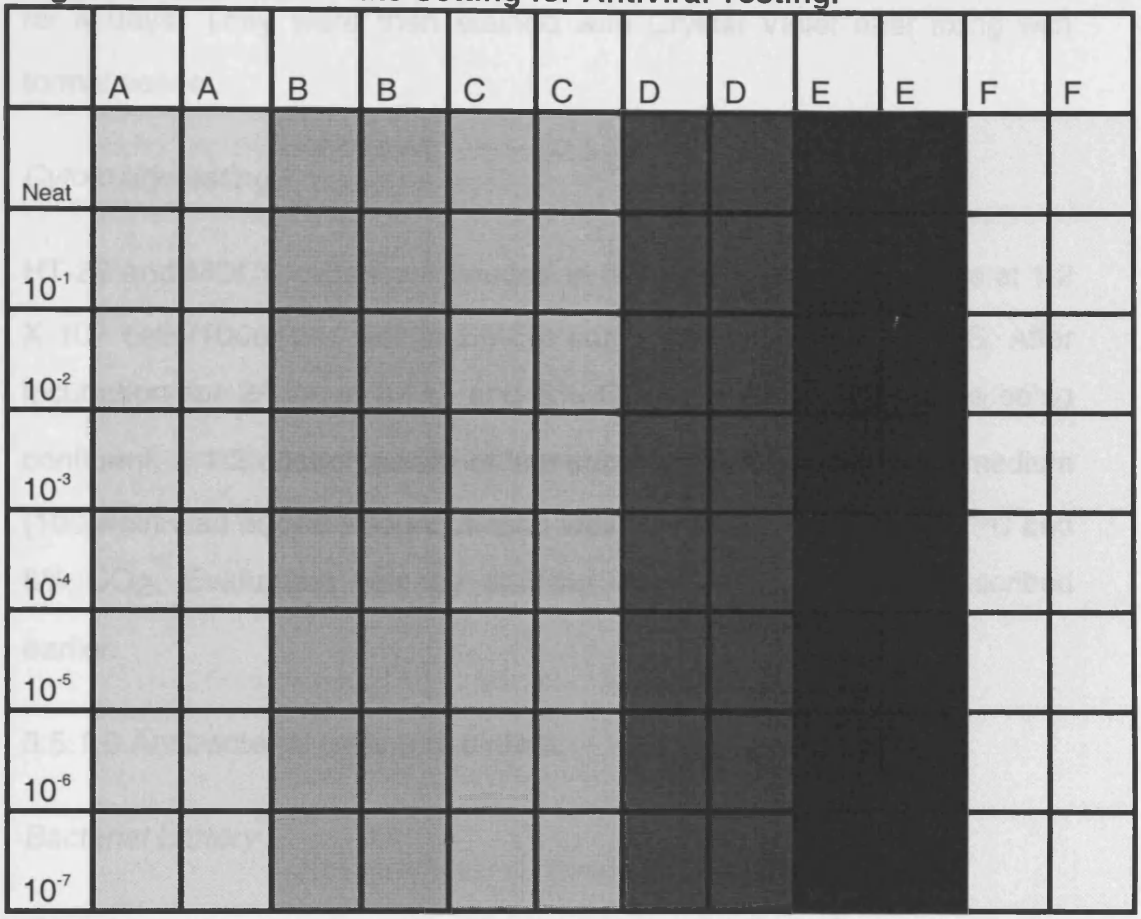
b. Virucidal test

Here, the virus was incubated with the extract for about an hour (37°C with 2% CO_2) before the mixture was introduced on to the cell line as described above (Hudson, 1990, Vanden Berghe *et al.*, 1996).

c. Antiviral test

The viral was added to the cell line and incubated for an hour (37°C with 2% CO_2). Plant extract was then added as described above (Vanden Berghe *et al.*, 1996).

Figure 2: Microtitre Plate Setting for Antiviral Testing.



KEY:

A__ Growth medium only (Cell Control).

B__ Plant extracts only (Cytotoxicity Test).

C__ Virus only (Viral Control)

D__ Virus + Extract (Neat).

E__ Virus + Extract (10^{-1}).

F__ Virus + Extract (10^{-2}).

All plants and viruses were diluted in medium 199 with 2% foetal calf serum (FCS). The test plates were then incubated at 37°C (with 5% CO_2) for 4 days. They were then stained with Crystal Violet after fixing with formal saline.

Cytotoxity testing

HT-29 and MDCK cells were seeded in flat bottom microtitre plates at 1.2 X 10^5 cells/100ul per well in EMEM supplemented with 10% FCS. After incubation for 24 hr at 37°C and 5% CO_2 and the seeded cells being confluent, a 1:2 dilution series of test substances in maintenance medium (100/well) was added and incubation was continued for 48 hr at 37°C and 5% CO_2. Evaluation was by staining with crystal violet as described earlier.

3:5:1:6 Antibacterial testing of extract

Bacterial battery

Pseudomonas aeruginosa (multiresistant to antibiotics) (Gram negative)

Enterococcus faecalis (Gram positive)

Enterobacter species (Gram positive)

Corynebacterium species (Gram positive)

Streptococcus pneumoniae (Penicillin-resistant) (Gram positive)

Staphylococcus aureus (Methicillin-resistant *Staphylococcus aureus*) (Gram positive)

Escherichia coli (Gram negative).

All bacteria were from clinical cases obtained from the Leicester Royal Infirmary.

Antibacterial testing

Plate-hole diffusion method was used for the antibacterial testing of plant extracts (Ieven et al., 1979). Mueller-Hinton diagnostic agar was used for the test. The hole was made using a cork borer (6mm diameter) on the set agar plates. Cooled molten agar was mixed with 200µl of bacterial culture and was allowed to set. A 6mm hole was bored on the agar and 100ul of plant extract was added per hole and the plates were first incubate at 4^{o}C for 30 minutes and after at 37^{o}C overnight. The zone of inhibition was then measured.

3.6 CHEMICAL DETECTION OF EXTRACT COMPOUNDS

Plants contain some substances that act individually or collectively, to prevent certain infection and infestations. Some of these substances are; Alkaloids, which are a very heterogeneous group of substances, containing one or more nitrogen atoms in combination as part of a cyclic system. They occupy an important position in phytomedicine (*Havesteen, 1983; Pathak et al., 1991*). More than 5000 alkaloids have been isolated and chemically characterized from plant sources. Many have biological activities although only few have been evaluated for antiviral and antibacterial activities. Saponins, which are steroid glycosides, contain 30 carbon in their structure derived from the basic 5-carbon isoprene unit. They are known to have membrane-damaging agents. They reduce surface tension and thereby exhibit the tendency to form stable, persistent foam (detergent-like) from aqueous alcohol solutions. Flavonoids with their secondary metabolite such as catechins, chalcones, flavonone and isoflavanones, are non-selective tyrosine inhibitors and contain polyhydroxyl groups on some of their rings (Harborne, 1988, Harborne and Mabry, 1982, *Tsuchiya et al., 1996*). Pholyphenols are vegetable tannins that cause contamination in most cases. Tannins are compounds that have astringent action afforded by their ability to precipitate proteins through the formation of multiple hydrogen bonds. They coagulate bacterial protoplasm, thereby destroying the microorganism (Bowman et al., 1968). Terpenes and their derivatives and sterols are glycosides that are associated with infective activity. They belong to terpenoid group but with less carbon atoms than saponins.

3.6.1 Saponin

Seven percent (7%) blood agar plates (Petridishes) were prepared and plant extract poured on them such that the entire plate is covered. The plates were incubated at 37^0C overnight. Haemolysis of the blood agar indicated the presence of saponins.

3.6.2 Alkaloid

Dissolve 8.0g of $Bi(NO_3)_3.H_2O$ in 30% w/v HNO_3 to obtain solution 1. Also dissolve 27.2g of KI in 50ml H_2O to give solution 2. Solution 1 and 2 were combined and allowed to stand at room temperature for 24 hr, then filtered and made-up to 100ml (Dragendorff reagent). The extract was acidified and a few drop of the reagent was added. Orange to brownish precipitate indicated the presence of alkaloids.

3.6.3 Flavonoids

The plant extract was dissolved in concentrated H_2SO_4. Colour changes giving a deep yellow solution indicated the presence of Flavones and Flavonols; red or red-bluish solution indicated the presence of chalcones and aurones and orange to red colours indicated the presence of Flavonones.

3.6.4 Sterols

The sample (1-2 mg) was dissolved in 1ml of chloroform and 1ml of concentrated H_2SO_4 added to the mixture. Red or yellow colour indicated the presence of sterols and methylated sterols.

3.6.5 Glycosides and Sesquiterpene

Solution 1 0.5% of freshly prepared sodium nitroprussiate in H_2O

Solution 2 0.2N sodium hydroxide (0.2N NaOH)

Two- (2) gram of sample was dissolved in pyridine (2-3 drops). To the mixture, 1 drop of solution 1 and 4 drops of solution 2 were added. A deep red colour indicated the presence of cardiac glycosides and pink indicated α ß- unsaturated lactone and some ß, y-lactones.

3.6.6 Polyphenols

A solution of 5% ferric chloride solution in water or ethanol was prepared and few drops added to a solution of plant extract. A brown precipitate indicated presence of other polyphenols.

3.7 PURIFICATION METHODS OF PLANT EXTRACTS

3.7.1 Preparation of sample

Freeze dried extracts were diluted (1g/28ml distilled sterile water) and filter sterilized. They were use immediately after reconstitution.

3.7.2 Performance liquid chromatography

In low-pressure column chromatography, the chromatographic packing materials consist of a densely packed adsorbent through which a mobile phase is "flowed". Depending on the choice of the packing material and mobile phase, several separation mechanism are available and include gel filtration (size exclusion), adsorption and partition chromatography. Separation of components of a mixture, or solute, occurs but selectively distributing the various components between a mobile phase and a stationary phase. A number of factors are involved in determining the distribution coefficient of solutes between a mobile phase and a stationary phase. These revolve around the chemical and physical nature of both phases (Cannel, 1998).

3.7.2.1 Gel filtration

In gelfiltration, molecules in solution are separated according to differences in their sizes as they pass through a column packed with a chromatographic medium (Le Maire *et al.*, 1989). The pores in the gel matrix, which are filled by the liquid phase, are comparable in size to the molecule to be separated. Relatively small molecules can diffuse into the gel from a surrounding solution, whereas relatively large molecules will be prevented by their size from diffusing into the gel to the same degree.

Sufficiently large molecules are unable to diffuse into the gel and are thus confines to the solution.

Column packing

The packing material (Sephadex G-25 Pharmacia Biotech®) was introduced into the column as slurry in a solvent, usually ethanol. Columns used for low pressure liquid chromatography are usually made out of heavy wall glass and designed for flash chromatography or rated for pressure, that is about three times the volume of silica needed. The amount of adsorbent used depended on sample loading. In general, 100-500g of packing material was used per gram of crude sample. A bed height of approximately 20-30 cm was desirable, with 40-60 cm headspace remaining to hold the solvent.

The desired amount of adsorbent was placed in a beaker and solvent added. The mixture was stirred and additional solvent added if needed, to obtained pourable slurry. The slurry must not be so thick thereby preventing air bubbles from being trapped in the column or so thin that the column could not be packed in one pour. Usually about 75% settled gel was suitable. For adsorbents that swell, sufficient time was allowed for the adsorbent to be fully absorbed the solvent to saturation.

The column was mounted on a stable laboratory stand. Locations which were exposed to draughts or direct sunlight, and which could cause temperature changes and the formation of bubbles in the packed column, were avoided. The top piece was fitted, thereby removing air via the air vent valve in the top piece. The flow was started soon after filling the column so as to obtain even sedimentation. The top piece air vent was closed and the column outlet opened to allow the packing to continue.

Equilibrating the column

The column was filled with the initial elute (1.0 M Tris buffer) composition without disturbing the silica bed. The pumps were runned to give a linear flow of approximately 1-2 cm/min. Continue equilibrating until the bed has a uniform appearance, i.e. no dry areas are visible, then introduce your sample. Two or three column volumes of eluent should be passed through the column in order to stabilised the bed and equilibrate with eluent buffer. A slightly higher flow rate than is to be used in the experiments should be used for packing.

Sample application

Sample loading can be effected in several ways depending on the type of column used and development mode used. A closed, pump system was used and the sample solution was introduced through injection into the valve, along the path of the mobile phase just ahead of the packing material bed.

Column development

Elution was carried out by pumping the mobile phase through the column at varying pressures. Columns usually run with 40- to 200 μm particles at flow rates that do not generate pressures significantly above atmospheric pressure. About 40 fractions were collected per column volume (5-10 ml fractions).

Cleaning gels and packed columns

After using the column for some time, it is necessary to remove precipitated proteins and other contaminants that have built up on the gel bed. Only fresh buffer solutions should be used in cleaning columns. For

Sephadex G types packed columns are cleaned with 2 column volumes of a non-ionic detergent solution. It may also be washed with NaOH (0.2M) on a Buchner funnel.

3.7.2.2 Ionexchange

Ion exchange chromatography is used for the separation of ionized or charged substances. Its use was first reported in the late 1930s and first applied to natural product research in the late 1940s as a technique to isolate and separate amino acids from protein hydrolysates. Electrostatic attraction between the ionized substances from the sample and the ionized centres of opposite charge on the stationary phase, are responsible for the separation (Dufresne, 1998).

Materials for Ion exchange

Packing materials consist of water-insoluble particles bearing covalently bound positively and negatively charged functional groups. The support matrices making up the insoluble particles can be based on a variety of polymeric materials including polysaccharides and synthetic resins. Physical properties of the ion-exchange column are determined by the nature of the parking materials.

Preparation of Gel

Q-Sepharose high performance and SP-Sepharose (Phamacia, Biotch®) high performance, which are strong ion exchangers, were used. The gel was poured into a graduated laboratory beaker and allowed to settle (the settled gel should have a volume of 1.25 x packed gel bed volume). The gel was washed with 5-10 gel volumes of distilled water on a glass filter and suspended in distilled water to a volume of 475 ml and 250μl of tween

20 was added. The tween 20 is added to decrease the surface tension, which makes the gel slurry more even.

Column Packing Method

P-1 peristaltic pump (Pharmacia®) was used in packing the column. XK 16/20 column was used.

Materials

The materials used include a peristaltic pump P-1 using 3.1 mm diameter PVC-tubing, packing reservoir RK 16, column XK 16/20, Q Sepharose high performance (Pharmacia®), distilled water, Tween 20, 20% Ethanol and 15% isopropanol in distilled water.

Packing Preparations

The packing reservoir (RK 16) was mounted at the top of the column and rinsed with distilled water. Then the filter and the bottom piece on the column were mounted. Injecting 20% ethanol through the effluent tubing wet the bottom filter. The column and packing reservoir were mounted vertically on a laboratory stand and rinsed with distilled water. Distilled water was applied 2 cm over the column piece and a tubing clamp on the effluent tubing was installed. The separation media slurry was poured into the column and packing reservoir and topped up carefully with distilled water. The gel bed was allowed to sediment without using the pump. When the level of the bed was stable, the column outlet was closed and the packing reservoir removed. The rest of the column was filled carefully with distilled water to form an upward meniscus at the top and then the flow adaptor was installed. The adaptor was then adjusted down to the bed surface. The flow adaptor was connected to the pump and the

column outlet opened then, 15% isopropanol was pumped through the column for 1 hour at maximum flow.

The position of the bed surface on the column was marked and the pump was stopped, the column outlet closed and the adaptor adjusted to the bed surface and then pushed a further 4-5 m. The column was then ready for use.

Equilibration of Packed Column

Before starting a run, the ion exchanger has to be charged with counter ions and then equilibrated. This was done by pumping one column volume of high ionic strength buffer followed by 5-10 column volumes of start buffer through the column until the conductivity and /or pH of the effluent is the same as for that of in-going solution. The column was then ready to use.

3.7.3 Thin layer chromatography

Planar chromatography involves the separation of mixtures on thin layers of adsorbents that are usually coated on glass, plastic or aluminium sheets. The most common form of planar liquid chromatography is thin layer chromatography (TLC) (Bauer *et al.*, 1991). TLC is the easiest, cheapest and most widely used method for the isolation of natural products

3.7.3.1 Methodology

Tubes containing fractions from the separation were combined around each peak identified. They were freeze-dried and re-suspended in 80% ethanol. The mixture was filtered using a 0.2μm sterile syringe filter (Gehman Sciences®). Excess solvent was evaporated *in vacuo* until

sediments were concentrated. Using a capillary tube, the sediments were spotted on the TLC paper 5 x 5cm (Silica gel 60 F_{254}, MERCK® KGaA, 64271 Darmstadt, Germany). The spot should not be more than 3mm in diameter. The spot is allowed to dry and the TLC paper placed in gel tank with developing solvent in it. The migration of the solvent was monitored until it reaches about a centimetre from the top end of the TLC paper. The paper was removed and then allowed to dry again before spraying with a developer.

Developing solvents

Five different combinations of solvents were used in developing the TLC plate. They are given in Table 7.

Table 7: Combination of Solvent used in TLC Tanks.

Solvent combination	Solvent 1 (ratio)	Solvent 2 (ratio)	Solvent 3 (ratio)
A	Butanol (4)	Acetic Acid (1)	Water (5)
B	Hexane (8)	Ethyl-acetate (2)	
C	Chloroform (95)	Acetone (5)	
D	Toluene (80)	Ethyl-acetate (18)	Acetic Acid (2)
E	Toluene (8)	Ethyl-acetate (2)	

Each combination is either a universal solvent or specific for a certain group of substances. For example, A is for flavonoids and glycosides, B is a universal solvent, C is general for medium polarity, D and E are for acidic metabolites. Each of the portions from the separation was run in all the solvent combination.

Developing Agents

Universal reagents from ACROS® were purchased from Fisher Scientific®. Six ready-to-use sprays comprising of Aniline phthalate (for identifying carbohydrates), Bromocresol green (for identifying carboxylic acids), Dimethylamino-benzaldehyde (for identifying amino acids and peptides), Ninhydrin (for identifying amines and amino acids), Phosphomolybdic acid (for identifying steroids e.g. phospholipids and substituted phenols) and Rhodamine B (for identifying lipids).

After developing plates in the gel tank and air drying them, they were sprayed with each of the above sprays, allowed to dry and developed in an oven (at 100^{o} C) until coloration appears. Blue coloration indicates terpenes, red indicates alkaloids and yellow indicates carboxylic acids.

CHAPTER FOUR: RESULTS

4.1 PRELIMINARY SCREENING OF EXTRACTS

Extracts of different parts of 50 medicinal plants from Nigeria, belonging to 11 different families, were screened for antiviral activity. These plants are being used for treating diarrhoea, febrile conditions, helminthiasis, dysentery and as surgical dressings (Table 8) in both humans and animals. The plants were extracted with 80% ethanol as described in the Materials and Methods, and evaporated to dryness before use.

Out of the total screened, 14 plant extracts from 10 families were found to have antiviral activity against both human and animal viruses used in this study. The parts of the plants used are also given in tables 8 and 13 and consist mainly of the leaf and bark.

The result of the plant extracts tested against astrovirus, polio virus 1, herpes simplex virus 1 (HSV 1), Equine herpes simplex virus (EHSV 1), canine and bovine parvovirus are given later in tables 10a, 10b, 10c and 11a, 11b, 11c). As demonstrated in these tables, some of the plant extracts inhibit all the human viruses and two of the animal viruses. Extracts of *Anacardium occidentale* (*Anacardiaceae*) and *Sterculia setigera* (*Sterculiaceae*) were found to inhibit both human and animal viruses used in this study.

Table 8: Local Clinical Uses of some Nigerian Medicinal Plants.

LOCAL NAME (HAUSA)	SCIENTIFIC NAME	PLANT PART USED	INDICATIONS (LOCAL).
MARKE	COMBRETACEAE *Anogeissus schimperi.* (Gull. & Per.) Collectors: Kudi & Ibrahim (651)	LEAF	Fever Diarrhoea Dressings
SABARA	COMBRETACEAE *Guiera* *senegalensis* (J.F.Gmelin) Collectors: Kudi & Demo. (661)	LEAF	Enteric problems. Worms
KALGO	LEGUMINOSAE *Bauhinia* *thonningii* (Schum.) Collectors: Kudi, Yayok & Ibrahim (691)	LEAF	Diarrhoea Fever
TAURA	LEGUMINOSAE *Detarium senegalense* (Gmel.) Cllectors: Kudi & Tula (691)	LEAF	Fever Dysentry Boils
RUMFU	LEGUMINOSAE *Cassia* *goratensis.* L. Collectors: Kudi & Demo (612)	LEAF	Fever general Worms
CASHEW	ANACARDIACEAE *Anacardium occidentale.* L. Collectors: Kudi & Haruna (613).	BARK	Enteric Cond. Worms
KADANYA	SAPOTACEAE *Butyrospermum parkii.* L. Collector: Kudi (644)	BARK	Fever Dressing Boils
MADACHI	MELIACEAE *Khaya senegalensis* (A. Juss.) Collectors: Kudi & Demo (644)	BARK	Helminths.
KIMBA	ANNONACEAE *Xylopia aethiopica* (A. Rich.) Collectors: Kudi & Ibrahim (621)	WHOLE PLANT	Diarrhoea

Table 8: Local Clinical Uses of some Nigerian Medicinal Plants.

KERANA	EUPHOBIACEAE *Euphobia kamerunica* (Ait.) Collector: Kuid & Ibrahim (671)	WHOLE PLANT	Fever
FARCHAN SHAFO	MIMOSACEAE *Acacia albida* (Hochst) Collector: Kudi & Ibrahim (632)	BARK	Enteric Conditions
DUNDU	MIMOSACEAE *Dichrostachys glomerata* (Chiev.) Collector: Kudi, Ibrahim & Demo. (642)	LEAF	Skin conditions Fever Diarrhoea
KURNA	RHAMNACEAE *Ziziphus mucronata* (Willd) Collector: Kudi. (682)	LEAF	Enteric Conditions
FARU	ANACARDIACEAE *Lannea humilis* (Oliv.) Collector: Kudi & Haruna. (623)	BARK	Diarrhoea Fever
KUKKUGI	STERCULIACEAE *Sterculia setigera* (Del.) Collector: Kudi & Ibrahim. (673)	BARK	STDs Fever
GWADAN JEJI	ANNONACEAE *Annona senegalensis* (PERS.) Collector: Kudi (611)	LEAF	Diarrhoea Fever Headache
HARARRABI	BURSERACEAE *Boswelia dalzielli*. L. Collectors: Kudi & Yau (624).	BARK	Diarrhoea Fever Dressing

4.2 CYTOTOXICITY

The result of the cytotoxicity test against HT-29 and MDCK cell lines (Table 9) showed that some of the plant extracts, such as *Anogeissus schimperi, Anacardium occidentale, Boswelia dalzeilii, Dichostachys glomerata* and *Sterculia setigera* (figures 3, 4, 5, 6, 7 and 8) were not cytotoxic even at 800μg/100μl concentration. However, most of the extracts show slight to complete cytotoxic effect above 200μg/100μl concentrations, examples include *Cassia gotarensis, Khaya senegalensis, Detarium senegalensis,* and *Ziziphus spina-chriti.* Most of the plant extracts were found to be active with no cytotoxicity to HT-29 and MDCK cells, at a dose of between 100-400μg/100μl. However, some of the plants brought from Nigeria were found to be cytotoxic at dilution of 50μg/100μl and hence were not used in the screening studies.

Fig 3a: Cytotoxocity and action of *Anogeissus schimperi*

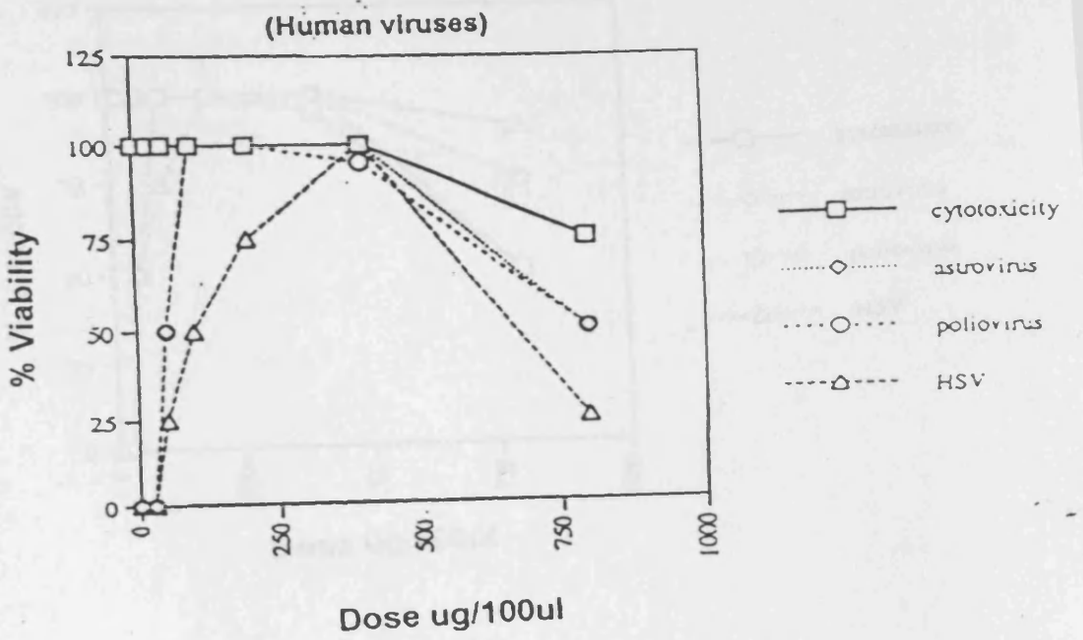


Fig 3b: Cytotoxicity and action of *Anogeissus schimperi*

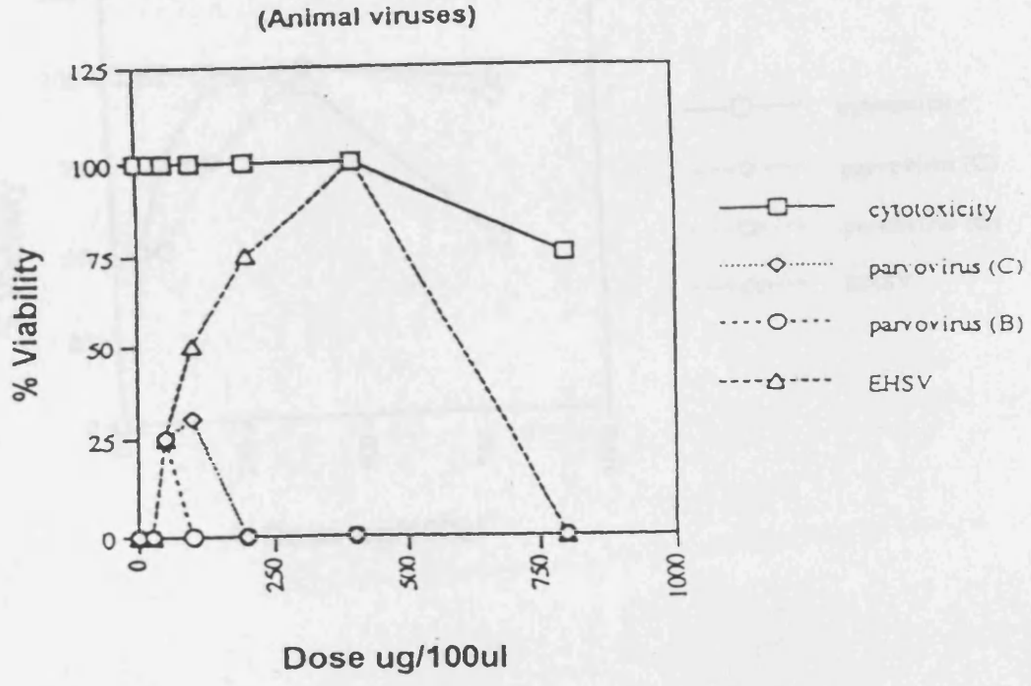


Fig 4a: Cytotoxicity and action of *Bauhinia thonningi*

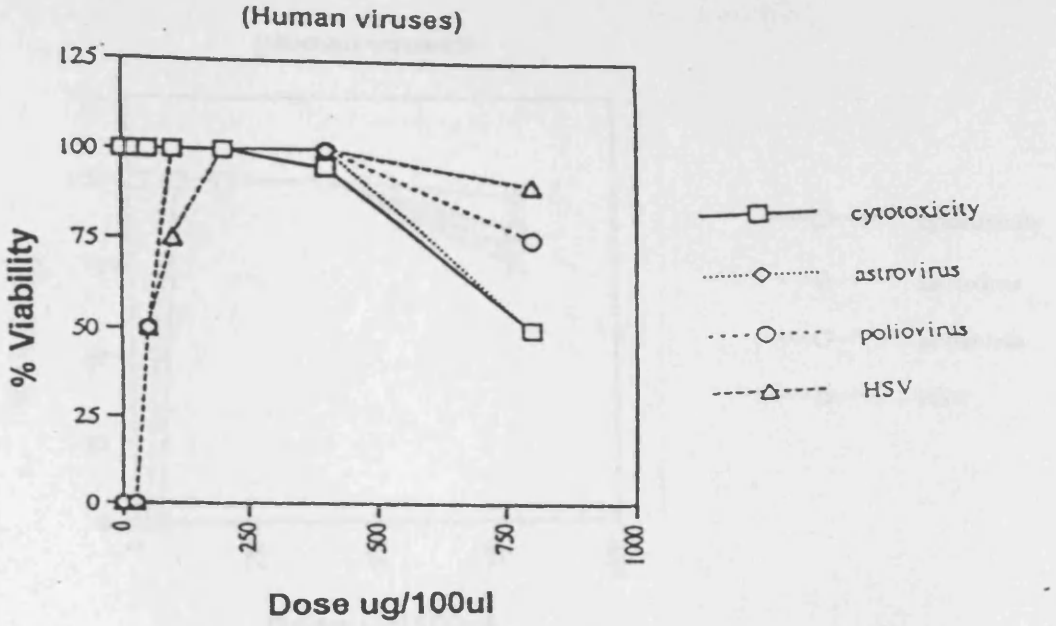


Fig 4b: Cytotoxicity and action of *Bauhinia thonningi*

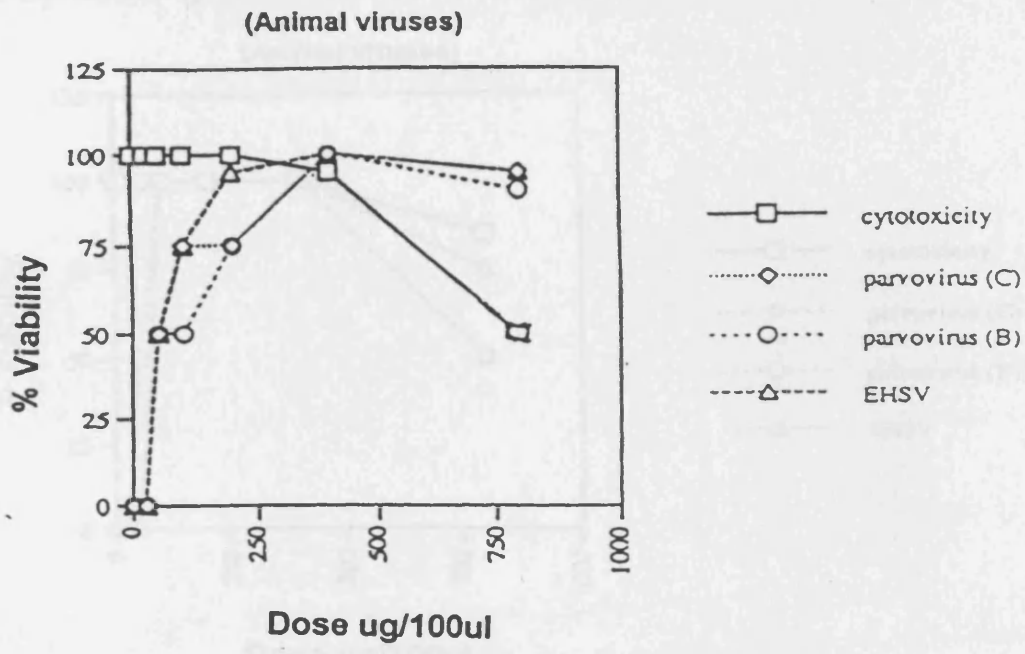


Fig 5a: Cytotoxicity and action of *Anacardium occidentale*

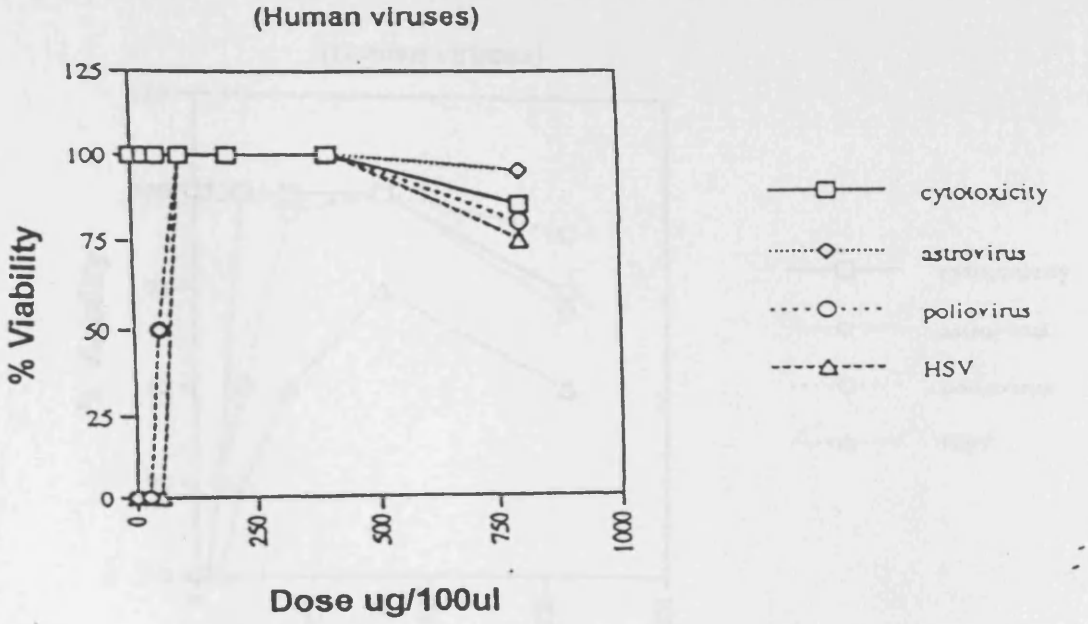


Fig 5b: Cytotoxicity and action of *Anacardium occidentale*

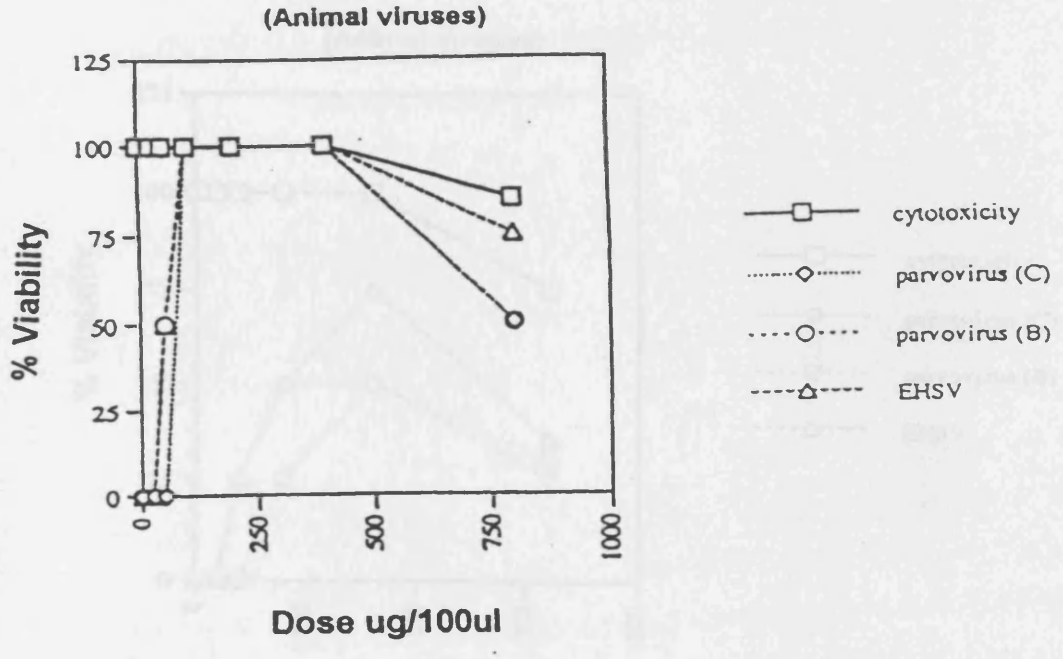


Fig 6a: Cytotoxicity and action of *Boswelia dalzeilii*

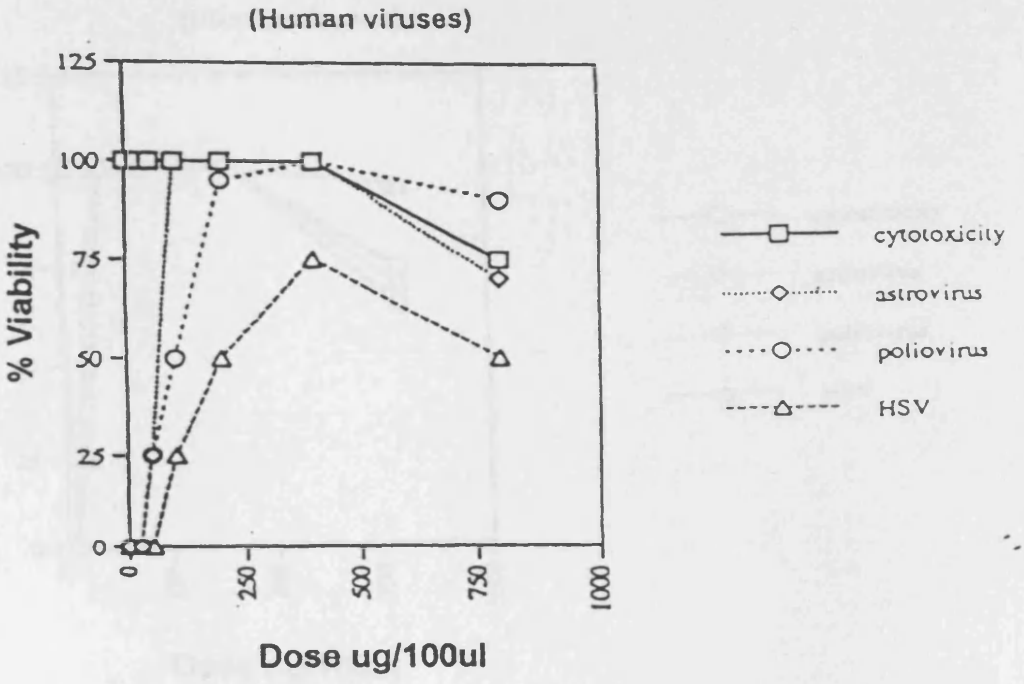


Fig 6b: Cytotoxicity and action of *Boswelia dalzeilii*

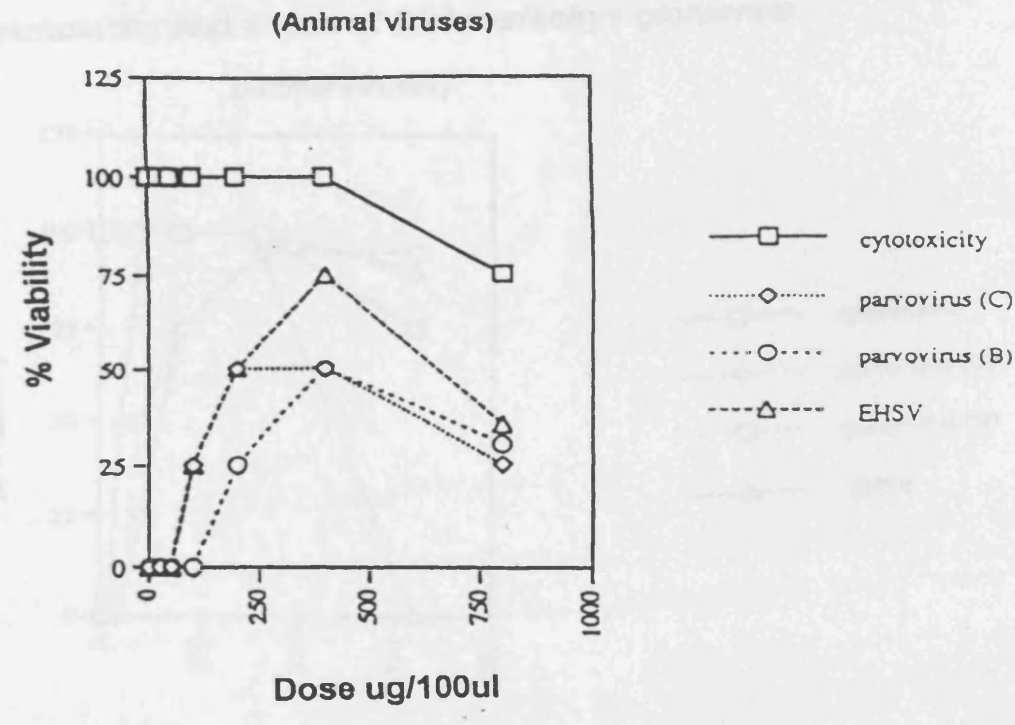


Fig 7a: Cytotoxicity and action of *Dichrostachys glomerata*

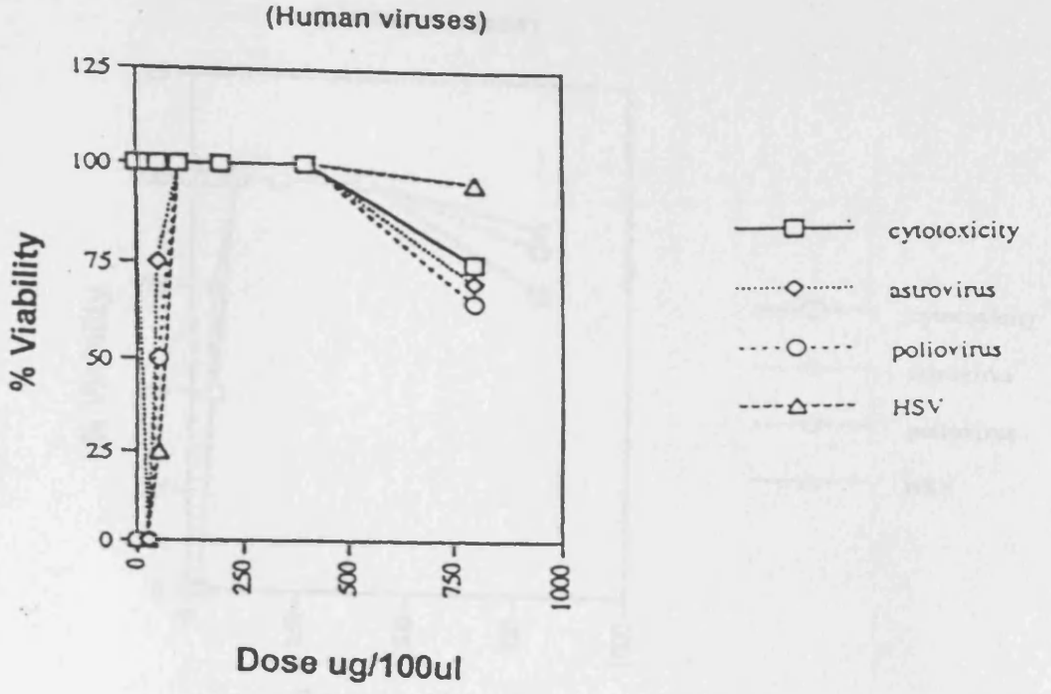


Fig 7b: Cytotoxicity and action of *Dichrostachys glomerata*

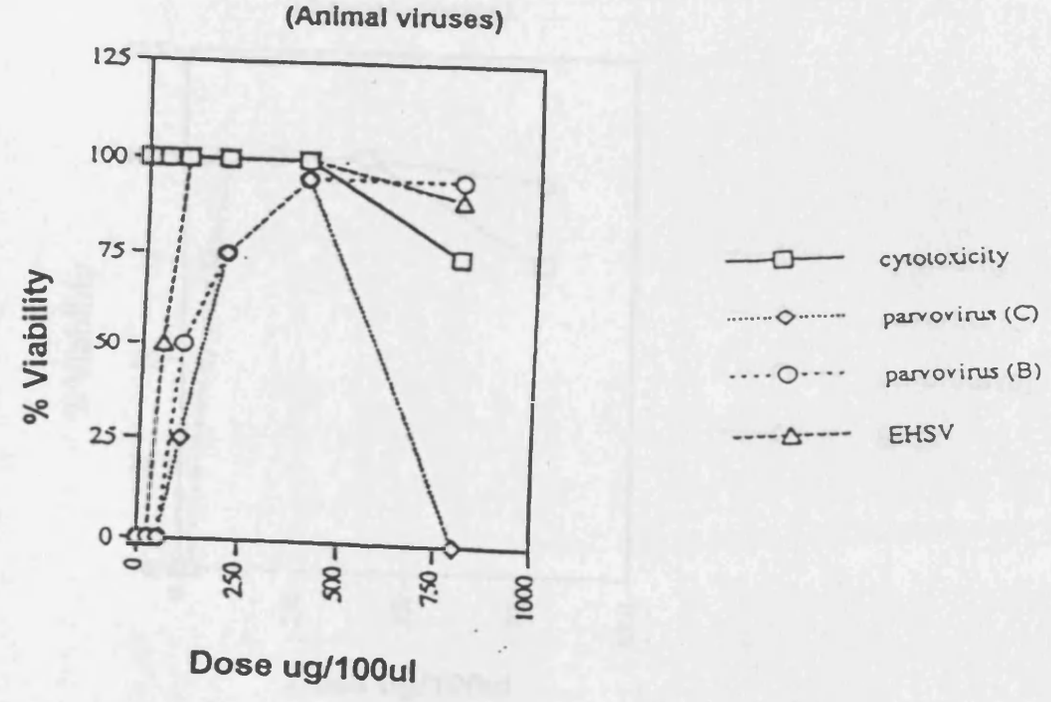


Fig 8a: Cytotoxicity and action of *Sterculia setigera*

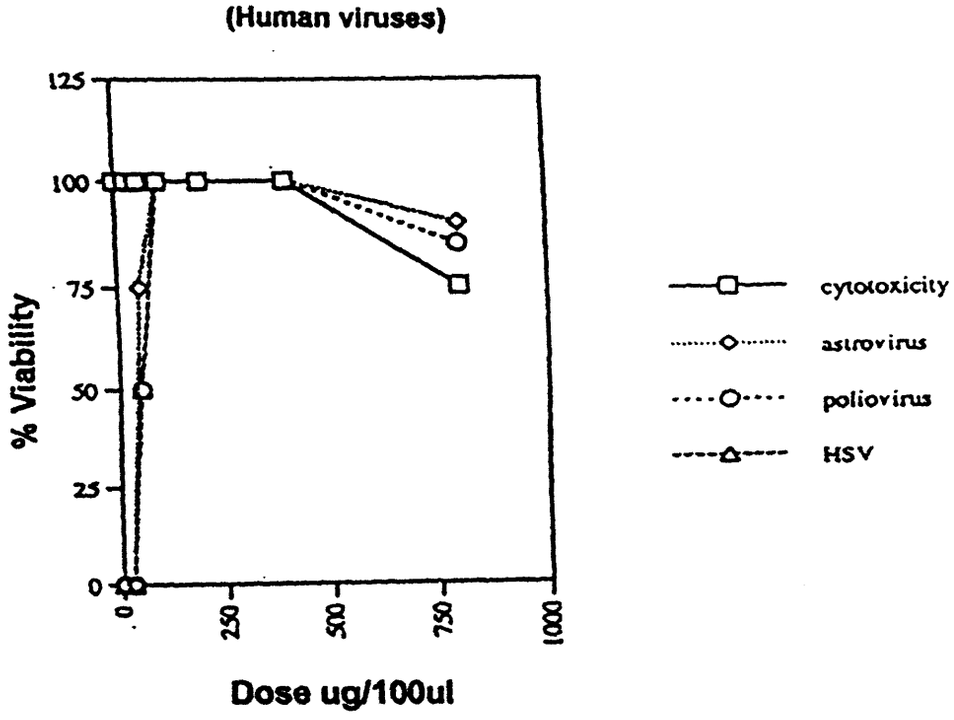


Fig 8b: Cytotoxicity and action of *Sterculia setigera*

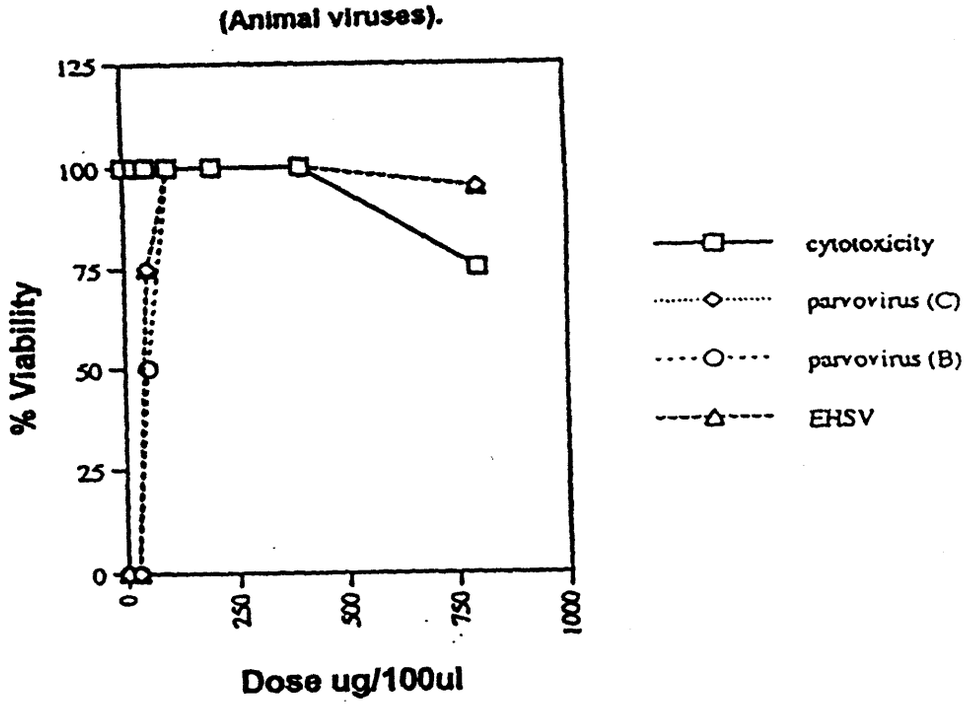


Table 9: Cytotoxicity of Some Nigerian Medicinal plant Extract on HT-29 and MDCK cells.

HAUSA NAME	SCIENTIFIC NAME	TOXICITY(ug/100ul)			
		100µg	200µg	400µg	800µg
Marke	*Anogeissus schimperi.*	-	-	-	+
Sabara	*Guiera senegalensis*	-	-	±	+
Kalgo	*Bauhinia thonningii*	-	-	-	+
Rumfu	*Cassia goratensis.*	-	+	+	+
Taura	*Detarium senegalensis*	±	+	+	+
Dundu	*Dichrostachys glomerata.*	-	-	-	±
Kurna	*Ziziphus spina-christi.*	-	-	+	+
Cashew	*Anacardium occidentale.*	-	-	-	±
Faru	*Lannea acida*	-	+	+	+
Kadanya	*Butryrospernum parkii.*	-	-	+	+
Kukkugi	*Sterculia setigera.*	-	-	-	±
Hararrabi	*Boswelia dalzeili.*	-	-	-	±

4.3 ANTIVIRAL EFFECTS OF EXTRACTS

4.3.1 Preinfection, Virucidal and Antiviral Tests

Three tests were conducted to determine the action of the plant extracts on human and animal viruses and they were preinfection, virucidal and antiviral actions.

In the preinfection tests (Table 10a and 11a), *Anogeissus schemperi, Guiera senegalensis, Dichrostachys glomerata* and *Boswelia dalzeilii* were found to be active against the human viruses used (polio virus, astrovirus and HSV1), *Anacardium occidentale* has partial activity on polio virus but complete on astrovirus and HSV1. The rest of the extracts were either partially active or completely inactive. For the animal viruses, *Dichrostachys glomerata*, and *Anacardium occidentale* were found to have complete inhibition against both canine and bovine parvovirus, while *Boswelia dalzeilii* has complete inhibitory action against the EHSV1. All the extracts of the other plant species had either no action or were only partially active.

In the virucidal test (Tables 10b and 11b), all the plant species show activity against all the viruses (human and animal). A similar picture was obtained for the antiviral testing especially on the human viruses (Tables 10c and 11c). As for the animal viruses, only *Anogeissus schimperi, Guiera senegalensis, Lannea acida* and *Butyrospermum parkii* showed inactivity especially against the parvovirus but active against EHSV 1. *Bauhania thonningii, Deterium senegalensis, Dichrostachys glomerata, Anacardium occidentale, Sterculia setigera* and *Boswelia dalzeili* have broad-spectrum activity against the 3 animal viruses in this study.

Plants with antiviral activity were heated at 100^0 C for 15 minutes and retested for antiviral activity. The result showed that *Anogeissus schimperi* and *Bauhania thonningii* lost activity, indicating that the active principle may be heat labile. *Cassia goratensis*, *Lannea acida, Sterculia setigera, Boswelia dalzeili* and *Khaya senegalensis* retain activity against the viruses, indicating that the active principle may be heat stable (Table 12).

Table 10a:Effects Of Some Nigerian Plants On Viruses (Preinfection Test)

Hausa Name	Scientific Name	Polio Virus	Astro Virus	Herpes Simplex Virus.
Marke	*Anogeissus schimperi.*	+	+	+
Sabara	*Guiera senegalensis*	+	+	+
Kalgo	*Bauhinia thonningii*	±	±	±
Rumfu	*Cassia goratensis.*	±	±	±
Taura	*Detarium senegalensis*	±	±	±
Dundu	*Dichrostachys glomerata.*	+	+	+
Kuma	*Ziziphus spina-christi.*	–	–	±
Lemu	*Citrus aurantifolia.*	–	–	–
Cashew	*Anacardium occidentale.*	±	+	+
Faru	*Lannea acida*	–	–	–
Kadanya	*Butryrospermum parkii.*	±	±	±
Madachi	*Khaya senegalensis*	±	±	±
Kukkugi	*Sterculia setigera.*	±	±	±
Hararrabi	*Boswelia dalzeili.*	+	+	+

Table 10b: Effects Of Some Nigerian Plants On Viruses (Virucidal Test)

Hausa Name	Scientific Name	Polio Virus	Astro Virus	Herpes Simplex Virus.
Marke	*Anogeissus schimperi.*	+	+	+
Sabara	*Guiera senegalensis*	+	+	+
Kalgo	*Bauhinia thonningii*	+	+	+
Rumfu	*Cassia goratensis.*	+	+	+
Taura	*Detarium senegalensis*	+	+	+
Dundu	*Dichrostachys glomerata.*	+	+	+
Kuma	*Ziziphus spina-christi.*	+	+	+
Cashew	*Anacardium occidentale.*	+	+	+
Faru	*Lannea acida*	+	+	+
Kadanya	*Butryrospermum parkii.*	+	+	+
Madachi	*Khaya senegalensis*	+	+	+
Kukkugi	*Sterculia setigera.*	+	+	+
Hararrabi	*Boswelia dalzeili.*	+	+	+

Table 10c: Activity of Some Nigerian Plant Extracts on Human Viruses.
(Antiviral Test)

Hausa Name	Scientific Name	Polio Virus	Astro Virus	Herpes Simplex Virus
Marke	*Anogeissus schimperi.*	+	+	+
Sabara	*Guiera senegalensis*	+	+	+
Kalgo	*Bauhinia thonningii*	+	+	+
Rumfu	*Cassia goratensis.*	+	+	-
Taura	*Detarium senegalensis*	+	+	-
Dundu	*Dichrostachys glomerata.*	+	+	+
Kuma	*Ziziphus spina-christi.*	+	+	+
Cashew	*Anacardium occidentale.*	+	+	+
Faru	*Lannea acida*	+	+	-
Kadanya	*Butryrospermum parkii.*	+	+	+
Kukkugi	*Sterculia setigera.*	+	+	+
Hararrabi	*Boswelia dalzeili.*	+	+	+

KEY: + = Plant active against virus

- =Plant not active against virus.

Table 11a: Effects Of Some Nigerian Plants On Viruses (Preinfection Test)

Hausa Name	Scientific Name	Canine Parvo Virus	Bovine Parvo Virus	Equine Herpes Simplex Virus
Marke	*Anogeissus schimperi.*	±	±	±
Sabara	*Guiera senegalensis*	±	±	±
Kalgo	*Bauhinia thonningii*	–	–	–
Rumfu	*Cassia goratensis.*	–	–	–
Taura	*Detarium senegalensis*	±	±	±
Dundu	*Dichrostachys glomerata.*	+	+	±
Kurna	*Ziziphus spina-christi.*	–	–	–
Cashew	*Anacardium occidentale.*	+	+	±
Faru	*Lannea acida*	–	–	–
Kadanya	*Butryrospermum parkii.*	–	–	±
Madachi	*Khaya senegalensis*	±	±	±
Kukkugi	*Sterculia setigera.*	±	±	–
Hararrabi	*Boswelia dalzeili.*	±	±	+

Table 11b: Effects Of Some Nigerian Plants On Viruses (Virucidal Test)

Hausa Name	Scientific Name	Canine Parvo Virus	Bovine Parvo Virus	Equine Herpes Simplex Virus
Marke	*Anogeissus schimperi.*	+	+	+
Sabara	*Guiera senegalensis*	+	+	+
Kalgo	*Bauhinia thonningii*	+	+	+
Rumfu	*Cassia goratensis.*	+	+	+
Taura	*Detarium senegalensis*	+	+	+
Dundu	*Dichrostachys glomerata.*	+	+	+
Kuma	*Ziziphus spina-christi.*	+	+	+
Lemu	*Citrus aurantifolia.*	–	–	–
Cashew	*Anacardium occidentale.*	+	+	+
Faru	*Lannea acida*	+	+	+
Kadanya	*Butryrospermum parkii.*	+	+	+
Madachi	*Khaya senegalensis*	+	+	+
Kukkugi	*Sterculia setigera.*	+	+	+
Hararrabi	*Boswelia dalzeili.*	+	+	+

Table 11c: Effects Of Some Nigerian Plants On Viruses (Antiviral Test)

Hausa Name	Scientific Name	Canine Parvo Virus	Bovine Parvo Virus	Equine Herpes Simplex Virus
Marke	*Anogeissus schimperi.*	–	–	+
Sabara	*Guiera senegalensis*	–	–	+
Kalgo	*Bauhinia thonningii*	+	+	+
Rumfu	*Cassia goratensis.*	+	+	–
Taura	*Detarium senegalensis*	+	+	+
Dundu	*Dichrostachys glomerata.*	+	+	+
Kurna	*Ziziphus spina-christi.*	–	–	–
Cashew	*Anacardium occidentale.*	+	+	+
Faru	*Lannea acida*	–	–	–
Kadanya	*Butryrospermum parkii.*	–	–	–
Madachi	*Khaya senegalensis*	–	–	±
Kukkugi	*Sterculia setigera.*	+	+	+
Hararrabi	*Boswelia dalzeili.*	+	+	±

Table 12: Plant Parts from Nigeria with Antiviral Activity (Result After Boiling).

HAUSA NAME	SCIENTIFIC NAME	ACTION AFTER BOILING
Fasa kumburi	*Portulaca oleracea.*	±
Marke	*Anogeeissus schimperi.*	+
Sabara	*Guiera senegalensis*	±
Kalgo	*Bauhinia thonningii*	+
Rumfu	*Cassia goratensis.*	-
Taura	*Detarium senegalensis*	±
Dundu	*Dichrostachys glomerata.*	±
Kurna	*Ziziphus spina-christi.*	±
Cashew	*Anocardium occidentale.*	±
Faru	*Lannea acida*	-
Kadanya	*Butryrospermum parkii.*	±
Kukkugi	*Sterculia setigera.*	-
Hararrabi	*Boswelia dalzeili.*	-
Madaci	*Khanya senegalensis.*	-

+ = Lost Activity

– = Retained Activity.

4.3.2 Chemical Testing of Extracts

The extracts of the active plants were tested chemically for the presence of some general chemicals found in plants (Hudson, 1990). These substances/chemicals are known to have some anti-infective properties (Atta-Ur-Rahman and Choudhary, 1995, Barnard *et al.*, 1993, Butler, 1988, Critchfield *et al.*, 1996), some of which were isolated as described in materials and methods and tested directly for antiviral activity. The result is shown in table 13 and showed that all the plants contain one or more of these chemicals/substances.

Plant extracts presummed to contain some of these chemicals, namely alkaloids, tannins, saponins, were tested, individually and combined, for antiviral activity against the viral battery (Table 14). Alkaloids were able to completely inhibit the parvoviruses and 75% inhibition of the rest of the viruses. Tannin showed 75% inhibition of all the viruses. Saponins were found to be less active compared to the 2 above. However, when all the three are combined, they totally inhibit poliovirus, astrovirus and the parvoviruses while they gave 75% inhibition for the herpes simplex viruses (HSV 1 and EHSV 1).

Table 13: Chemical Testing of Plant Extracts for known Antivirals in Plants:

Plant Local Name	Scientific Name	Alkaloids	Tannins	Phloba-tannins	Saponins
Kukkugi (bark)	*Steculia setigera*	+	+	-	+
Marke (leaf)	*Anogeissus schemperi*	+	-	-	-
Dundu (leaf)	*Dichrostachys glomerata*	+	+	+	+
Cashew (bark)	*Anacardium occidentale*	+	+	-	+
Hararrabi (bark)	*Boswelia dalzeili*	+	+	+	+
Kalgo (leaf)	*Bauhinia thonningii*	+	+	-	+
Taura (leaf)	*Detarium senegalensis*	+	+	-	-
Rumfu (leaf)	*Cassia goratensis*	+	+	+	+
Madachi (bark)	*Khanya senegalensis*	+	+	-	+
Sabara (leaf)	*Guiera senegalensis*	+	-	-	-
Kadanya (leaf)	*Butryspernum parkii*	+	+	-	-
Faru (bark)	*Lannea acida*	+	-	-	-
Kurna (leaf)	*Ziziphus spina-christi*	+	+	-	+

Table 14: Antiviral action of Alkaloids, Tannins and Saponins extracted from some medicinal plants from Nigeria.

Plant Compound	Viruses					
	Polio-virus	Astro-virus	HSV 1	Equine HSV	Bovine parvovirus	Canine Parvovirus
ALKALOID	+++	+++	+++	+++	++++	++++
TANNINS	+++	+++	+++	+++	+++	+++
SAPONINS	++	+++	++	++	++	++
COMBINED	++++	++++	+++	+++	++++	++++

++++= Total Inhibition, +++= 75% Inhibition, ++= 50% Inhibition, +=< 50% Inhibition,

4.3.3 Dialysis of Extract

The extracts of the 7 out of the 14 plants with activity were dialyzed to have an idea of the size of the molecule(s) responsible for the antiviral actions. Molecular weight cut off (MWCO) of 100, 500 and 5000 were used and the dialyzed portion of the extract tested for antiviral activity.

The results are given in tables 15 and 16. After dialysis at the molecular cut off, the extracts still retain activity against astrovirus and poliovirus for all the 7 plant extracts except *Anogeissus schimperi* where activity was lost after dialysis at 500 MWCO. In the case of herpes simplex virus 1 (HSV1), antiviral activity was retained at 5000 MWCO for *Anacardium occidentale, Sterculia setigera*, and *Boswelia dalzeili*, which happen to be the bark extracts. For the leaf extracts of *Anogeissus schimperi, Dichrostachys glomerata, Detarium senegalensis* and *Cassia goratensis,* activity against HSV1 was lost at 5000 MWCO.

After dialysis of the extracts, they were diluted 2 fold up to 10^{16} and tested against the viruses. Activity was detected from neat (500• g/ml) to 10^{4} (125• g/ml) for all the 7 plant extracts against human viruses. Against the animal viruses, only *Anacardium occidentale, Anogeissus schimperi, Sterculia setigera, Boswelia dalzeili* and *Dicrostachys glomerata* have activity from 500• g/ml to 125• g/ml (Table 17).

Table 15: Action Of Plant Extract On Virus After Dialysis (Astrovirus and Polivirus)

NAME OF PLANT*	5000 MWCO	500 MWCO	100 MWCO
CASHEW *(Anacardium occidentale)*	+	+	+
MARKE *(Anogeissus schimperi)*	-	+	+
KUKKUGI *(Sterculia setigera)*	+	+	+
HARARRABI *(Boswelia dalzeili)*	+	+	+
DUNDU *(Dichrostachys glomerata)*	+	+	+
TAURA *(Detarium senegalensis)*	+	+	+
RUMFU *(Cassia goratensis)*	+	+	+

Table 16: Action Of Plant Extract On Virus After Dialysis (Herpes Simplex Virus)

Name of Plant**	5000 MWCO	500 MWCO	100 MWCO
Cashew *(Anacardium occidentale)*	+	+	+
Marke* *(Anogeissus schimperi)*	-	+	+
Kukkugi *(Sterculie setigera)*	+	+	+
Hararrabi *(Boswelia dalzelli)*	+	+	+
Dundu* *(Dichrostachys glomerata)*	-	+	+
Taura* *(Detarium senegalensis)*	-	+	+
Rumfu* *(Cassia goratensis)*	-	+	+

* Leaf part of the plant.

** local hausa names.
+ = active against virus
- = lost activity against virus

Table 17: Action Of Plant Extract Dilution On Viruses After Dialysis (Astrovirus, Polivirus and Herpes Simplex Viruses)

NAME OF PLANT*	NEAT (500µg/mL)	10^2	10^4	10^8	10^{16}
CASHEW *(Anacardium occidentale)*	+	+	+	+ -	-
MARKE *(Anogeissus schimperi)*	+	+	+	+ -	-
KUKKUGI *(Sterculia setigera)*	+	+	+	+ -	-
HARARRABI *(Boswella dalzelll)*	+	+	+	+ -	-
DUNDU *(Dichrostachys glomerata)*	+	+	+	+ -	-
TAURA *(Detarium senegalensis)*	+	+	+	+ -	-
RUMFU *(Cassia goratensis)*	+	+	+	+ -	-

4.3.4 Quantitative Analysis

Quantitative analysis of the effect of plant extracts on the different viruses gave a general log reduction of 5 at neat (undiluted extract) and 10^2 dilution ($TCID_{50}$= 10^5) and among 4.0 to 0 log reduction at 10^4 to 10^6 dilution (Table 5 and 6). Most plants have different reduction as shown in tables 18 and 19. However, *Anogeissus schimperi, Boswelia dalzeilii, Dichrostachys glomerata, Anacardium occidentale* and *Sterculia setigera* (bark) were found to have a log reduction of 5 even at 10^4 dilution for both human and animal viruses.

Table 18: Quatitative Analysis of Plant Extracts on Poliovirus (Log Reduction at TCID 10^5)

PLANT	LOG REDUCTION AT NEAT DILUTION	LOG REDUCTION AT 1/2 DILUTION	LOG REDUCTION AT 1/4 DILUTION	LOG REDUCTION AT 1/6 DILUTION
Boswelia dalzeili	5	5	5	4
Guera senegalensis	5	4	1	0
Khanya senegalensis	5	4	1	1
Dichrostachys glomerata	5	5	4	1
Detarium senegalensis	5	4	2	0
Bauhinia thonnongii	5	5	5	2
Ziziphus spina-christi	5	4	3	1
Butyrospemu m parkii	5	3	1	0
Anogeissus schimperi	5	5	5	3
Cassia goratensis	5	3	1	0
Anacardium occidentale	5	5	5	3
Steculia setigera (Root)	5	4	2	0
Steculia setigera (Leaf)	5	4	1	0
Steculia setigera (Bark)	5	5	5	3
Lannea acida	5	3	1	0

Table 19: Quatitative Analysis of Plant Extracts on Equine Herpes simplex virus (Log Reduction at $TCID_{50}$ of 10^5).

PLANT	LOG REDUCTION AT NEAT DILUTION	LOG REDUCTION AT 1/2 DILUTION	LOG REDUCTION AT 1/4 DILUTION	LOG REDUCTION AT 1/6 DILUTION
Bosweiia dalzeili	5	5	4	3
Guera senegalensis	4	3	1	0
Khanya senegalensis	4	2	1	0
Dichrostachys glomerata	5	5	3	1
Detarium senegalensis	5	4	2	1
Bauhinia thonnongii	5	5	4	2
Ziziphus spina-christi	4	2	1	0
Butyrospemu m parkii	3	1	0	0
Anogeissus schimperi	5	5	4	2
Cassia goratensis	3	1	0	0
Anocardium occidentale	5	5	4	3
Steculia setigera (Root)	4	2	1	0
Steculia setigera (Leaf)	3	1	0	0
Steculia setigera (Bark)	5	5	4	2
Lannea acida	3	2	1	0

4.3.5 Chromatography (FPLC), TLC and Trials of Separated Extracts

Extracts of 5 plants from the 14 that have antiviral activity were chosen for chromatography. They include *Anogeissus schimperi, Anacardium occidentale, Sterculia setigera, Dichrostachys glomerata* and *Boswelia dalzeilii.* Fast Pressure Liquid Chromatography (FPLC) was used for the separation. Charges of the active portions of the extracts were determined by mixing the extracts with Q-sepharose (Fisher), allowing it to stand and after that, testing for antiviral activity. Binding to the Q-sepharose determines whether it is positively or negatively charged, before chromatography so as to tell what method to use. The result showed that 4 were found to be positively charged (Cation exchange) except *Boswelia dalzeili* that was negatively charged (Anion exchange) (Table 20)

Table 20: Charges Of Active Plant Extract Component(S)

NAME OF PLANT*	ANION EXCHANGER (Q_SEPHAROSE)	CATION EXCHANGER (SP_SEPHAROSE)
CASHEW *(Anacardium occidentale)*	±	±
MARKE *(Anogeissus schimperi)*	+	–
KUKKUGI *(Sterculia setigera)*	+	+
HARARRABI *(Boswelia dalzelli)*	–	+
DUNDU *(Dichrostachys schimperi)*	+	–
TAURA *(Detarium senegalensis)*	+	–
RUMFU *(Cassia goratensis)*	+	+

+ Extract no antiviral activity

_ Extract with antiviral activity

4.3.5.1 *Anogeissus schimperi*

Chromatography and trial of fractions

The result obtained for *Anogeissus schimperi*, using both Ion exchange and Gel filtration are given in fig 9a and 9b respectively. A range of peaks was identified in each case and included A (1-9), B (10-24), C (25-35) and D (36-45). Elutes, from the peaks, were combined and tested for antiviral activity. The result obtained from elutes of ion-exchange chromatography showed no activity against the viruses when tested individually, however, when combined, a less than 50% inhibition was found at $TCID_{50}$ of 10^5 (Table 21). After gel filtration, peak B (Tubes with nº 10-24) was found to have a 50% inhibition against the Herpes simplex Viruses (HSV) and less than 50% inhibition for the other viruses in the battery (Table 22). When all the samples are combined, activity against HSV increased to 75% while that against the other viruses increased to 50% inhibition.

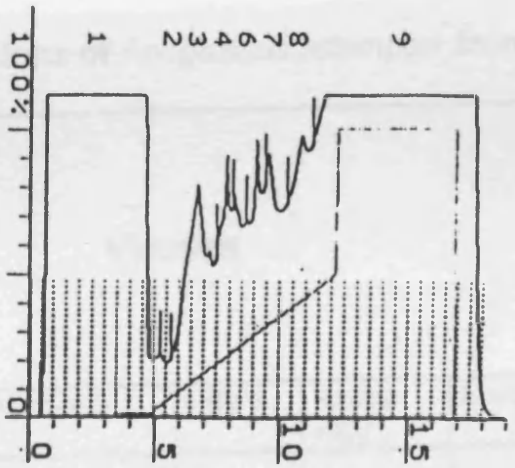


Fig 9b: Chromatograph of *Anogeissus schimperi*

(Gel-Filtration)

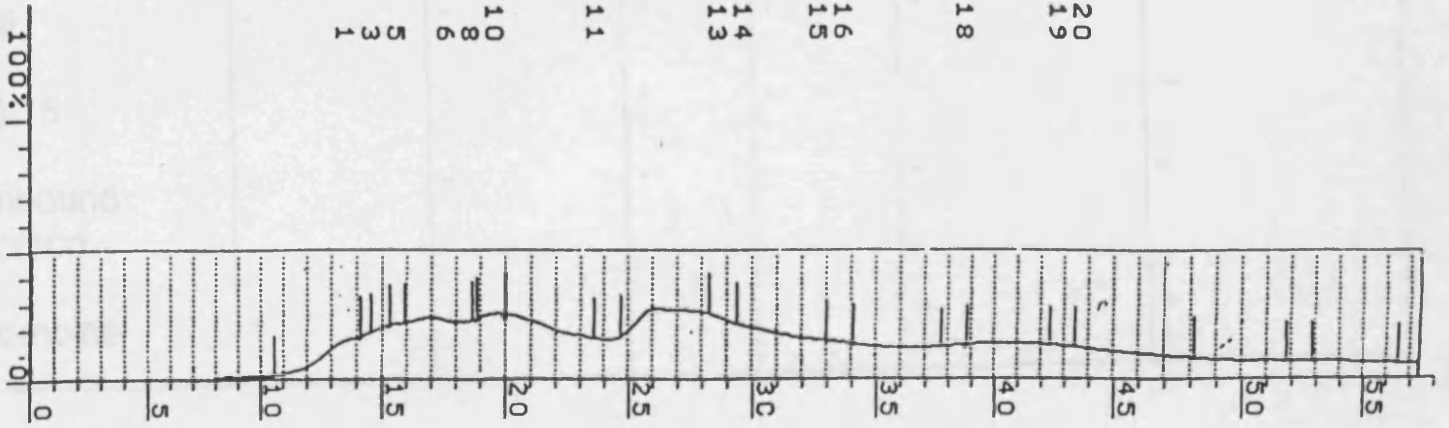


Table 21: Antiviral action of Fractions of *Anogeissus schimperi* from FPLC (Ion-Exchange)

Fractions	Viruses					
	Polio-virus	Astro-virus	HSV 1	Equine HSV	Bovine parvovirus	Canine Parvovirus
0-5	-	-	-	-	-	-
6-7	-	-	-	-	-	-
8-9	-	-	-	-	-	-
10-15	-	-	-	-	-	-
Unbound Portion	-	-	-	-	-	-
Combine	+	+	+	+	+	+

++++= Total Inhibition, +++= 75% Inhibition, ++= 50% Inhibition, +=< 50% Inhibition, - = Negative

Table 22: Antiviral action of Fractions of *Anogeissus schimperi* from FPLC (Gel Filtration)

Fractions	Viruses					
	Polio-virus	Astro-virus	HSV 1	Equine HSV	Bovine parvovirus	Canine Parvovirus
1-19	-	-	-	-	-	-
20-24	+	+	++	++	+	+
25-35	-	-	-	-	-	-
36-45	-	-	-	-	-	-
Unbound Portion	-	-	-	-	-	-
Combine	++	++	+++	+++	+	+

++++= Total inhibition, +++= 75% inhibition, ++= 50% inhibition, +=< 50% inhibition, - = Negative

TLC of Separated Fractions of Anogeissus schimperi

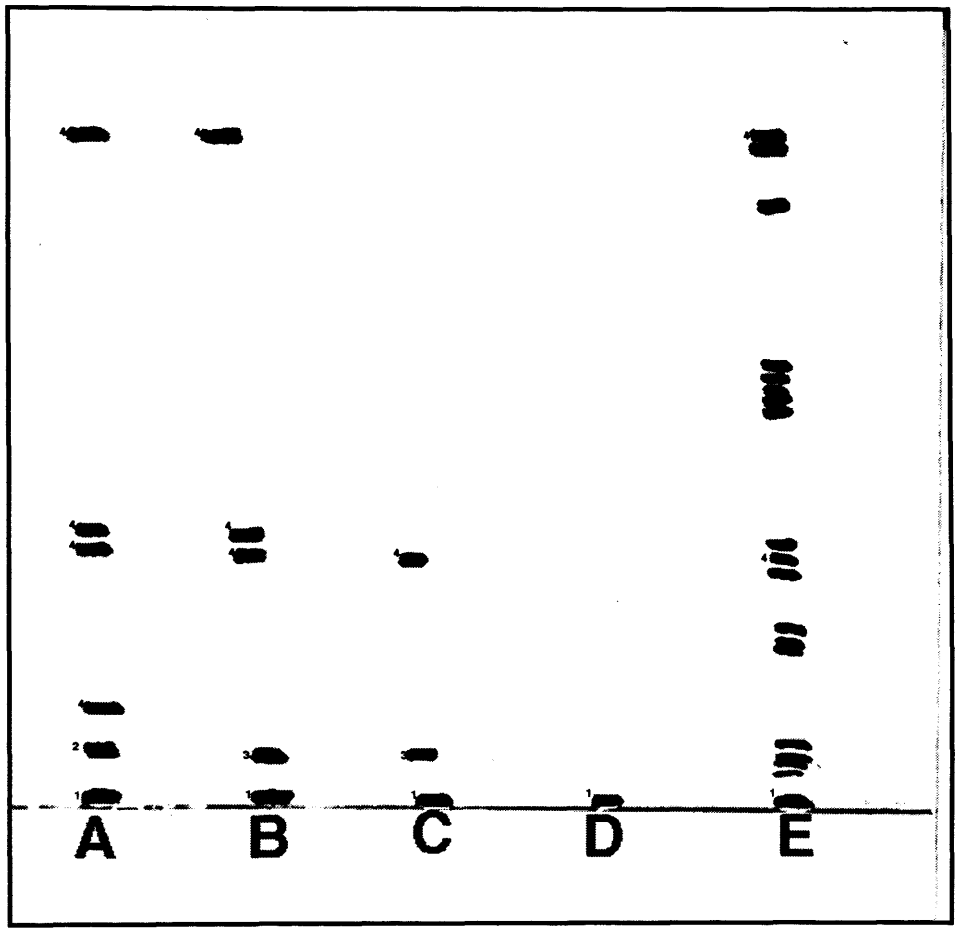


Figure 10: TLC of *Anogeissus schimperi.* A (1-19), B (20-24), C (25-35), D (36-45), E (neat), 1- Alkaloid, 2- Flavonoids, 3- Terpenes, 4- Polyphenols.

Result of the TLC of the gel filtration fractions of *Anogeissus schimperi* (Fig 10) showed that portion A (1-19) and B (20-24) have substances in the following groups; 1 Alkaloids, 2 - Flavonoids, 3 - Terpenes and 4 - Polyphenols. Portion B however does not contain Flavonoids. Portion C contain same chemical grouping as that of portion B but with only one class of Terpenes. Portion D contain only Alkaloid. The TLC plate was scanned immediately because the colour reaction faded after some time. E portion is the complete extract.

4.3.5.2 *Anacardium occidentale*

Chromatography and trial of fractions

Ion-Exchange chromatography (Fig 11a) showed that none of the peaks has activity against any of the viruses, however, when combined, there was a less than 50% inhibition for all the viruses (Table 23).

Gel filtration (Fig. 11b) gave a better result. Peaks A (10-24 tubes) and B (25-35 tubes) have less than 50% inhibition on all the viruses while peak C (35-45 tubes) have no activity on any of the viruses. When the 3 peaks are combined, 75% inhibition was seen on HSV 1 and EHSV 1, 50% inhibition of polio virus and astrovirus and less than 50% for the canine and bovine parvovirus (Table 24).

(Ion-Exchange)

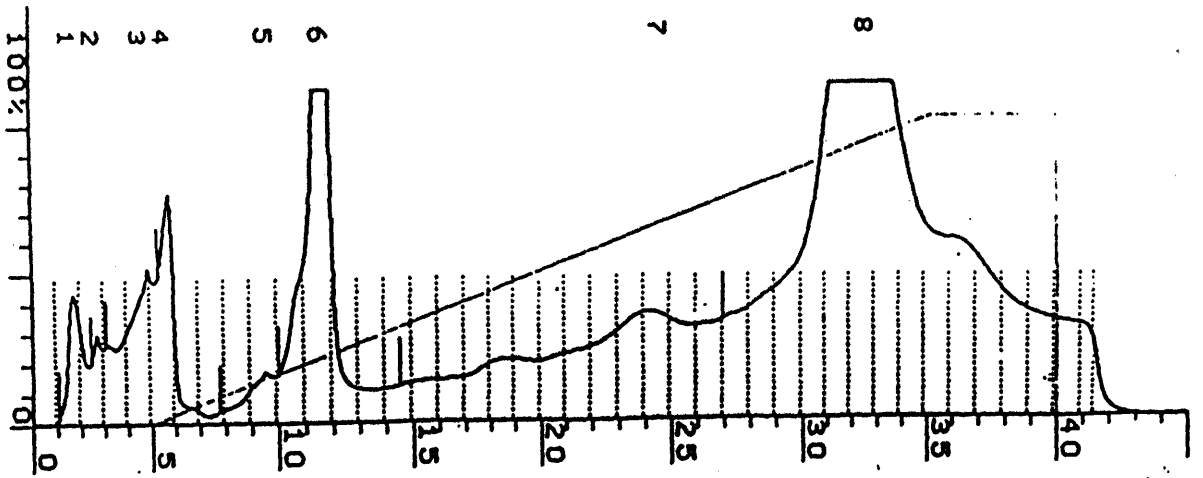


Fig 11b: Chromatograph of *Anacardium occidentale*

(Gel-Filtration)

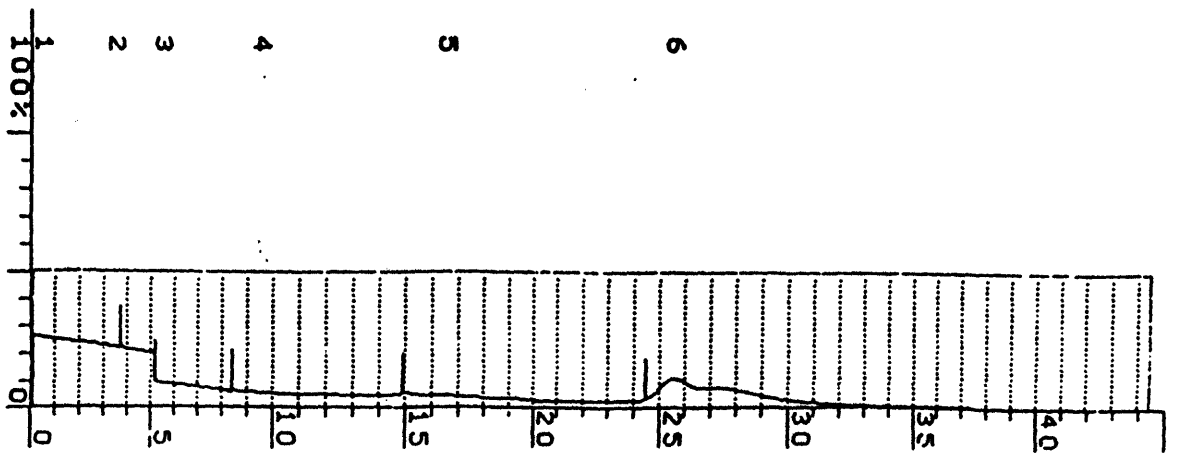


Table 23: Antiviral action of Fractions of *Anacardium occidentale* from FPLC (Ion-Exchange)

Fractions	Viruses					
	Polio-virus	Astro-virus	HSV 1	Equine HSV	Bovine parvovirus	Canine Parvovirus
8-14	-	-	-	-	-	-
15-27	-	-	-	-	-	-
28-42	-	-	-	-	-	-
Combine	+	+	+	+		
Unbound Portion	-	-	-	-	-	-

++++= Total Inhibition, +++= 75% Inhibition, ++= 50% Inhibition, +=< 50% Inhibition, - = Negative

Table 24: Antiviral action of Fractions of *Anacardium occidentale* from FPLC (Gel Filtration)

Fractions	Viruses					
	Polio-virus	Astro-virus	HSV 1	Equine HSV	Bovine parvovirus	Canine Parvovirus
10-24	+	+	+	+	+	+
25-35	+	+	+	+	+	+
36-45	-	-	-	-	-	-
Combine	++	++	+++	+++	+	+

++++= Total inhibition, +++= 75% inhibition, ++= 50% inhibition, +=< 50% inhibition, - = Negative

TLC of separated fractions of Anacardium occidentale

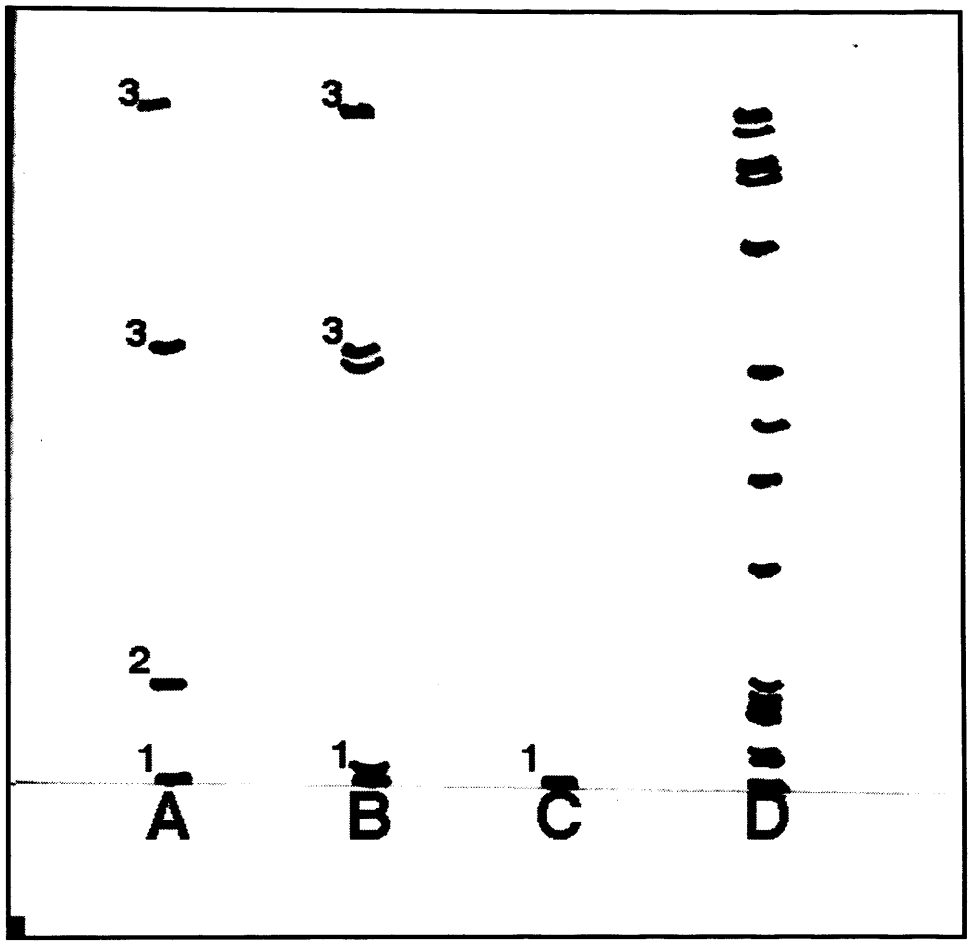


Figure 12: TLC of *Anacardium occidentale* Fractions. A (10-24), B (25-35), C (35-45) D (neat), 1- Alkaloids, 2- Flavonoids, 3- Terpenes.

TLC of *Anacardium occidentale* fractions revealed that fractions A (10-24) contain 1 - Alkaloids, 2 - Flavonoids and 3 - Terpenes. Only Alkaloids and Terpenes were found in fraction B (25-35). Fraction C (35-45) contained only Alkaloids. Plates were scanned because of colour fading from them after a while. D is the complete plant extract.

4.3.5.3 *Boswelia dalzeili*

Chromatography and trial of fractions

From the graph of *Boswelia dalzeili* extract separation (Fig. 13a), five portions were identified with ion-exchange separation. Portion A (7-13 tubes), B (14-19), C (20-28 tubes), D (29-41 tubes) and the unbound portion. Table 25 showed the inhibition or lack of inhibition of the above listed portions. Portion A showed a less than 50% inhibition for polio virus, astrovirus and canine and bovine parvovirus with no inhibition of HSV 1 and EHSV 1 viruses. Portion B and C showed no inhibition against any of the viruses used. Portion D showed inhibition of less than 50% on poliovirus, astrovirus, HSV 1 and EHSV 1. The unbound portion and the combined portion showed no inhibition against any virus.

Gel filtration separation graph was divided into 5 portions (Fig. 13b); A (1-12 tubes), B (13-18 tubes), C (19-24 tubes), D (25-32 tubes) and the combination of all above. No inhibition was seen on all viruses for portions A, B and C. Portion D gave a less 50% inhibition on poliovirus, astrovirus, HSV 1 and EHSV 1. When combined, inhibition was seen only on HSV 1 and EHSV 1 (Table 26).

(Ion-Exchange)

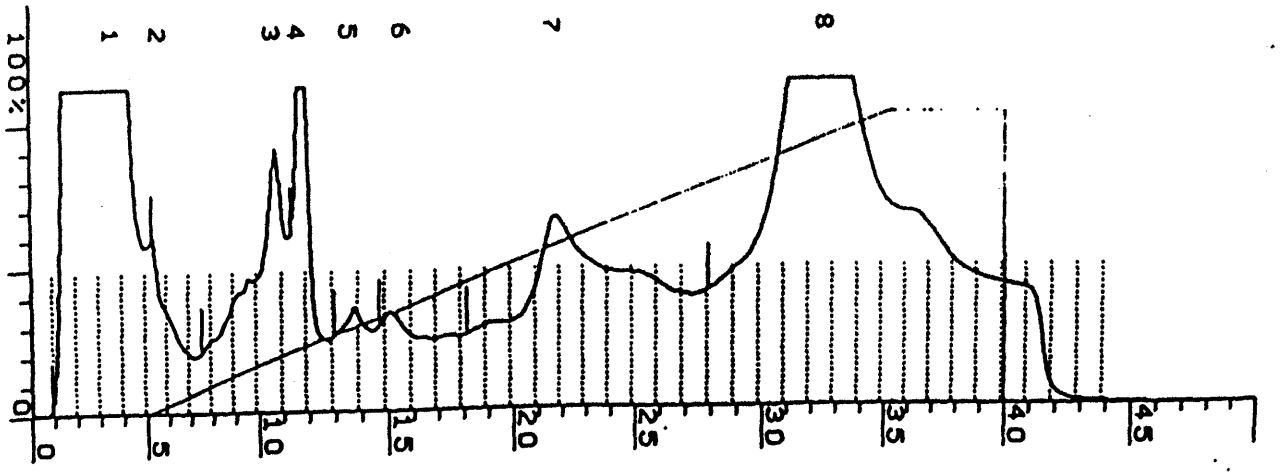


Fig 13b: Chromatograph of *Boswelia dalzeilii*

(Gel-Filtration)

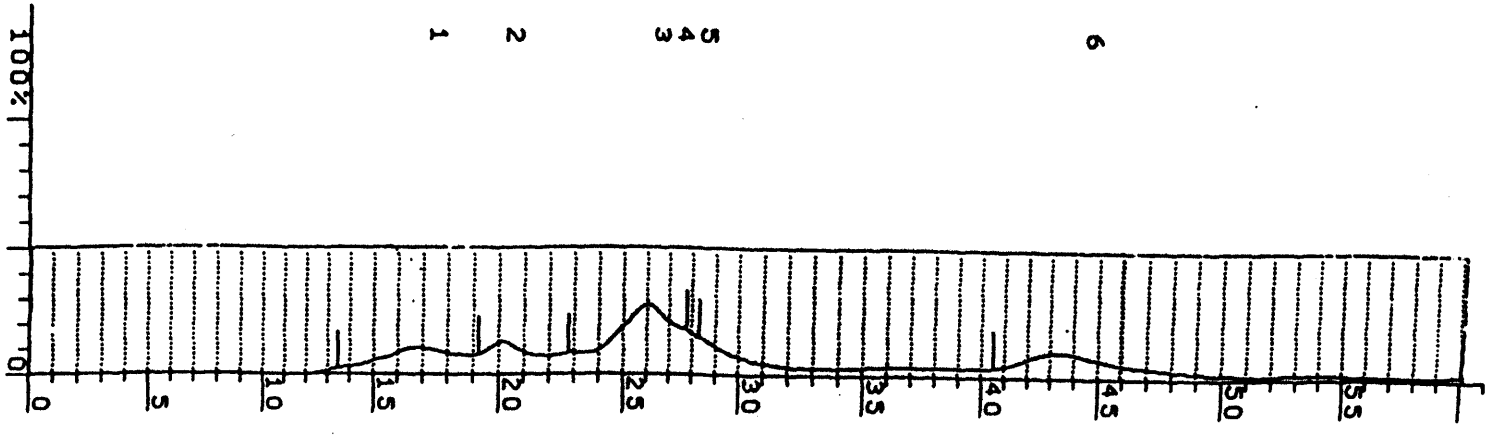


Table 25: Antiviral action of Fractions of *Boswelia dalzeili* from FPLC (Ion-Exchange)

Fractions	Viruses					
	Polio-virus	Astro-virus	HSV 1	Equine HSV	Bovine parvovirus	Canine Parvovirus
7-13	+	+	-	-	+	+
14-19	-	-	-	-	-	-
20-28	-	-	-	-	-	-
29-41	+	+	+	+	-	-
Unbound Portion	-	-	-	-	-	-
Combine	-	-	-	-	-	-

++++= Total inhibition, +++= 75% inhibition, ++= 50% inhibition, +=< 50% inhibition, - = Negative

Table 26: Antiviral action of Fractions of *Boswelia dalzeili* from FPLC (Gel Filtration)

Fractions	Viruses					
	Polio-virus	Astro-virus	HSV 1	Equine HSV	Bovine parvovirus	Canine Parvovirus
1-12	-	-	-	-	-	-
13-18	-	-	-	-	-	-
19-24	-	-	-	-	-	-
25-32	+	+	+	+	-	-
Combine	-	-	+	+	-	-

++++= Total inhibition, +++= 75% inhibition, ++= 50% inhibition, +=< 50% inhibition, - = Negative

TLC of separated fractions of Boswelia dalzeili

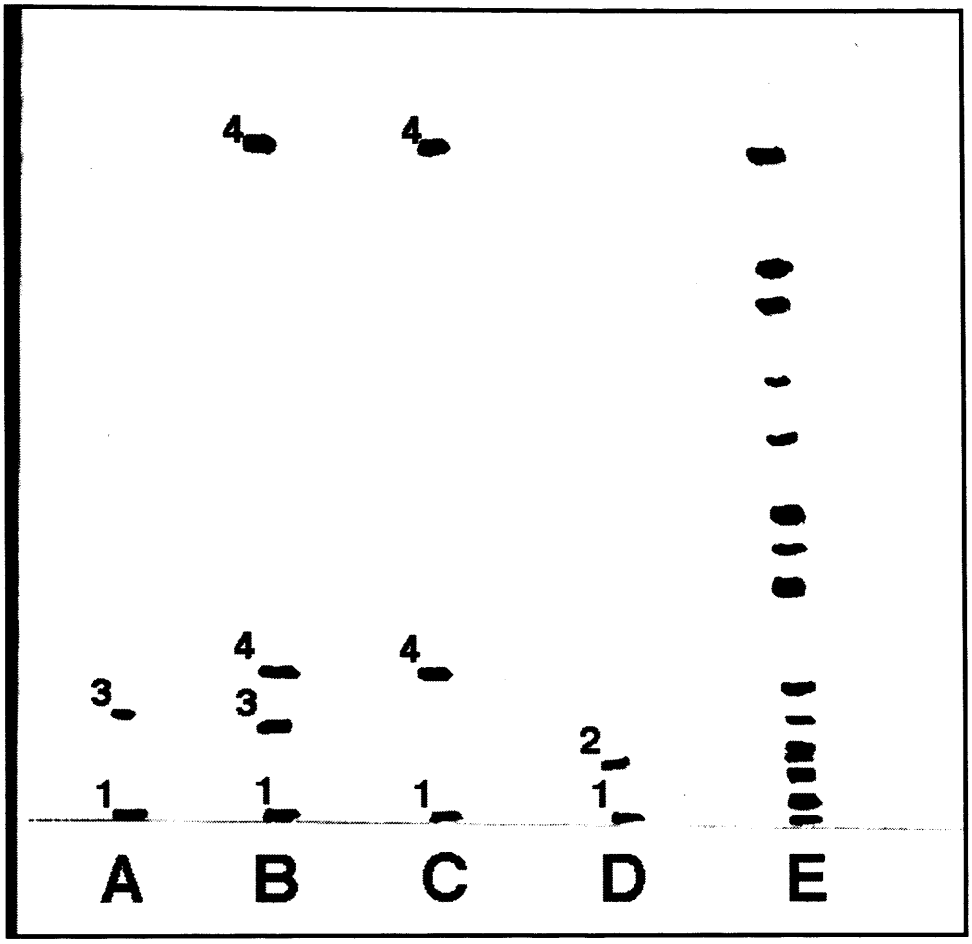


Figure 14: TLC of *Boswelia dalzeili* separated portion. A (1-12), B (13-18), C (19-24), D (25-32), E (neat), 1- Alkaloid, 2- Flavonoids, 3- Terpenes, 4- Polyphenols.

Fractions of *Boswelia dalzeili*, after TLC gave for fraction A (1-12) 1 - Alkaloids and 3 - Terpenes. Fractions B (13-18) gave 1 - Alkaloids, 3 - Terpenes and 4 - Polyphenols. Fraction C (19-24) gave 1 - Alkaloids, 4 - Polyphenols and fraction D (25-32) gave 1 - Alkaloids and 2 - Flavonoids. Plate was scanned to prevent loss of bands with time after exposure. E is the complete extract.

4.3.5.4 *Dichrostachys glomerata*

Chromatography and trial of fractions

Figure 15a show the graphs of the separation of *Dichrostachys glomerata*. The ion-exchange separation was divided into 5 portions, A, B, C, D, and the unbound portion. The test for inhibition conducted for each portion is given in table 27. Portions A, B, C and D showed no inhibition against all the viruses in the battery. However, the unbound portion gave an inhibition of 50 % with poliovirus, astrovirus, HSV 1 and EHSV 1 and less than 50 % for the canine and bovine parvoviruses. When all the portions were combined, 50 % inhibition was seen for all the viruses.

Gel filtration graph (fig 15b) was divide into 4 portions, A, B, C, and D. Portion A (10-18) and B (19-23) gave an inhibition of less than 50% for all the viruses (Table 28). No inhibition was seen with portions C (24-30) and D (31-40) against all the viruses. When the whole 4 portions were combined, less than 50% inhibition was seen for all the viruses.

(Ion-Exchange)

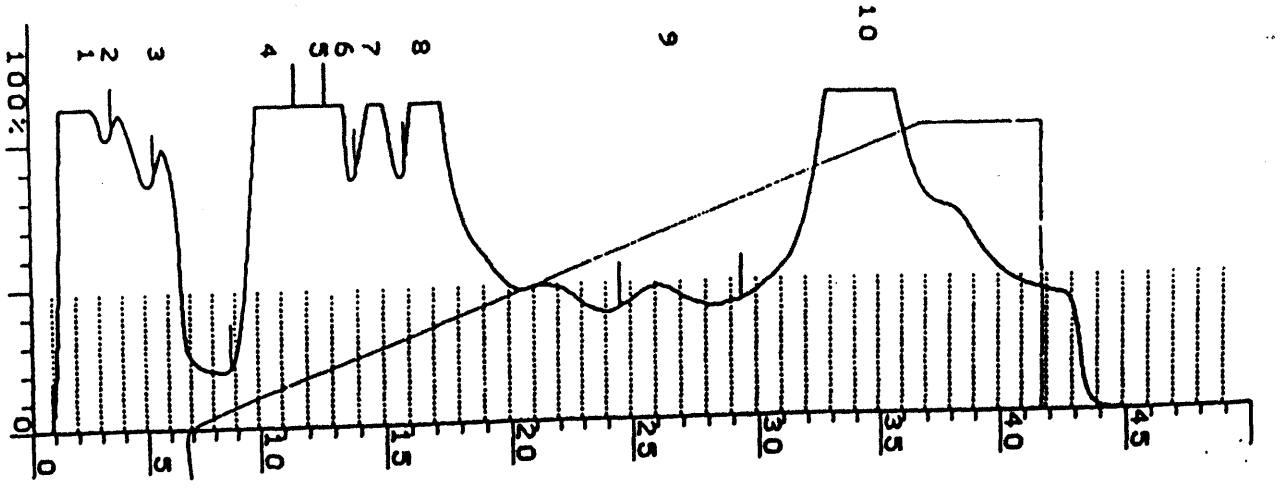


Fig 15b: Chromatograph of *Dichrostachys glomerata*

(Gel-Filtration)

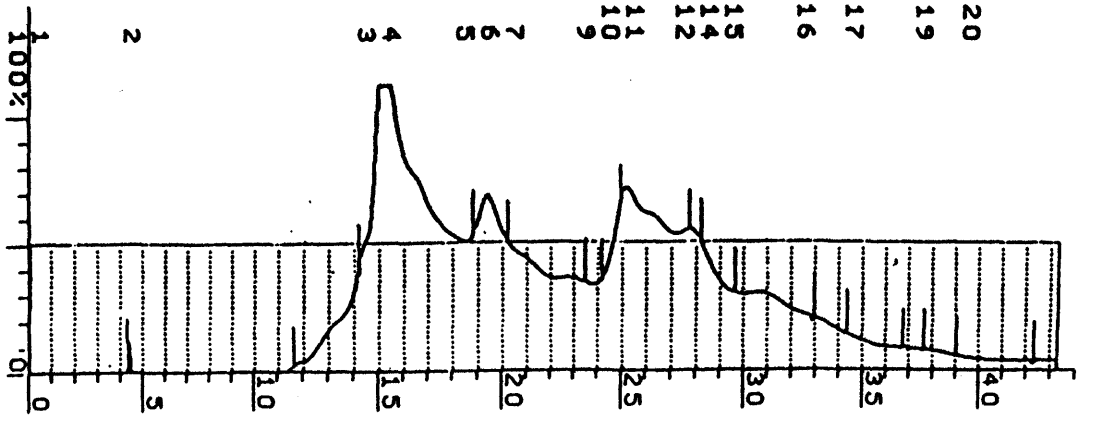


Table 27: Antiviral action of Fractions of *Dicrostachys glomerata* from FPLC (Ion-Exchange)

Fractions	Viruses					
	Polio-virus	Astro-virus	HSV 1	Equine HSV	Bovine parvovirus	Canine Parvovirus
9-13	-	-	-	-	-	-
14-16	-	-	-	-	-	-
17-30	-	-	-	-	-	-
31-45	-	-	-	-	-	-
Unbounded Portion	++	++	++	++	+	+
Combine	++	++	++	++	++	++

++++= Total Inhibition, +++= 75% Inhibition, ++= 50% Inhibition, +=< 50% Inhibition, - = Negative

Table 28: Antiviral action of Fractions of *Dicrostachys glomerata* from FPLC (Gel Filtration)

Fractions	Viruses					
	Polio-virus	Astro-virus	HSV 1	Equine HSV	Bovine parvovirus	Canine Parvovirus
10-18	+	+	+	+	+	+
19-23	+	+	+	+	+	+
24-30	-	-	-	-	-	-
31-40	-	-	-	-	-	-
Combine	+	+	+	+	+	+

++++= Total inhibition, +++= 75% inhibition, ++= 50% inhibition, +=< 50% inhibition, - = Negative

TLC of separated fractions of Dichrostachys glomerata

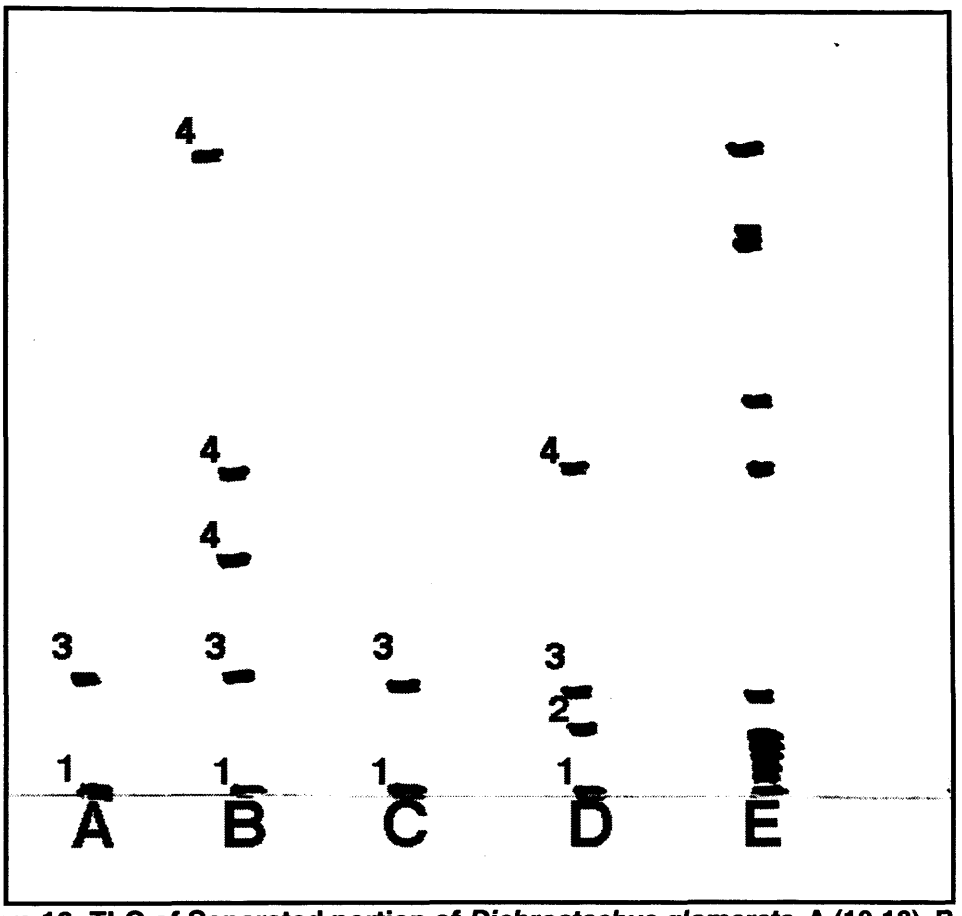


Figure 16: TLC of Separated portion of *Dichrostachys glomerata*. A (10-18), B (19-23), C (24-30), D (31-40), E (Neat), 1 - Alkaloid, 2 - Flavonoids, 3 - Terpenes and 4 - Polyphenols.

TLC of *Dichrotachys glomerata* showed that fractions A (10-18) has 1 - Alkaloids, 3 - Terpenes; portion B (19-23) have 1 - Alkaloids, 3 - Terpenes and 4 - Polyphenols. Fraction C (24-30) has 1 - Alkaloids and 3 - Terpenes while frction D has 1 - Alkaloids, 2 - Flavonoids, 3 - Terpenes and 4 - Polyphenols. Plates were scanned to prevent loss of coloured bands that occurred after exposure to the developers. E is the complete extract.

4.4 ANTIBACTERIAL ACTIVITY

The results of the screening are listed in Table 29. Out of the 16 samples, representing 8 plant species, 10 showed activity against one or more Gram-positive organisms. Four of the plants; *Bauhania thonnongii, Butryspernum parkii, Anogeissus schimperi* and *Anacardium occidentale* showed activity against the Gram-negative bacteria *Escherichia coli* and the last two against *Pseudomonas aeruginosa.* All the bacteria were clinical isolates from human cases from Leicester Royal Infirmary. The negative results obtained against the Gram-negative bacteria by the rest of the plants were not surprising as, in general, these bacteria are more resistant than Gram-positive bacteria (Martin, 1995; Paz et al., 1995; Vlietinck et al., 1995). Specific fractions were not tested against bacteria. Thepreliminary results presented here were not followed up for time reasons.

The results of this study support, to a certain degree, the traditional medicinal uses of the plants evaluated both for human and animal disease therapy (Sofowora, 1982) and reinforces the concept that the ethnobotanical approach (Cox and Balick, 1994) to screening plants as potential source of bioactive substances is successful. Several of the plants tested are now under investigation to isolate the active principles.

TABLE 29:Antibacterial Properties of Some Nigerian Medicinal Plants Used in Traditional Medicine:

PLANTS	Minimum Inhibition Concentration(ug/ml)									
	PART OF PLANT	*EC*	*OSA*	*ES*	*SP*	*CP*	*EF*	*MSA*	*AS*	*PA*	*MPA*
Detarium senegalensis	LEAF	>100	6.25	>100	>100	>100	50	50	25	>100	>100
Dichrostachys glomerata	LEAF	50	6.25	3.13	3.13	6.25	1.56	50	6.25	50	50
Ziziphus spina-christi	LEAF	50	25	25	25	25	>100	>100	6.25	>100	>100
Lannea acida	LEAF	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Sterculia setigera	BARK	6.25	25	50	25	50	25	25	25	>100	>100
Khaya senegalensis	BARK	>100	25	>100	>100	>100	>100	>100	>100	>100	>100
Cassia goratensis	LEAF	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Boswella dalzalli	BARK	>100	6.25	>100	>100	6.25	>100	>100	6.25	>100	>100
Bauhinia thonningi	LEAF	6.25	6.25	>100	>100	>100	50	50	50	>100	>100
Butryospernum parkli	BARK	25	6.25	25	>100	>100	50	>100	>100	>100	>100
Guiera senegalensis	LEAF	>100	50	>100	>100	3.13	6.25	>100	>100	>100	>100
Anogeissus schimperi	LEAF	3.13	1.56	3.13	1.56	3.13	1.56	3.13	3.13	3.13	0.78
Anacardium occidentale	BARK	6.25	3.13	6.25	6.25	6.25	1.56	6.25	6.25	6.25	3.13

EC= *Escherichia coli*; OSA= Oxoid *Staphylococcus aureus*; ES= *Enterobacter species*; SP= *Streptococcus pneumoniae*;
CP= *Corynebacterium pyogenes*; EF= *Enterococcus faecalis*; MRSA= Multiresistant *Staphylococcus aureus*;
AS= *Acenotobacter species*; PA= *Pseudomonas auregenosa*; MPA= Multiresistant *Pseudomonas auregenosa.*

CHAPTER FIVE: DISCUSSION

5.1 ANTIVIRAL ACTION OF EXTRACTS

The plants investigated here originated from Northern Nigeria and are employed in the treatment of a variety of ailments (Watt and Breyer-Brend, 1962, Kerharo and Adam, 1974). The result of the present investigation provided further evidence of the importance of using ethnopharmocology as a guide to the screening of biologically active plant materials (Farnsworth and Kaas, 1981, Frei *et al.*, 1998).

In this study, 14 plant extracts from 10 families were found to have activity against some or all of the viruses used. Most of these plant extracts were found to be active, with no cytotoxicity to the cell lines used, at a dose of between 100-400 μg/100 μl. Some of the plant extracts; i.e. *Anogeissus schimperi, Anacardium occidentale, Boswelia dalzeili, Dichrostachys glomerata* and *Sterculia setigera* were not cytotoxic even at concentrations above 400 μg/100 μl.

Virus replication was also inhibited by pre-treatment of cells with the extract. This was notable for all of the human viral battery with *Anogeissus schimperi, Guiera senegalensis, Dichrostachys glomerata,* and *Boswelia dalzeili.* Other plant extracts have partial or no activity (Table 10a). For the animal viral battery, activity was seen for the canine parvovirus with *Dichrostachys glomerata* and *Anacardium occidentale* and EHVS 1 by *Boswelia dalzeili* (Table 11a). Pre-infection efficacy is a favourable finding whereby uninfected cells, *in-vitro,* will synthesize less virus if they become

infected in the course of disease. A number of plant extracts from many parts of the world have been found to inhibit viral replication (Irvin *et al.*, 1980, Aron and Irvin, 1980, Barbieri *et al.*, 1982).

The irreversible denaturation of virus proteins or glycoproteins, so that infectivity of the virus is completely lost, is called virucidal activity. A virucidal extract may not only inactivate the virus extracellularly, so that only non-infectious virus particle remained, but it may also attack one of the replication steps in an irreversible way. In this way, virucidal substances may behave as real antiviral, since they act irreversibly on the viruses and cure the cells completely. This was noticed in this study, indicating that the virucidal activity was the same as that of the antiviral activity for the human viruses (Tables 10b and 10c). The same goes for the following plant extracts with the animal viruses; *Bauhania thonningii, Detarium senegalensis, Dichrostachys glomerata, Anacardium occidentale, Sterculia setigera,* and *Boswelia dalzeili* (Tables 11b and 11c).

This study has indicated that extracts of *Anogeissus schimperi, Guiera senegalensis, Dichrostachys glomerata, Bauhania thonningii, Detaruim senegalensis, Anacardium occidentale, Sterculia setigera* and *Boswelia dalzeili* will inhibit replication of a number of DNA and RNA viruses. The antiviral activity against the DNA virus HSV was promising for 8 plant extracts and moderate for another 4.

The greatest incidence of overlap (Tables 18 and 19) was recorded by samples active against astrovirus and poliovirus, and also between human and animal HSV. This is not surprising since both groups of viruses belong to the same group i.e. non-envelope and envelope viruses respectively. Many examples of overlap were found for the DNA and RNA

viruses. Thus, it would appear that selection of samples on the basis of ethnomedical and ethnoveterinary medical consideration gives a higher hit-rate than a screening program of general synthetic products. This is a conclusion supported by other research groups (Farnaworth and Kaas, 1981, Vanden Berghe *et al*, 1986, Vlietnick and Vanden Berghe, 1991, Vanden Berghe and Vlintnick, 1991).

Some of the plant extracts, *Cassia goratensis, Lannea acida, Sterculia setigera, Boswelia dalzeili* and *Khaya senegalensis* resisted boiling (Table 14) suggesting that two functions are mediated, in all the plant extracts, at least in part by different compounds. From this study then, the contention that the breadth of efficacy of an antiviral agent embracing DNA and RNA indicates "non-specificity" is called into question, as it is quite conceivable that an antiviral agent will operate on a specific macromolecular event which is general to viruses of different groups. Indeed, it has been known for many years that a number of antiviral agents have efficacy against both DNA and RNA viruses (Sandstrom et al., 1985, Price et al., 1986, Chrisp and Clissard, 1991). Secondly, breadth of efficacy is essentially a favourable attribute of any extract. The mechanism of action of the active components of the extracts is presently unknown. Moderate to complete antiviral properties, i.e. reduction in the viral titre from 10^5 to 0, were exhibited by the 17 plant species. Notable ones with complete reduction, for the human and animal viral titre, were *Anogeissus schimperi, Boswelia dalzeili, Dichrostachys glomerata, Anacardium occidentale* and *Sterculia setigera* (Tables 18 and 19).

Following successful detection of active plant extracts, isolation and characterization of the substances responsible for the bioactivity follows. Constituents of medicinal plants with antiviral properties come from a whole range of substance classes (Vanden Berghe et al., 1986) which

include alkaloids, lignans, phenols, quinones, flavonoids, phenolic glycosides, sesquiterpenes, triterpenes and saponins. This study was able to determine some of the group of substances contained in each extract (Table 13 and 14). Most of these substances, however, belong to the group of antivirals with an activity on extracellular virus, including denaturing agents, detergents, lipids and solvents, acids, alcohol, phenolics, urea, polysaccharide etc. Their activity could be due to an irreversible denaturation of the virus or glycoproteins, so that infectivity of the virus is completely lost.

5.2 RESOLUTION OF ACTIVE COMPONENTS

5.2.1 Separation of sample using FPLC

Method of separation was found to be important in this study. Even though only two methods were used due to logistics (Ion-exchange and Gel filtration chromatography), the difference obtained were in favour of gel filtration chromatography (except in the case of *Dichrostachys glomerata*). The reason why most individual fractions in Ion exchange have no activity may be due to the separation method. Possibly, most of the active components were bound to the silica during elution. However, the combined fractions that showed very weak antiviral activity could be explained by synergy between some of the substances present.

Gel filtration was able to identify individual fractions with activity against most of the viruses in the viral battery. In all the plant extracts, combination of the fractions enhanced the antiviral action. This could be due to synergistic effect between the fractions or due to increased concentration of active substances within the combined fractions. The only exception was the combined fractions of *Boswelia dalzeili* that gave no activity against the viruses even though two of the fractions (A and D) showed weak activity against most of the viruses in the viral battery. Lack of minerals, like iron, or marsking effect due to other compounds could be responsible for this action.

In all, the fractions, whether individually or combined, appeared to have stronger activity against the herpes simplex viruses followed by astrovirus and poliovirus and then the parvoviruses. When compared with the activity of the crude extracts, the indication was that most of the activity of

the crude extracts could have been potentiated by the presence of other substances like detergents, acids, alcohol or other polysaccharides.

5.2.2 Thin layer chromatography (TLC)

Attempts were made to identify the constituents of the fractions that had antiviral activity. They were found to contain a variety of substances most of which were common to the different plant extracts. The major grouping of chemicals were alkaloids, flavonoids, terpenes and polyphenols (Fig. 10, 12, 14, and 16). From the Gel Filtration FPLC chromatograph of *Anogeissus schimperi*, fraction B was the only one with activity (Table 22) but when all fractions were separated by TLC, fractions A and B have similar bands except for the presence of flavonoid in fraction A (Fig. 10). The lack of flavonoid in fraction B could be responsible for the action noted. In fraction A, flavonoid may be forming a complex that may hinder activity. The result of the combined fractions that showed activity may be due to the independent action of fraction B. A different picture was seen in the case of *Anacardium occidentale*, where, even though fraction A contain flavonoid, both fractions (A and B) have activity. The flavonoid in this case may possibly be different in structure to the one above (Osawa et al., 1992). *Boswelia dalzeili* had activity with fraction D only and from the TLC, it was shown (fig. 14) to contain flavonoid in addition to alkaloid. The flavonoid in this situation may also be different with that of *Anogeissus schimperi.* Flavonoids occupy an important position in phytomedicine (Havesteen, 1983; Pathak et al., 1991). Previously noted antiinfective flavonoids are secondary metabolites and their analogues such as catechins, chalcones, flavanones and isoflavones (Sakanaka et al., 1989; Masaru et al., 1996). The picture for *Dichrostachys glomerata* is difficult to explain especially as fraction A and C contained the same bands (fig. 16) with only fraction A having activity. The explanation here

could be that the detailed structure of the chemical substances may be different and hence the lack of activity of fraction C.

It is possible that the presence of other components such as metals in the fractions played part in the activity against the viruses. Another possible explanation is that there may be some strong synergistic activity between the fractions to warrant activity. There is also the possibility of synergism between two or more compounds that together could provide useful antiviral activity (Amoros *et al.*, 1992b). If such synergism were separated during purification, then the antiviral activity could be lost. Synergism could operate at the level of the animal model or the real disease situation. This may explain the success of many medicinal plant extracts, which could be therapeutically useful for several apparently unrelated syndromes by virtue of the synergistic effects of two or more components that complement each other *in vivo* (Cannel, 1998). In fact, a combination of extract may be even more potent for the same reason.

Antiviral activity could also disappear during the course of fractionation and repeated extraction, and this could be due to masking, sequestering by another compound, dilution, or loss of protective substances, or real inactivation (Hudson, 1990).

5.3 ANTIBACTERIAL ACTION OF CRUDE EXTRACT

The minimum inhibitory concentration of the action of the crude extract was determined and the most active extract was found to be *Anogeissus schimperi, Anacardium occidentale* and *Dichrostachys glomerata* (Table 29). Their actions were against a wide range of both Gram positive and Gram negative bacteria (Cowan, 1999, Himejima and Kubo, 1991, Silva *et al.*, 1996). Most of the other plant extracts are found to be active against mainly Gram positive organisms. The finding is not very surprising especially when their usage is considered by the traditionalist, who prefer the use of the 3 plant extract mentioned earlier with broad spectrum action. This to some extent confirms the claim by the traditional animal practitioners of the usefulness of these plants as antibacterial compounds (Afolayan and Meyer, 1997, Ivanovska *et al.*, 1996, Mendoza *et al.*, 1997, Navarro *et al.*, 1996, Sato *et al.*, 1996, Scalbert, 1991, Tsuchiya *et al.*, 1996). Considering that the bacteria used in this study were clinical isolates from Leicester Royal Infirmary, these plant extracts could be a potential source of antibacterial compounds of the future.

5.4 CONCLUSION

The study was able to show that some of the plants screened have both antibacterial and antiviral inhibiting ability when in the crude form. Four of these plants were further exploited through fractionation (Fast Performance Liquid Chromatography) and when the fractions were tested, they were found to contain a range of classes of substances that may be responsible or contribute to the actions seen with the crude extract. However, reduction in activity was noted when fractions were tested for antiviral activity, individually or in combination. This could be due to loss of other compounds or minerals that potentiate activity or act synergistically with the isolated compounds. Hence, the separation could have destabilised the compound responsible for the full activity seen with the crude extract.

Tables 30 and 31 show the different groups of compounds that were identified in this study. Further purification and isolation of these compounds was not carried out because of time and resources constraint.

Table 30: Chemical grouping of some of the compounds present in the Extracts.

Plant Species	Alkaloids	Flavonones	Tannins	Saponins
Anogeissus schimperi	+	+	-	+
Anacardium occidentale	+	+	+	+
Boswelia dalzeilii	+	+	+	+
Dicrostachys glomarata	+	+	-	+

+ = Presence of the compound.

- = Absence of the compound.

Table 31: Presence of chemicals in fractions of the Extracts.

Plant Extracts	Fractions from the Extracts after FPLC															
	A				B				C				D			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Anogeissus schimperi	+	+	-	+	+	-	-	+	+	-	+	+	+	-	-	-
Anacardium occidentale	+	+	+	-	+	-	+	-	+	-	-	-	n	n	n	n
Boswelia dalzeilii	+	-	+	-	+	-	+	+	+	-	-	+	+	+	-	-
Dichrostachys glomerata	+	-	+	-	+	-	+	+	+	-	+	-	+	+	+	+

+ = present in the extract

- = absent in the extract

n = No extract D

1 = Alkaloids; **2** = Flavonones; **3** = Terpenes; **4** = Polyphenols

The above tables indicated many of the chemical groupings that are known to have antiviral activity. However, it should be noted, for example for alkaloids, that there are more than 5000 types identified and the ones found could be any one of them (Hudson, 1990). A similar situation applies to Flavonoids and Terpenoids and the activity noted could be one or a group of them that were isolated already or perhaps a new one. This could be ascertained only when proper isolation and identification is done; hence further studies are required in this case. Also in the future, the possibility of finding plant extracts combination that will act synergistically to give a better and stronger action against microbes should be sought as this is the main practice in traditional medicine.

CHAPTER SIX: FIELD-TESTING

6.1 INTRODUCTION TO THE PROBLEMS

Herds are subject to numerous health problems and about 35 recognized diseases plus some 26 other conditions that are specific to given sexes or ages of animals or that result from accidents or other misfortunes, are known today. For each of these health problems herders could identify their contagiousness, characteristic symptoms, seasonal prevalence and causes or predisposing factors (Diallo, 1989).

6.1.1 Contagious diseases

Herders are and very knowledgeable about the contagious diseases of cattle including the speed with which such diseases strike their mode and rate of spread and their sensitivity. They classify six diseases as contagious: pleuro-pneumonia, foot-and-mouth disease, blackleg, anthrax, rinderpest and a form of chronic botulism. Herders say these diseases are mainly spread by infected animals mixing with healthy ones at watering places, along cattle routes, in pastures and in the cattle camps at night. These modes of transmission are of particular concern because they can introduce unfamiliar contagious diseases from other regions or countries. They also recognise that wild animals like insects and birds can also carry contagion. Also, wind and other certain odours borne on the wind (< biblio >) could spread infection.

6.1.2 Non-contagious diseases

Non-contagious diseases are classified by herders into 5 broad categories and they include fever, swellings, diseases attributed to the environment (e.g. bloats and diarrhoea, mastitis and malnutrition).

Fever: a complex of such fevers is identified by symptoms like aches and pains, general fatigue and the inability to stand or walk. These ailments can be fatal if they leave an animal unable to graze.

Diseases attributed to the environment: a good environment promotes good livestock production. However, environment can also be the source of livestock diseases and disorders, whether via grazing sites and forages, seasonal changes or wildlife. Bloat, for example, is attributed to the fodder or other items that the animal ate, while diarrhoea is caused by poor grazing habits. Herders know diarrhoea can be fatal, especially when it leads to dehydration.

Mastitis: this is a disease associated with the hot dry season and the herders recognize it as an important production disease that is affected by season.

Malnutrition: Herders consider all clinical syndromes involving malnutrition and general weakness very important. They realize that most animals are subject to seasonal or annual cycles of malnutrition that can prejudice growth, reproductive capacity, milk production and disease resistance. Herders distinguish many forms and degrees of malnutrition depending on the quantity and quality of forage available, the season of the year, the types of animals affected and their clinical signs. Herders also linked malnutrition to lack of salt (Scillhorn Van Veen, 1996)

6.1.3 Health problems associated with sex or age

All herders recognize that a number of health problems do affect only or mainly specific sex or age groups. Cows may have abortions, calving problems, mastitis, infertility, uterine prolapsed or impaired milk production. These problems are important since they affect herd reproduction and productivity directly and hence the wellbeing of herders and their families (Kudi et al., 1998).

Newborn calves are particularly susceptible to severe and fatal diarrhoea and to intestinal parasitism, which can lead to potentially fatal conditions (Kudi et al., 1998). They are also known to suffer from swollen tongue that prevents proper nursing (a condition caused by over-consumption of colostrum and sometimes leads to fevers). A swollen and inflamed navel (Navel ill) contaminated by dirt or insects (a condition that can block urination and eventually kill the calf) and a frothy cough that is thought to be due to ingestion of the dam's hair while suckling.

Two genital disorders commonly afflict mature bulls. One produces infertility as a result of severe damage to the scrotum. In a venereal infection, the penis may fail to retract, causing urinary and mating difficulties.

6.1.4 Accident and sorcery

Animals frequently suffer broken legs, intestinal obstructions, repeated snake bites, falls and other unpredictable problems. Herders believe that some animals and even whole herd are 'fated' to be especially vulnerable or prone to accident and disease. Animals subjected to sorcery also share

this condition and accounts for animal health problems that find no other explanation (Sofowora, 1983).

6.2 ANIMAL HEALTHCARE PRACTICES

Herders have access to local healers, most of who are specialist in treating certain unusual or complex livestock diseases. But even ordinary herders know many ways to prevent, control and cure common animal health problems themselves. Some of the most common techniques and treatments are outlined below.

6.2.1 Preventive care

For certain diseases (e.g. rinderpest and chronic botulism) quarantine may be instituted. The carcasses of animals that succumb to infectious diseases are buried so as to forestall further contagion. To dispel or repel contagion, herders may also fumigate their kraals by burning medicinal plants (leaves, roots or barks). At the same time, some incantations may be said to provide added protection. Branding is also done to prevent a number of contagious diseases that are believed, by the herders, to be afraid of fire.

Most preventive measures are simply a normal part of judicious herding and grazing strategies. Chief among these is herd movement, which is the herder's main strategy for providing their animals with the best and most varied diet, and therefore with greater resistance to disease in general. As part of their preventive healthcare regime, herders add minerals to their herds' diet whenever under-nourishment threatens. The type of supplement varies with the season and with animal health and age.

For contagious bovine pleuro-pneumonia (CBPP) and some other diseases, herders practice a form of vaccination that builds resistance by exposing animals to a mild case of the disease in question. They prepare the vaccine by removing a small piece of lung from an animal that died of

CBPP and steeping the tissue overnight in milk or millet mixture. This procedure is said to reduce the toxicity. The next morning the piece of lung is placed in a small incision made on the nose in such a way that the animal is not able to lick it. The vaccinated stock, usually run a fever for two or three days and then recover. Occasionally, though, if the lung tissue is "too hot" (i.e. virulent), an animal may contract a full-blown case of CBPP and die.

To prevent or combat sorcery against animals, herders often hire a special type of traditional healer to prepare and or administer protective medicines, fetishes and incantations. These may consist of a mixture of herbs, a liquid to put on the ground in cattle camp or an amulet made of a cord knotted while reciting protective formulae.

6.2.2 Curative care

Herders use a variety of curative treatments to treat, cure or heal animal diseases. Some involve the administration of drenches or ointment prepared from roots or leaves; others require physical manipulation; still others make extensive use of fire or water. Treatments may be applied simultaneously or successively. And since herders do not draw a sharp distinction between the natural and the supernatural world, medical interventions are often mixed with ritual acts and incantations. Thus, science and ceremony reinforce each other to promote herd health, well-being and security (Leeflang, 1993).

Herders generally administer medicines in one of two ways: Orally or externally. Some examples include: for diarrhoea or FMD, drenching or force feeding of some plant mixtures, to relieve bloat, feeding fresh milk or a sugar mixture; or to induce vomiting, forcing the animal to drink soap suds using the locally-made black soap.

External treatments take various forms involving massage or rubbing, sometimes with medicaments and sometimes without. Examples include application of a sort of plaster of butter and leaves or roots in the form of a cream or thick liquid to wounds, sores, swellings or pain. Medicaments typically administered in these ways include for example, butter, cream, herbal mixtures of leaves, ash and dirt or mud (especially the earth from termite hills).

Common surgical techniques include bloodletting to remove "blackblood" or "deadblood" and piercing or incising the skin. The latter techniques are used to treat bloat and blackleg. Cutting or piercing the skin is always accompanied by other treatments. Wounds and abscesses are often opened, cauterized and/or anointed. Herders are also adept at setting bones and adjusting dislocations. In the case of a fracture, the limb is wrapped and tied, usually in a mat. In dislocations, the limb is pulled and reset in its proper position. Fire is used for branding or scarring, cauterizing and fumigating and these techniques are usually accompanied by other treatment. Branding is used for a variety of conditions, including anthrax, FMD, fevers, myiasis, phlebitis, hoof diseases and inflamed ticks (Brisebarre, 1990).

6.3 PRACTICE OF ETHNOVETERINARY MEDICINE IN NORTHERN NIGERIA

Whether in conceptualizing, classifying, preventing, controlling or treating livestock health problems, veterinary concerns among herders focus on the practical implications for human well-being and food security. They judge the severity of a problem mainly in terms of its effects on live weight, milk yields, fertility and reproduction in their animals. They have a vast array of strategies and treatments-ecological, nutritional, ethnopharmacological, manipulative, immunological, surgical and magico-religious- to counter health problem in their herds.

These series of experiments are designed to test the ethnopharmacological activity of the medicinal plants from northern Nigeria already described in previews chapters in treating cases of diarrhoea in cattle.

6.4 EXPERIMENTAL DESIGN

6.4.1 Selection of farms

Farms were selected randomly within the guinea Savannah from a group of farms willing to participate in the study. The breed of cattle used were the Zebu (indigenous to Northern Nigeria). A total of ten farms were used from which 100 animals with diarrhoea and fevers were selected at random.

6.4.2 Selection of Animals

In the farms, 100 cases of cattle with diarrhoea and fever were selected and divided into five groups consisting of 20 animals each, for each of the plant extracts, and the last group as control. The first four groups were treated with the plant extracts and the last group was treated with antibiotics (neomycin sulphate), however, if the case becomes very serious in the groups especially the first four, then conventional antibiotics and symptomatic treatment was instituted. Treatment with the crude plant extracts was given at a dose of 50ml/100kg-body weight (1g plant material/ml of distilled water) orally, twice a day (mornings and evenings), for 7 days in conformity with the way the traditionalist used the extracts. All groups were monitored daily for improvement and recovery from high body temperature and the diarrhoea.

6.4.3 Plant Extracts Used

When the plants brought from Nigeria were screened, *Anacardium occidentale* (Cashew), *Boswelia dalzeilii* (Hararrabi), *Anogeissus schimperi* (Marke) and *Dichrostachys glomerata* (Dundu) were found to be

the best plant extracts with broad-spectrum action against a wide range of bacteria and viruses hence were selected for this trial.

6.4.4 Statistical Analysis

Data collected were subjected to the Analysis of Variance (ANOVA) using the Minitab® software (Eddison, 1999). The data collected for each plant sample were compared with the control (Group treated with antibiotic).

6.5 RESULTS OF FIELD TRIALS

The result obtained after 7 days of treatment are given in Table 32 and showed that, for the plant extracts, effective treatment was achieved after the fifth day of therapy. For the antibiotic that was used, many of the animals showed recovery after the third day.

Analysis of variance (ANOVA) results is given in Table 33 and it compared the effect of treatment with the different plant extract and antibiotic. The result showed that there is no any significant difference between the extracts and the antibiotic in efficacy of treatment (**P=0.778**, **F= 0.44**). A Boxplot shows that there is not much difference between the means of the time of recovery (Fig 17).

Table 32: Cumulative Number of animals showing Recovery by day of treatment.

Days	ANTIBIOTICS	CASHEW	DUNDU	MARKE	HARARRABI
1	0	0	0	0	0
2	3	1	3	1	2
3	10	4	7	3	6
4	14	8	10	5	9
5	17	15	12	9	12
6	19	17	16	14	15
7	20	19	19	16	18

Table 33: One-way Analysis of Variance of the Extracts Compared with Antibiotics.

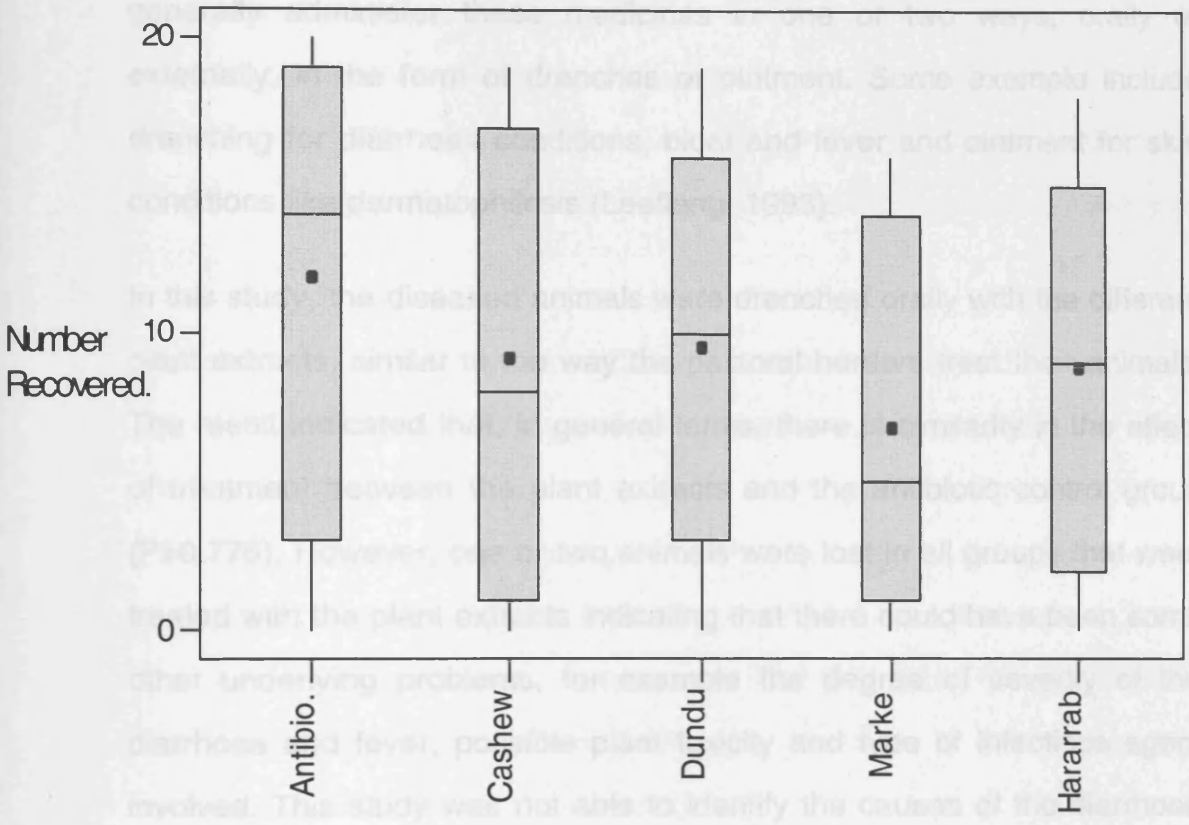
Source of Variation	DF	SS	MS	F	P
Treatment	4	89.5	22.4	0.44	0.778
Error	30	1523.1	50.8		
Total	34	1612.7			

Means

Level	N	Mean	StDev
Antibiotic	7	11.857	7.862
Cashew	7	9.143	7.862
Dundu	7	9.571	6.803
Marke	7	6.857	6.309
Hararrabi	7	8.857	6.644

Fig 17: Boxplot of the means of number of Recovery after treatment.

(means are indicated by solid circles)



6.6 DISCUSSION AND CONCLUSION

African livestock producers especially the pastoral groups have, before the advent of modern (allopathic) veterinary intervention, relied exclusively on the use of locally available plants and herbal forage in coping with various animal health problems. The pastoral herders use a variety of curative treatments to treat, cure or heal various animal diseases. They generally administer these medicines in one or two ways; orally or externally, in the form of drenches or ointment. Some example include drenching for diarrhoea conditions, bloat and fever and ointment for skin conditions like dermatophilosis (Leeflang, 1993).

In this study, the diseased animals were drenched orally with the different plant extracts, similar to the way the pastoral herders treat their animals. The result indicated that, in general terms, there is similarity in the effect of treatment between the plant extracts and the antibiotic control group ($P=0.778$). However, one or two animals were lost in all groups that were treated with the plant extracts indicating that there could have been some other underlying problems, for example the degree of severity of the diarrhoea and fever, possible plant toxicity and type of infectious agent involved. This study was not able to identify the causes of the diarrhoea and fever in the animals selected because of logistics. However, because of the fever the conditions were regarded as being caused by infectious agents.

In the opinion of the pastoral herders, the severity of a problem is judged mainly in terms of its effect on live weight, milk yields, fertility and reproduction and inability to graze or stand. In only a few cases do they suspect a particular infectious disease from previous experience or knowledge. To further treat any of the conditions that did not respond to

ethnopharmacology, a vast array of strategies and treatment including ecological, nutritional, manipulative, immunological, surgical and magico-religious procedures could have been used to counter the problem (Sofowora, 1982). None of the above methods were employed during the trial.

In conclusion, one can say that the results of this trial, when compared with the earlier *in-vitro* study, indicated that the plant extracts have good antibacterial and/or antiviral when used *in vivo* in the crude form. This result confirms the claim by the pastoral herders that the plants are of medicinal value and can be relied upon for treating diarrhoeal conditions in livestock.

The result of this trial could also help to stimulate vigorous research in ethnopharmcology especially into many claims, by pastoral herders, of the efficacy of many plants in the treatment of various livestock and human diseases. The use of these plant extracts may, perhaps, have the advantages of use in antibiotic resistance cases. The advantages are that they have low toxicity and are cheap. There is also the potential of isolating a novel compound that may open the door to the discovery of new anti-infective agents in the near future.

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Journal of Ethnopharmacology 68 (1999) 289–294

Journal of ETHNO-PHARMACOLOGY

www.elsevier.com/locate/jethpharm

Short communication

Antiviral activity of some Nigerian medicinal plant extracts

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Received 3 February 1999; received in revised form 25 March 1999; accepted 25 March 1999

Abstract

Plants from Northern Nigeria with a history of use in both human and veterinary traditional medicine have been investigated for their antiviral activity and their cytotoxicity determined. Extracts were tested against poliovirus, astrovirus, herpes simplex viruses and parvovirus, using the microtitre plate inhibition tests. Most of the extracts have activity against more than one virus at a dose rate of between 100 and 400 μg/100 μl.

Keywords: Plant extract; Traditional medicine; Cytotoxicity; Antiviral; Nigeria

1. Introduction

Traditional medical and veterinary medical practices still play an important role in many areas of Nigeria (Kerharo and Adam, 1974; Sofowora, 1982). A large number of plants are use on the African continent for the treatment of various and different diseases and complaints (Kerharo and Adam, 1974). As a result, a number of reports concerning the antiviral activity of African medicinal plants have appeared (Watt and Breyer-Brandwijk, 1962; Sofowora, 1982; Oliver-Bever, 1986; Vanden Berghe et al., 1986) but the studies have not been extensive and thousands of plants still need to be investigated.

Today, there is an increasing need for substances with antiviral activity since the treatment of viral infections with most antivirals is often unsatisfactory due to the problem of, amongst other things, viral latency and the likelihood of new viral agent being discovered. The objective of the present work was to assess the antiviral effect of native plants with some ethnobotanical indications of use for treating viral conditions of both humans and animals in Nigerian folk medicine. The plants were selected on the basis of their uses for the treatment of diarrhoea conditions and wounds in humans and animals.

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0378-8741/99/$ - see front matter
PII: S0378-8741(99)00049-5

2. Methodology

2.1. Plant collection

Plants were collected from the savannah region of Northern Nigeria during the rainy season, corresponding to the time when most of the plant species were available and growing well. The sample collection was done with the aid of four traditional medical practitioners. A voucher specimen was made for each collection after confirmation of identification. Plant materials collected were gently pounded using pistil and mortar, oven dried (50°C), ground and stored in a cool dry place until use.

2.2. Preparation of extract

Dried, ground plant material (10 g) was mixed with 200 ml of 80% ethanol at room temperature, left overnight and then filtered through Whatman number 1 filter paper. The filtrate was evaporated in vacou and the sediments were then partitioned in equal volume of hexane/methanol mixture (9:1). The hexane portion was discarded and the methanol portion evaporated in vacuo and then freeze-dried. The freeze-dried extracts were dissolved in maintenance tissue culture medium (Medium 199 Gibco®) (1 g in 28 ml), filter sterilised using 0.2 μ Acrodisc filters (Gelman Sciences®) and used for antiviral testing.

2.3. Cell culture and cytotoxicity assays

The cell lines used were HT-29 (human colonic cancer cells) for growing astrovirus, poliovirus (type 1) and human herpes simplex virus and MDCK (monkey kidney cell) for equine herpes simplex virus, bovine parvovirus and canine parvovirus. All cells were grown in monolayer in a 5% carbon dioxide and 95% atmosphere at 37°C, in Medium 199 (Gibco®) (Modified Earl's salt) with 10% foetal bovine serum (FBS) (Gibco®) and 10 ml procaine streptomycin and 5 ml fungizone. For MDCK cells, 1% of non essential amino acid (NEAA) (Gibco®) was added.

To test for cytotoxicity, monolayers of the cell lines were grown in 96-well microtitre plates (Falcon 3072), and exposed to twofold serial dilution (dilution factor) of the extracts, starting from neat (400 μg/100 μl) to 10^8 (50 μg/100 μl). The treated cells were then incubated at 37°C for 3 days after which they were checked for cytopathologic effect (CPE) and stained with crystal violet to show effect on cells if any. CPE was indicated by clearance of cells in the wells.

2.4. Antiviral assay

Three human and three animal viruses were used. They were poliovirus (type 1), astrovirus, human herpes simplex virus (type 1), equine herpes simplex virus, bovine parvovirus and canine parvovirus. The ability of dilute plant extract to inhibit virus specific CPE was used as a measure of antiviral activity.

To screen for antiviral activity, monolayer of the cells described earlier were grown in 96-well microtitre plates. Twofold serial dilutions of the extract were made just as in the cytotoxicity assay. Tissue culture medium infective dose (TCID 50) of 10^5 viral particles (100 μl) were added to each test well. The cultures were then incubated at 37°C in 5% CO_2 for an hour to allow for viral adsorption after which 100 μl/well of the plant extracts (from neat to threefold dilutions) were added and the plates reincubated at the same temperature and 5% CO_2 (usually for 3–4 days) to allow development of CPE if any. Controls were set consisting of only cells and cells with virus only.

An extract was said to have antiviral activity if there was absence of viral CPE. Partial inhibition of the virus (marked reduction in infectivity of the virus exposed to an extract when compared to the virus only control) was also recorded.

3. Results

Different parts of 17 medicinal plants belonging to 11 different families (Table 1) were tested for their antiviral activity. Six plant extracts, each from a different plant species, were found to have antiviral activity against more than one of the viruses, at a concentration non-toxic to the cell lines used.

The result of the 17 plant extracts tested against astrovirus, poliovirus 1, herpes simplex virus 1, equine herpes simplex virus, bovine parvovirus and canine parvovirus are given in Table 2. As demonstrated in Table 2, four of the plant extracts inhibited all the human viruses and two all the animal viruses. A total of six plant extracts inhibited astrovirus and poliovirus. Two of the extracts; *Anacardium occidentale* (Anacardiaceae) and *Sterculia setigera* (Sterculiaceae), were found to inhibit both human and animal viruses used in this study.

Most of the plants have partial activity at the lowest concentration of 100 μg/100 μl (dilution factor of three) but full activity at 400 μg/100 μl (neat) against some of the viruses. Examples are *Anogeissus schimperi* against Herpes simplex virus, *Guiera senegalensis* against human viruses and one of the animal virus (EHSV), *Bauhinia thonningii* against parvovirus, *Boswelia dalzielii* against the animal viruses, *Detarium senegalensis* against all the viruses and *Dichrostachys glomerata* against parvovirus (Table 2). Some of the plant extracts were found to be cytotoxic at the neat concentration (400 μg/100 μl) but not at twofold dilution. Others are toxic even at the highest dilution (50 μg/100 μl) (Table 2).

Table 1
Local indications of some Nigerian medicinal plants

Local name (Hausa)	Scientific name	Plant part used	Indications (local)
Marke	Combretaceae *Anogeissus schimperi* (Gull. & Per.) Collectors: Kudi and Ibrahim (651)	Leaf	Fever, diarrhoea, dressings
Sabara	Combretaceae *Guiera senegalensis* (J.F. Gmelin) Collectors: Kudi and Demo (661)	Leaf	Enteric problems, worms
Kalgo	Leguminosae *Bauhinia thonningii* (Schum.) Collectors: Kudi, Yayok and Ibrahim (691)	Leaf	Diarrhoea, fever
Taura	Leguminosae *Detarium senegalense* (Gmel.) Collectors: Kudi and Tula (691)	Leaf	Fever, dysentery, boils
Rumfu	Leguminosae *Cassia goratensis* L. Collectors: Kudi and Demo (612)	Leaf	Fever, worms
Cashew	Anacardiaceae *Anacardium occidentale* L. Collectors: Kudi and Haruna (613)	Bark	Enteric conditions, worms
Kadanya	Sapotaceae *Butyrospermum parkii* L. Collector: Kudi (644)	Bark	Fever, dressing, boils
Madachi	Meliaceae *Khaya senegalensis* (A. Juss.) Collectors: Kudi and Demo (644)	Bark	Helminths.
Kimba	Annonaceae *Xylopia aethiopica* (A. Rich.) Collectors: Kudi and Ibrahim (621)	Whole plant	Diarrhoea
Kerana	Euphobiaceae *Euphobia kamerunica* (Ait.) Collector: Kudi and Ibrahim (671)	Whole plant	Fever
Farchan Shafo	Mimosaceae *Acacia albida* (Hochst) Collector: Kudi and Ibrahim (632)	Bark	Enteric conditions
Dundu	Mimosaceae *Dichrostachys glomerata* (Chiev.) Collector: Kudi, Ibrahim and Demo (642)	Leaf	Skin conditions, fever, diarrhoea
Kurna	Rhamnaceae *Ziziphus mucronata* (Willd) Collector: Kudi (682)	Leaf	Enteric conditions
Faru	Anacardiaceae *Lannea humilis* (Oliv.) Collector: Kudi and Haruna (623)	Bark	Diarrhoea, fever
Kukkugi	Sterculiaceae *Sterculia setigera* (Del.) Collector: Kudi and Ibrahim (673)	Bark	STDs, fever
Gwadan Jeji	Annonaceae *Annona senegalensis* (Pers.) Collector: Kudi (611)	Leaf	Diarrhoea, fever, headache
Hararrabi	Burseraceae *Boswelia dalzielii* L. Collector: Kudi and Yau (624)	Bark	Diarrhoea, fever, dressing

Table 2
Antiviral action, cytotoxicity and effective concentration of some medicinal plant extracts from Nigeria[a]

Plant species	Cytotoxicity (μg/100 μl)	Viruses						Effective concentration (mg/ml)	Dilution factor[b]
		Poliovirus	Astrovirus	HSV 1	Equine HSV	Bovine parvovirus	Canine parvovirus		
Anogeissus schimperi	NT	++++	++++	++	++	–	–	2	2
Guiera senegalensis	NT	+++	+++	+++	+++	–	–	2	2
Bauhinia thonningii	NT	++++	++++	++++	+++	+++	+++	1	3
Cassia goratensis	400	+++	+++	–	–	++	++	2	2
Anacardium occidentale	NT	++++	++++	++++	++++	++++	++++	1	3
Butyrospermum parkii	200	++	++	–	–	–	–	1	2
Boswelia dalzielii	NT	++++	++++	++	++	+++	+++	1	3
Khaya senegalensis	400	+++	+++	+	+	–	–	2	2
Xylopia aethiopica	100	–	–	–	–	–	–	Toxic	3
Euphobia kamerunica	100	–	–	–	–	–	–	Toxic	3
Acacia albida	100	–	–	–	–	–	–	Toxic	3
Detarium senegalensis	400	+++	+++	+++	++	++	++	2	2
Dichrostachys glomerata	NT	++++	++++	++++	++++	+++	+++	1	3
Ziziphus mucronata	400	+++	+++	–	–	–	–	2	3
Lannea humilis	100	++	++	+	+	–	–	1	3
Sterculia setigera	NT	++++	++++	++++	++++	++++	++++	1	3
Annona senegalensis	100	–	–	–	–	–	–	Toxic	3

[a] ++++, Total inhibition; +++, 75% inhibition; ++, 50% inhibition, +, <50% inhibition; –, no inhibition; NT, not cytotoxic at 400 μg/100 μl.
[b] 1, Neat; 2, 10^2 dilutions; 3, 10^4 dilutions of the extract.

4. Discussion

in this study, six of the 17 plant extracts of Nigerian medicinal plants species, used by the Hausa and other tribes of Northern Nigeria, for symptoms possibly indicative of viral illness were found to have antiviral activity. Some of the traditional medicinal uses recorded for the plant species which these extract were derived from are given in Table 1.

The results of the present investigation provide further evidence of the importance of ethnopharmacology as a guide to the screening for biologically active plants materials (Farnsworth and Kaas, 1981; MacRae et al., 1988; Vanden Berghe et al., 1986; Vlietinck et al., 1995). The plants investigated here originated from Northern Nigeria and are employed in the area for the treatment of a variety of human and animal ailments (Kerharo and Adam, 1974; Odebiyi and Sofowora, 1978; Nwude and Ibrahim, 1980).

While this study used 100% inactivation to define an antiviral extract, it is important to note that many extracts had partial antiviral activity. These extracts could contain compounds that are true antivirals but that were present at quantities insufficient to inactivate all infectious viruses in the standard virus preparation. It should be pointed out, however, that different cell lines were used in the two antiviral assays, and there may be some variation in the way plant compounds behave in the different cell types (Hudson, 1990). One of the inherent drawbacks of in vitro antiviral testing is the environmental sensitivity of cell lines in culture. Preparations which exert antiviral effects in vivo may not be detected in in vitro assays because of the extremely low concentrations of extract tolerated by cells in the artificial system (McCutcheon et al., 1995). Even with this limitation, 12 of the 17 extracts screened exhibited some antiviral activity.

Vanden Berghe et al. (1993) have suggested that the antiviral activity of crude plant extract should be detectable in at least two subsequent dilutions of the maximum non-toxic concentration to ensure that the activity is not directly correlated with the toxicity of the extract. Most of the active, non-toxic plant extracts found in this study were found to have antiviral activity after two subsequent dilutions. The most active extracts were those effective against poliovirus and astrovirus. They are non-enveloped, single stranded, RNA virus, belonging to the picornaviridae.

Given the pressing need for new antiviral agents and the inherent limitations of in vitro antiviral testing for such agents, the results of this screening were promising. It is possible that the elucidation of the active constituents in these plants may provide useful leads in the development of antiviral therapeutics (Vanden Berghe and Vlietinck, 1991; Vlietinck and Vanden Berghe, 1991).

Acknowledgements

The authors wish to thank the World Bank and Japanese Government for providing the funds for this study.

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ELSEVIER

Journal of Ethnopharmacology 67 (1999) 225–228

Journal of ETHNO-PHARMACOLOGY

www.elsevier.com/locate/jethpharm

Short communication

Screening of some Nigerian medicinal plants for antibacterial activity

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Received 18 May 1998; received in revised form 18 November 1998; accepted 18 November 1998

Abstract

Crude extracts from eight Nigerian medicinal plants, used traditionally in the treatment of infectious and septic diseases in both humans and animals were screened in vitro for antibacterial activity, using the hole-plate diffusion method. Most of the extracts were active against Gram-positive bacteria. Two of the plant, *Angeiossus schimperi* and *Anacardium occidentale*, had good antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* which are Gram-negative bacteria.

Keywords: Antibacterial; Medicinal plant; Nigeria

1. Introduction

Infectious diseases are usually characterised by clear symptoms, so it is likely that traditional healers have been able to recognize such diseases and have developed effective therapies. Moreover, as antibiotics mostly have clear effects, the chance of finding antimicrobially active traditional medicine is considered high (Sofowora, 1984; Elmi et al., 1986).

Traditional medicine is practised by a large proportion of the Nigerian population for their physical and psychological health needs. Medicinal plants have become the focus of intense study recently in terms of conservation and as to whether their traditional uses are supported by actual pharmacological effects or merely based on folklore (Cunningham, 1988; Locher et al., 1995; Williams, 1996).

This study was designed to investigate Nigerian medicinal plants for potential antibacterial activity by preliminary bioassay screening. The selec-

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0378-8741/99/$ - see front matter
PII: S0378-8741(98)00214-1

tion of plants for evaluation was based on traditional uses (Sofowora, 1984) for treatment of symptoms such as wounds, boils, purulent sores and diarrhea among other things. A total of 20 plant extracts, some form different parts of the same plants, were tested for antibacterial activity using the hole-plate diffusion method (Ieven et al., 1979).

2. Materials and methods

2.1. Plant material

All plants were collected in the Bauchi district (Northern Nigeria) during the rainy season, when their leaves are fresh and well grown. The collection was done by the main author with the help of four traditional medicine men. Indications of these plants, collectors and voucher numbers are given in Table 1. Plant materials collected were gently pounded using a pestle and motar, oven-dried (50°C), grounded and stored in a cool dry place until use.

2.2. Preparation of extract

Dried, ground plant materials (5.0 g) was mixed with 50 ml of 80% ethanol at room temperature, left overnight and then filtered under vacuum using 0.45 acrodisc filter (Sartorius®). The filtrate was evaporated in vacuo and the sediments dissolved in water and freeze-dried. The freeze-dried extract were dissolved in 28 ml of distilled water to give a concentration of 36 mg/ml.

Table 1
Local indications of some Nigerian medicinal plants

Local name (Hausa)	Scientific name	Indications (local)
Marke	Combretaceae *Angeiossus schimperi* (Gull. & Per.) Collectors: Kudi & Ibrahim (651)	Fever Diarrhea Dressings
Sabara	Combretaceae *Guiera senegalensis* L. Collectors: Kudi & Demo (661)	Enteric problems Worms
Kalgo	Leguminosae *Bauhinia thonningii* (Schum.) Collectors: Kudi, Yayok & Ibrahim (691)	Diarrhea Fever
Rumfu	Leguminosae *Cassia goratensis* L. Collectors: Kudi & Demo (612)	Fever general Worms
Cashew	Anacardiaceae *Anacardium occidentale* L. Collectors: Kudi & Haruna (613)	Enteric condition Worms
Kadanya	Saptaceae *Butyrospernum parkii* L. Collector: Kudi (633)	Fever Dressing Boils
Madachi	Meliaceae *Khaya senegalensis* (A. Juss.) Collectors: Kudi & Demo (644)	Helminths
Hararrabi	Burseraceae *Boswellia dalzeili* L. Collectors: Kudi & Yau (624)	Diarrhea Fever Dressing

Table 2
Properties of some Nigerian medicinal plants used in traditional medicine[a]

Plants	Part of plant	EC	OSA	ES	SP	CP	EF	MSA	AS	PA	MPA
Khaya senegalensis	Leaf	6	8*	8*	6	6	6	6	6	6	6
	Bark	6	12*	6	6	6	6	6	6	6	6
Cassia goratensis	Leaf	6	8*	6	6	6	6	6	6	6	6
	Bark	6	6	12*	8*	6	6	6	6	6	6
Boswellia dalzielli	Leaf	6	8*	6	8*	6	6	6	6	6	6
	Bark	6	10*	6	6	12*	6	6	8*	6	6
Bauhinia thonningi	Leaf	10*	8*	6	6	12*	6	8*	10*	6	6
	Bark	10*	8*	6	6	6	6	8*	8*	6	6
Butyrospernum parkii	Leaf	8*	16*	8*	6	6	8*	6	6	6	6
	Bark	8*	12*	8*	6	6	8*	6	8*	6	6
Guiera senegalensis	Leaf	6	12*	6	6	18*	12*	6	6	6	6
	Bark	6	10*	6	6	16*	10*	6	6	6	6
Anogeissus schimperi	Leaf	18*	18*	18*	18*	14*	18*	20*	18*	16*	22*
	Bark	16*	18*	16*	18*	16*	16*	18*	16*	18*	20*
Anacardium occidentale	Leaf	10*	12*	12*	14*	6	6	10*	6	6	12*
	Bark	8*	12*	10*	12*	10*	8*	12*	8*	8*	10*

[a] EC, *Escherichia coli*; OSA, oxoid *Staphylococcus aureus*; ES, *Enterobacter* species; SP, *Streptococcus pneumoniae*; CP, *Corynebacterium pyogenes*; EF, *Enterococcus faecalis*; MRSA, multiresistant *Staphylococcus aureus*; AS, *Acinetobacter* species; PA, *Pseudomonas aeruginosa*; MPA, multiresistant *Pseudomonas aeruginosa*.
* Values greater than 6 mm shows some activity.

2.3. Antibacterial activity

The plate-hole diffusion assay as described by Ieven et al. (1979) was used to determine the growth inhibition of bacteria by the plant extracts. The following bacteria, obtained from human clinical cases at the Leicester Royal Infirmary (except for *Staphyloccocus aureus*, Oxford) were used: *Staphylococcus aureus*, multiresistant *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Enterobacter* species, *Acinetobacter* species and *Corynebacterium pyogenes*. Bacteria were maintained at 4°C on nutrient agar plates before use.

Nutrient agar was prepared and 25 ml of each was poured into sterile universals. The universals with the broth were inoculated with the different species of bacteria and incubated at 37°C overnight. A total of 25 ml of molten Mueller–Hinton (MH) agar (Oxoid) held at 40°C was poured into sterile universals maintained at 40°C in a water bath. Each universal was inoculated with 200 μl of the different bacteria species, mixed well with the HM agar and poured into sterile petri dishes and allowed to set. Using a sterile cork-borer of 6 mm diameter, four holes per plate were made into the set agar containing the bacteria culture. A total of 200 μl of plant extracts were poured into three wells and one contained distilled water; the plates were placed in the incubator at 37°C overnight. Antibacterial activity was recorded if the zone of inhibition was greater than 6 mm (Vlietinck et al., 1995).

3. Results and discussion

The results of the screening are listed in Table 2. Out of the 16 samples, representing eight plant species, ten showed activity against one or more Gram-positive organisms. Four of the plants; *Bauhania thonnongii*, *Butryspernum parkii*, *Angeiossus schimperi* and *Anacardium occidentale*

showed activity against the Gram-negative bacteria *Escherichia coli* and the last two against *Pseudomonas aeruginosa*. All the bacteria were clinical isolates from human cases from Leicester Royal Infirmary. The negative results obtained against the Gram-negative bacteria by the rest of the plants were not surprising as, in general, these bacteria are more resistant than Gram-positive bacteria (Martin, 1995; Paz et al., 1995; Vlietinck et al., 1995).

The results of this study support, to a certain degree, the traditional medicinal uses of the plants evaluated both for human and animal disease therapy (Sofowora, 1984) and reinforce the concept that the ethnobotanical approach (Cox and Balick, 1994) to screening plants as potential sources of bioactive substances is successful. Several of the plants tested are now under investigation in order to isolate the active principles.

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