African Journal of Biotechnology Vol. 5 (19), pp. 1785-1788, 2 October, 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2005 Academic Journals

Full Length Research Paper

Virucidal potential of some edible Nigerian vegetables

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Accepted 22 August, 2006

Extracts from four leaves: *Ceratotheca sesamoides* (Endl.), *Lactuca taraxacifolia* (Willd.), *Euphorbia lateriflora* (Schum. & Throes) and *Amaranthus viridis* (Linn.) used in Nigeria as edible vegetables were tested for antiviral activities using the measles virus on Human Epidermoid carcinoma (HEP-2) cell line. The efficacy of each extract in preventing viral penetration into a living cell as well as its effect on the virus itself and the toxicity of same on the cell were all determined. All the extracts showed dose-dependent antiviral activities. The inhibitory action of the extracts from *C. sesamoides* and *L. taraxacifolia* were greatest at high concentrations of 15 mg/ml when the virus had already penetrated the cell. *E. lateriflora* only inhibited the virus activity at very high concentrations of 15 mg/ml when the virus was treated with the extract and also when the HEP-2 cell was treated with the extract before the virus suspension was added. *Amaranthus viridis* concentrations of 10 and 15 mg/ml were contaminated but the extract at a low concentration of 5 mg/ml was able to exhibit antiviral activity when the cell was treated with the extract before the virus was added. In view of the efficacy of these extracts in inhibiting the cytopathic effect (CPE) of measles virus without harming the host, the utilization of the extracts in the formulation of new antiviral drugs is strongly recommended especially when the availability and the very low cost of these vegetables are considered.

Key words: Nigerian vegetables, virucidal, potential.

INTRODUCTION

Lack of effective curative measures against some severe and deadly viral diseases such as measles, AIDS, hepatitis, rabies, polio and ebola is of serious concern to health practitioners the world over. Antiviral drugs are available to treat only a few viral diseases. The reason for this may be the fact that viral replication is so intimately associated with the host cell that any drug that interfered significantly with viral replication, is likely to be toxic to the host (Hardie, 1999). Present control of viral infections depends largely on the application of chemotherapeutic drugs and/or vaccines.

To a large extent, costs of some of these antiviral agents are beyond the reach of the masses that constitute a greater part of our population. Worse still, those currently available, like most other drugs, are bedeviled with either side effects and/or loss of efficacy due to the development of resistance to them by these microbial agents. These factors in concert with the twin factors of low literacy level and economic crises have forced many Nigerians today to patronize tradomedical preparations most of which are untested concoctions. Some, however, seem to possess some efficacy. It is these promising plant materials that now form part of herbal preparations called “supplements” or “nutriceuti-cals” which are fast gaining global recognition. The general notion in Nigeria (Sofowora, 1982) that for every illness known to man nature has provided an answer in form of one herb or a combination of herbs may therefore right.

A recent unpublished Harris Poll (www.naturaldatabase.com) conducted by the Dietry Supplement Education Alliance in July 2001 found that 12% of those over age 65 use herbal supplements and 9% use specialty supplements regularly. Also the World Health Organization estimates that 85% of the earths population uses some type of herbal remedy as their main form of medical treatment. In 1997, there were about 629 million visits to

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alternative medicine providers in U.S., almost 243 million more visits than visits to all U.S. primary care doctors that same year.

Worldwide, roughly one in four prescriptions is likely to contain plant-based ingredients and currently in Europe there is herbal renaissance. Medicinal plant consumption in Germany has risen by 20%. It is reported that the harvest of and trade in medicinal and aromatic plants is huge and threaten the population of some species (http://www.epocrates.com). Despite their unquantifiable importance, medicinal plants still face danger and are grossly understudied. In our research group, we recently identified four edible vegetable plants: *Lactuca taraxacifolia, Amaranthus viridis*, *Ceratotheca sesamoides* and *Euphorbia lateriflora* with antibacterial and antiviral activities *in vitro.* The aim of the present work was to study, in more detail, the antiviral activities of these edible vegetables found in our locality using the measles virus with a view to provoking interest in them considering their availability, their low cost and the familiarity of the rural folk with them.

L. taraxacifolia also taxonomically recognized as *Launaea taraxacifolia* (Willd.) Amin ex C. Jeffery is a plant species that is cultivated mainly in the Western part of Nigeria. It has a basal rosette of leaves with erect stems up to 1.3m high from a woody rhizome. The leaves are eaten fresh as a salad or cooked in soups and sauces. The leaves are fed to cows-in-milk in Northern Nigeria to increase the yield and to sheep and goats mixed with natron to produce multiple births. The leaves mixed with fine ash are rubbed into the sores of yaws (Burkill, 1985; Schippers, 2000).

A. viridis (Linn.) is an annual herb, small and stunted to rather tall and erect, up to 100 cm tall, stem simple or branched or glabrous. The leaves are arranged spirally, simple without stipules and the petiole 1-10 cm long. It belongs to family of plants called *Amaranthaceae*. The genus comprises of about 70 species including at least 17 species with edible leaves. The African or Asian cultivated types are generally much smaller than the Mediterranean types, more strongly branched and erect or prostrate. Like maize and sugar cane, the genus *Amaranthus* is characterized by C_4 cycle photosynthetic pathway. Generally, *Amaranthus* species are used as vegetables or as ingredients in sauces. Dishes with *Amaranthus* are eaten with the main dish of cereals or tubers (Grubben and Denton, 2004). The leaves of *A. viridis* contain tannin, reducing sugars and resin. The plant is also called green amaranth or African spinach. It is an erect or ascending annual or short-lived perennial herb up to 1 m tall with a slender stem, branched, angular, glabrous to sparsely pubescent in upper parts with multicellular hairs. The leaves are alternate and simple with petiole up to 10 cm long (Grubben and Denton, 2004; Schippers, 2000). *C. sesamoides* (Endl.) also known as *C. melanosperma* and false sesame is indigenous in Africa. The leaves and flowers are consumed as vegetable. The leaves are finely chopped and used in sauces. They are also pounded and mixed with ground flour, salt and a little hot water or milk with onions and tomatoes and then used as a sauce. The plant is an annual herb up 100-120 cm tall, sometimes with woody rootstock, with prostrate, ascending, or erect pubescent stems. The leaves are opposite or nearly opposite, simple, without stipules. The petiole is up to 6 cm long in lower leaves but very short in upper leaves. The leaves can also be steeped and the slimy liquid dropped into the eye to treat conjunctivitis. The mucilage is occasionally used as an emollient and lubricant (Grubben and Denton, 2004). *E. lateriflora* (Schum. and Thonn.) is a shrub reaching 1.7 m in height with near-vertical succulent branches. The latex is used in Northern Nigeria as a remedy for head-lice and also for ringworm in the scalp. It is also taken with milk or cereals or liver to provoke purging and sometimes vomiting (Burkill, 1994).

MATERIALS and METHODS

Sourcing and collection of specimens:

The herbal extracts used in this study were obtained from a local market and then identified and prepared in the Pharmacognosy Department of the College of Medicine, University of Lagos, Nigeria. The plants were dried to a constant weight at 65°C in an oven and then weighed, blended into tiny bits from where portions of 5, 10 and 15mg/ml were prepared by soaking the respective weights in the equivalent of 1 ml of physiological saline for 12 h before filtering with Millipore filter into khan tubes. The filtrates were then stored in the freezer at 35°C until needed for the various studies.

Preparation of cell culture suspension

The continuous cell line of human epidermoid carcinoma HEP-2 was used according to the method of Levy et al. (1997). A confluent monolayer was washed twice with prewarmed phosphate buffered saline (PBS) and then layered with 1-5 ml of prewarmed 0.25% trypsin for 5 min to dislodge the cells. As soon as there was evidence of cell rounding, the trypsin was tipped off and 1-2 ml of growth medium (Eagles minimum essential medium was added) to neutralize the action of trypsin. With a sterile 5 ml pipette, the cells were dislodged by pipetting the medium against the wall of the bottle. Viable cells were estimated using the 1:10 suspension of cells in neutral red in a haemocytometer. The cells were seeded into tubes using 100,000 cells/ml. They were allowed to form a monolayer before being used for viral assay.

Viral stocks were prepared as aliquots of culture medium from HEP-2 cells infected at multiplicity of one virion per 10 cells and cultured for 3 days according to the method of Levy et al. (1997). They were stored at – 80°C. Working stocks were prepared by serial dilution of viral stock in culture medium and assayed in triplicate on HEP-2 cells monolayer in the wells of microtitre tray. The working stock suspensions were then stored at 4°C until used.

Antiviral assay of plant extracts

For the antiviral investigation, each plant extract in graded doses of (5, 10 and 15 mg/ml) were mixed with equal volumes of 100 TCID50/0.1 ml of measles virus stock. The mixture was incubated for I h and agitated at intervals of 15 min. One hundred microtitres

Table 1. Antiviral activity of *Ceratotheca sesamoides* extract on measles virus.

Assay	Concentration (mg/ml) of *C. sesamoides* extracts		
	5 mg/ml	10 mg/ml	15 mg/ml
Virus + Extract + Cell	0	0	0
Virus + Cell + Extract	0	+	++
Cell + Extract + Virus	0	0	±

+ = Inhibition 0 = No inhibition ± = Faint inhibition

Table 2. Antiviral properties of *L. taraxacifolia* on measles virus.

Concentration (mg/ml) of *L. taraxacifolia* extracts			
Assay	**5 mg/ml**	**10 mg/ml**	**15 mg/ml**
Virus + Extract + Cell	0	0	±
Virus + Cell + Extract	0	0	++
Cell + Extract + Virus	0	0	±

+ = Inhibition 0 = No inhibition + = Faint inhibition

Table 3. Antiviral properties of *Euphorbia lateriflora* extract on measles virus.

Assay	Concentration (mg/ml) of *E. lateriflora* extracts		
	5 mg/ml	**10 mg/ml**	**15 mg/ml**
Virus + Extract + Cell	0	0	+
Virus + Cell + Extract	0	0	toxic
Cell + Extract + Virus	0	0	++

+ = Inhibition 0 = No inhibition ± = Faint inhibition

of extract-virus mixture were inoculated into HEP-2 cells in a 96-well tissue culture plate. For the control, in the same plate, the last row of wells was reserved for controls that were not treated with extract or not treated with virus. The culture was incubated with the un-inoculated controls at 37°C. The entire setup was examined daily for seven days.

The reactions in the three different experimental sets: virus and extract before cell line; virus and cell before extract and cell and extract before virus were stopped by adding 70% methanol into the wells using multichannel micropipette. The mixtures were allowed to stand on the bench for 10 min for complete inactivation of cells to take place after which they were discarded and crystal violet solution added. After about 3 min, they were rinsed with water, and examined visually. Antiviral activity was identified as confluent, relatively unaltered monolayer of stained HEP-2 cells that were previously treated with the virus. Cytotoxicity was identified as the loss of the cell monolayer as a result of the plaques formed by the measles virus (Levy et al., 1997).

RESULTS AND DISCUSSION

All the four plant extracts exhibited antiviral activity as indicated by earlier trial studies. As shown in Table 1, when the virus in the cell line was mixed with *C. sesamoides* extract, no antiviral activity was observed at all the concentrations. When the extract was treated with the virus and the cell, again no antiviral activity was observed at the concentration of 5 mg/ml. However, as the concentration increased to 10 mg/ml antiviral activity was observed reaching its peak at 15 mg/ml. When the cell was treated with the extract before adding the viral stock, there was no antiviral activity at both the 5 and 10 mg/ml concentrations, but at 15 mg/ml, the extract was observed to exert a mild antiviral activity. *C. sesamoides* is therefore proven to be efficacious against the measles virus and so should be further studied especially as it is generally used in ethnopharmacognosy in the management of another viral infection – conjunctivitis (Grubben and Denton, 2004) locally called “Apollo” in Nigeria.

In Table 2, there was no antiviral activity at 5 and 10 mg/ml when the virus was mixed with *L. taraxacifolia* extract before the cell was added, but at 15 mg/ml the extract was noticed to mildly exert antiviral activity. When the extract was treated with the virus and the cell, a marked antiviral activity was exhibited at 15 mg/ml while there was no activity at low concentrations of 5 mg/ml and 10 mg/ml. Similarly, there was no antiviral activity at 5 and 10 mg/ml, but there was a mild antiviral activity at 15 mg/ml when the cell was treated with the extract before the virus stock was added.

In Table 3, *E. laterifolia* showed no antiviral activity when the virus was mixed with the extract at 5 and 10 mg/ml but at 15 mg/ml, the extract was observed to exhibit an inhibitory effect on the virus. When the virus was mixed with the cell and later treated with the extract, there was no inhibition at 5 and 10 mg/ml, but at 15mg/ml

Table 4. Antiviral properties of *Amaranthus viridis* extract on measles virus.

Assay	Concentration (mg/ml) of *A. viridis* extracts		
	5mg/ml	10mg/ml	15mg/ml
Virus + Extract + Cell	0	0	contaminated
Virus + Cell + Extract	0	contaminated	contaminated
Cell + Extract + Virus	+	contaminated	contaminated

+ = Inhibition 0 = No inhibition ± = Faint inhibition

the extract was observed to be toxic to the cell. Also at the concentration of 15 mg/ml there was a marked inhibitory activity when the cell was mixed with extract and then the virus but the extract could not block the activity of the virus at low concentration of 5 and 10 mg/ml. The observed cytopathic effect of *E. laterifolia* should be noted in spite of its desirable antiviral efficacy. It has been earlier reported to be effective against ring worm and head lice (Burkill, 1994). The same principle may be at work in this place.

In Table 4, contamination of the *A. viridis* extract occurred and prevented observation of antiviral activity at concentration of 10 and 15 mg/ml. however antiviral activity was observed at 5 mg/ml when cell was treated with extract before addition of the virus stock. This is a strong indication of desirable antiviral activity especially for a plant that is commonplace and is used widely in soups and as sauce for yam and cocoyam as well as cereals. It may also be useful in the management of some common viral infections such as hepatitis B which we (Obi et al., 2006) have found to be prevalent even among pregnant women attending an antenatal clinic in Nigeria.

The plants investigated in this study are all commonly used in Nigeria as edible vegetables. In a part of the study, the virus was treated with the extract before the cell line was introduced. This was to test if the extract on its own could block viral receptocytes. When a viral receptocyte is blocked, the virus is neutralized and so infection is prevented. All the extracts did this at 15 mg/ml confirming their possible utility as potent antiviral agents. In another instance, the virus was allowed to infect a living cell and this mixture was treated with the extract to test if the extract could dislodge the virus after attachment to and penetration into a cell. From the results, the activity of the extract was greatest when the virus was within the cell. This confirms the potency of these extracts and so makes them possible drugs for the treatment and management of already established viral infections. Finally, the cell was treated with the extract before the virus was added in order to test if the extract could block viral receptors on the cell. The results also revealed the ability of the extracts (especially *E. lateriflora* and *A. viridis* extracts) to block some viral receptors. On the whole, these results indicate that constant consumption of these vegetables could, to a large extent, prevent infection, or even if infection has occurred, could prevent further replication of the virus.

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