

Short Communication

Antiviral properties of two Nigerian plants

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Ethanollic extracts were prepared from *Bambusa vulgaris* and *Aframomum melegueta*. They were analysed for antiviral activities against three human viruses namely: measles, yellow fever and polio viruses by standard laboratory tests. Both extracts showed antiviral activities against one or two viruses. *B. vulgaris* resulted in inhibition only on measles virus at MIC of 62.5 µg/ml while *A. melegueta* inhibited measles and yellow fever viruses at MICs of 125 and 250 µg/ml respectively. Polio virus type 1 was not susceptible to any of these extracts.

Key words: *Bambusa vulgaris*, *Aframomum melegueta*. antiviral measles, yellow fever and polio viruses.

INTRODUCTION

The widespread of viral infections in Africa and the limited number of available drugs which are effective against them led to investigations on antiviral potentials of plants easily grown in Africa (Anani et al., 2000; Gbeassor et al., 1996; de souza et al., 1993, 1995).

There are still many viral infections that are of public health importance in Nigeria. These include measles, yellow fever and polio viruses. Regardless of the socio-economic status of the population at large, the major focus on the control of some of these viral infections has been that of prophylaxis, whereby every effort has been made to prevent infections through the judicious use of vaccines.

Studies conducted in laboratories around the world revealed that traditional medicinal plants can provide a rich source of antiviral activities (Yip et al., 1991; Vietinck and Vanden-Berghe, 1991; De-souza et al., 1993, 1995; Taylor et al., 1996; Gbeassor et al., 1996; Lipipun et al., 2003; Chiang et al., 2003a, 2003b). Such types of study have often been justified in the context of phytochemical leads for pharmaceutical development. In the African context, however, bioactive extracts can also be considered as "ethical phytomedicines", if appropriate phytochemical standardization and toxicology investigations are undertaken (Gbeassor et al., 1996). However, it is desirable to use flexible test protocols that can be modified to suit the test materials under study in order to optimize the detection of these bioactivities (Hudson et al.,

1994).

Materials from both plants are used by Nigerian herbal practitioners in the preparation of concoction for the treatment of viral diseases. Extracts from *Bambusa vulgaris* are traditionally very effective in the treatment of measles and helminthic infections while extracts from *Aframomum melegueta* are used in the treatment of infections such as cholera, smallpox and chicken pox (Table 1). This present study was carried out to assess the antiviral activities of *A. melegueta* and *B. vulgaris* against measles, yellow fever and polio viruses.

MATERIALS AND METHODS

Plant collections

The plant materials were collected in Ado-Ekiti, a southwestern town of Nigeria. They were air-dried. Voucher specimens were deposited in the herbarium unit, Plant Science Department, University of Ado-Ekiti, Nigeria. Identifications were made by this unit (Table 1).

Preparation of plant extracts

Dried specimens were taken to the Microbiology Laboratory, University of Ado-Ekiti for processing. Powdered samples (50 g) were soaked for five days at 27 °C in 250 ml of 70% ethanol. These were filtered through Whatman #1 filter paper. The filter was rinsed with another 250 ml of 70% ethanol and the combined filtrates were evaporated and freeze-dried. Each dried extract was redissolved in 70% ethanol to make 100 mg/ml.

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Table 1. Experimental medicinal plants.

Family, genus and species	Part of plant used	Treatment
1. <i>Poaceae</i> , <i>Bambusa</i> , <i>Bambusa vulgaris</i>	Leaf	Measles infections and antihelmintic
2. <i>Zingiberaceae</i> , <i>Aframomum</i> , <i>Aframomum melegueta</i>	Seed	Cholera, smallpox and chicken pox

Table 2. Cytotoxicity assays.

Plant specie	Cell type	Cytotoxicity ($\mu\text{g/ml}$)	230	235	240	245	250	255	260	260	265
<i>Bambusa vulgaris</i>	Vero		-	-	-	-	+	+	+	+	+
	L20B		-	-	-	-	+	+	+	+	+
<i>Aframomum melegueta</i>	Vero		-	-	-	-	+	+	+	+	+
	L20B		-	-	-	-	+	+	+	+	+

Absence of cytotoxicity = -
Presence of cytotoxicity = +

Table 3. Antiviral activities.

Plant specie	Measles virus	Yellow fever virus	Poliovirus type 1
1. <i>Bambusa vulgaris</i>	1 - 14	0	0
2. <i>Aframomum melegueta</i>	1 - 7	1	0

Antiviral assays

The technique described is a modified form of procedures previously described in detail (Marles et al., 1992; Taylor et al., 1996). Vero cells (for measles and yellow fever virus) and L20B cell (for polio virus) were grown in Dulbecco's Modified Eagle Medium (MEM) containing 5% fetal bovine serum (all cell culture reagents were obtained from GIBCO Life Sciences, Ontario) in 96-well microtest trays (Falcon), 0.2 ml per well. When the cells formed confluent monolayers, they were used for the assays.

Each plant extract was diluted 1:100 in MEM plus 0.1% serum (Hudson et al., 1994) and filtered through a sterile syringe filter 0.2 μ pore diameter.

The filter equivalent to 1,000 $\mu\text{g/ml}$ dried plant material and 1% of the 70% ethanol was the starting test material.

In the standard procedure, serial 2-fold dilution of the extract were made (in duplicate) in MEM plus 0.1% serum across a row of wells in an empty 96-well microtest tray. With the aid of a multipipettor, these diluted extracts were transformed to the aspirated vero and L20B cell monolayers of another 96-well tray, 0.1 ml per well. The cultures were incubated at 37°C for 60 min and examined microscopically for possible immediate cytotoxic effects. Then 0.1ml of virus (measles, yellow fever and polio virus type 1), comprising 100 pfu in MEM + 0.1% serum was added to each well. Controls induced cells with no virus and cells infected with untreated virus. Cultures were inspected periodically in the microscope for viral CPE.

Cytotoxicity assays

The procedure was similar to the antiviral assay except that no virus was added to the wells and following light exposure, the trays were returned to the incubator for periodic microscopic assessment of changes in cell morphology or visible toxic effect.

RESULTS AND CONCLUSION

The outcome of the antiviral screenings of *A. melegueta* and *B. vulgaris* was impressive as the extracts possess activity against two of the viruses which were tested. Table 1 lists the plants used in this study with information on their traditional applications. They were used to treat infections. The results of the cytotoxicity assays are shown in Table 2. This protocol involving continuous exposure of the cells to extract for 5 days, permits detection of cytotoxic effects leading to cell death as well as more subtle effects on the cells that may not be deleterious e.g. alteration of cell shape to a more rounded morphology. The extracts produced such changes in cell morphology at concentration greater 250 $\mu\text{g/ml}$.

Table 3 summarizes the results of the antiviral assays involving three different animal viruses (all of which can infect humans). Activity was found in extracts of the two plants. Out of the three viruses, only measles virus was susceptible to the extract of *B. vulgaris*. The other two viruses were resistant to the extract. Extract of *A. melegueta* was however inhibitory on both measles and yellow fever viruses. Measles virus was inhibited at a minimum concentration of 125 $\mu\text{g/ml}$ and yellow fever virus was inhibited at a minimum concentration of 250 $\mu\text{g/ml}$. Polio virus was resistant to extracts of both plants. This is characteristics of this virus (Lipipun et al., 2003). Polio-virus is a non-envelop virus infecting through the gastrointestinal tract and causes poliomyelitis due to its ability

to evade the acidic nature of this tract. This is a kind of natural defense mechanism. It is therefore not surprising that extracts of the two plants had no effect on the poliovirus type 1. Although, extract of *B. vulgaris* was only potent on measles virus in-view of medicinal plant applications, extracts with broad-spectrum activities and minimal cytotoxic effects might be more important.

It is interesting to attempt to correlate the traditional applications of the plant extracts with the microbial targets. In the case of *B. vulgaris* this is feasible since the extract of this plant had activity only on measles virus. Antiviral activities observed with extract from *A. melegueta* had a broader activity since it inhibited the growth of both measles and yellow fever viruses. The antiviral activities observed with these plants are most probably due to the phytochemicals in the plants. Such phytochemicals, according to Ekwete (1992) include tannin, phenolic compounds, saponins, flavonoids and protocatectic acid among others. These phytochemicals are known to activate the lymphocytes of infected individual and prevent forming of resistance in viruses and also virus replication (Chiang, 2003b). It should therefore be recommended that application of extracts from these plants could help in the treatment of measles and yellow fever infections.

Antiviral activities were measured as complete or partial inhibition of viral CPE (cytopathic effects) at concentrations of 250µg/ml (1), 230 µg/ml (2), 210 µg/ml (3), 190 µg/ml (4), 170 µg/ml (5), 150 µg/ml (6), 125µg/ml (7), 100 µg/ml (8), 90 µg/ml (9), 80 µg/ml (10), 70 µg/ml (11), 65 µg/ml (12), 62.5µg/ml (13), 60 µg/ml (14), 0 = no detectable activity.

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