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Full Length Research Paper

Antiviral effect of *Hibiscus sabdariffa* and *Celosia argentea* on measles virus

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Studies on extracts and biologically active compounds isolated from medicinal plants have doubled in the last two decades. The leaves of *Hibiscus sabdariffa* (red and green leaved) and *Celosia argentea* were studied for their antiviral activities against Measles Virus (MV) as well as the effects of the extracts on Hep-2 cells. Ethanol extract of the leaves of each of these plants showed no toxicity to the Hep-2 cells at all concentrations used (5, 10 and 15 mg/ml). The pre-inoculative treatment of Hep-2 cells with plant extracts showed that *C. argentea* had no antiviral activities on MV at all concentrations (5, 10, 15 mg/ml) while *H. sabdariffa* had antiviral activities only at 10 and 15 mg/ml on MV. The post-inoculative treatment of Hep-2 cells with plant extracts showed that at 5, 10 and 15 mg/ml concentrations, *H. sabdariffa* had antiviral activities on MV while the antiviral activity of *C. argentea* could not be established at 10 and 15 mg/ml but did not show any antiviral activity at 5 mg/ml.

Key words: *Hibiscus sabdariffa*, *Celosia argentea*, antiviral, toxic, Hep-2 cell, herb, medicinal plant.

INTRODUCTION

Many natural plants have been sourced and used as valuable medicinal agents for many years with proven potentials of treating infectious diseases and with lesser side effects compared to synthetic agents (Beuscher et al., 1994; Ekpa, 1996; Gbile and Adesina, 1986). Medicine constitutes one of the many resources of the forest on which the health of the average African population depended since the time of creation. Herbs have usually served as the repository of healing materials and have been acknowledged to generally save without or with minimum side effects (Gbile and Adesina, 1986). Herbs, apart from healing, provide the necessary nutrients for health and development of the human body. Many vegetable crops particularly the leafy vegetables are mainly consumed for their nutritional values without much consideration for their medicinal importance. There are several varieties of these leafy vegetables either in the wild or under cultivation in the rural areas.

The potential of forest flora as a veritable source for pharmaceuticals and other therapeutic materials have been emphasized (Gbile and Adesina, 1986). Moreover, the last decade has witnessed intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine (Collins et al., 1997; Nascimento et al., 2000; Rios and Racio, 2005).

However, much problem is associated with synthetic drugs. These problems include high cost, increasing adulteration and side effects, coupled with their inadequacies in disease treatment as found most especially in developing Countries of the World (Sheriff, 2001).

Two of the major plants that have been used for medicinal purposes in Africa include *Hibiscus sabdariffa* and *Celosia argentea* (The Guardian, 2009). *H. sabdariffa* belongs to the family Malvaceae. It is commonly known as 'Sobarodo' or 'Yakuwa' among the Hausa people and 'Isapa' or 'Isakun' among the Yoruba people in Nigeria. *C. argentea* belongs to the family

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Amaranthaceae. It is commonly known as 'Sokoyokoto' or 'Ajefawo' amongst the Yoruba It is an edible vegetable, very rich in vitamin (Altshull, 1973).

Therefore, this work was aimed to study the antiviral potentials of *H. sabdariffa* and *C. argentea* on Measles virus (MV).

MATERIALS AND METHOD

Collection of plant materials

The leaves of *H. sabdariffa* (Red and green leaves) and C. *argentea* were purchased from the open market in Lagos and authenticated in both the Pharmacology and Botany Department of the University of Lagos in 1999.

Preparation of extract

The leaves of each plant were oven-dried at 40 - 60°C. The dried leaves were powdered using Laboratory milling machine. Each powdered plant material (70 g) was extracted using 500 ml of 99% analar grade ethanol for 72 h in a Soxhlet extractor using the modified Alade and Irobi (1993) method. Each extract was heated to remove residue of the solvent. Respective weights of 5, 10 and 15 mg of each extract was transferred into twelve different vials. To the content of each vial was added 1 ml of distilled water and allowed to stand for a few hours at room temperature with shaken at regular intervals. The contents were later filtered using Uniflo 0.2 µm filter units into labeled screw cap tubes. The filterate of each extract was diluted 10 folds and stored at refrigeration temperature of 4°C for later use.

Tissue culture material

A growth medium supplemented with 10% fetal calf serum (FCS) was prepared. A continuous cell line of Human Larynx epidermoid carcinoma cells (Hep-2) were used for virus stock, back titration and antiviral assay. A confluent monolayer of Hep-2 cell culture was treated before use by washing twice with pre-warmed phosphate buffered saline which was then layered with 1.5 ml of pre-warmed 0.25% trypsin for 5 min to dislodge the cells. About 2 ml of growth medium was added to neutralize the action of the trypsin on the cell with a sterile disposable pipette. The cells were further disintegrated by pipetting the medium down the wall of the flask.

Cytotoxicity assay

The highest non-toxic concentration of each of the extracts was determined by inoculating different volumes (5, 10 and 15 mg/ml) of each extract into Hep-2 cells. Inoculated cells were incubated at 37°C in CO_2 incubator and monitored each day using inverted microscope for any changes in morphology compared with the control cells containing no extract.

Titration of stock virus

Serial tenfold dilutions of MV were made from 10^{-1} to 10^{-8} for each virus. To the Hep-2 cells in a microtitre tissue culture plate were added 10 µl each of the different dilutions of the MV serotype. The plate was incubated at 37°C. Infected cells were monitored daily and observations were terminated when the virus titre was determined. The titre was regarded as the dilution of the virus that caused cytopathic effect (CPE) in 50% of inoculated wells.

Antiviral screening

To MV was added different concentrations (5, 10 and 15 mg/ml) of the extract of each plant, and later incubated at 37°C for 1 h in CO_2 incubator. Into each well of a 96-well microtitre tissue culture plate was added in duplicate 200 µl of the virus-extract mixture and 100 µl Hep-2 cells. The cells were incubated for 5 days in CO_2 incubator and monitored with the aid of an inverted microscope for CPE. Control wells had no Hep-2 cells and extracts.

Pre-innoculative treatment of Hep-2 cells with plant extract

Hep-2 cells were added to each of the 96-wells of a microtitre plate. Hundred microlitres (100 µl) of different concentrations (5, 10 and 15 mg/ml) of each extract was added to it and incubated at 37°C for 30 min. The extracts were removed after incubation to prevent any interaction with MV when it was added. Measles virus at 10^{-5} dilution was added to the wells together with growth medium supplemented with 10% FCS. This was incubated for 7 days for CPE.

Post innoculative treatment of Hep-2 cells with plant extract

Hep-2 cells were dispensed into each well of a 96-well microtitre tissue culture plate, and to this was added 10 fold serial dilution of viral stock. The plate was incubated in a CO_2 incubator for 30 min after which 100 µl of different concentrations (5, 10 and 15 mg/ml) of the extract of each plant was added to it. This was incubated for 7 days for CPE.

Staining

The contents of the plates were carefully decanted. Into each well was added 70% alcohol and left for 10 min, after which the content was decanted. Into each of the wells was added crystal violet-formaldehyde solution, and left to stand for 3 min. The plates were then rinsed with distilled water and left to dry.

RESULT

This study showed that neither *H. sabdariffa* (red and green leaved) nor *C. argentea* extract had toxicity effect on Hep-2 cells at all concentrations (5, 10 and 15 mg/ml) used.

The pre-inoculative treatment of Hep-2 cells with plant extracts showed that *C. argentea* had no antiviral activities on MV at all concentrations (5, 10, 15 mg/ml) while *H. sabdariffa* had antiviral activities at 10 and 15 mg/ml on MV (Table 1).

The post-inoculative treatment of Hep-2 cells with the plant extracts showed that *H. sabdariffa* had antiviral activities on MV at all concentrations (5, 10 and 15 mg/ml) used. The antiviral activity of *C. argentea* could not be determined at 10 and 15 mg/ml but it had no antiviral activity on MV at 5 mg/ml (Table 2).

Table 1. The effect of pre-inoculative treatment of Hep-2 cells with plant extracts on MV.

Plant species	Extracts (mg/ml)	MV dilutions	Morphology of Hep-2 cell after incubation
C. argentea	5	10^{-5}	R
	10	10^{-5}	R
	15	10^{-5}	R
H. sabdariffa (green leaves)	5	10^{-5}	R
	10	10^{-5}	N
	15	10^{-5}	N
H. sabdariffa (red leaved)	5	10^{-5}	R
	10	10^{-5}	N
	15	10^{-5}	N

N: Cells were normal in shape (that is, extract had inhibitory effect on virus).
R: Cells were rounded (that is, extracts had no inhibitory effect on virus).

Table 2. The effect of post-inoculative treatment of Hep-2 cells with plant extracts on MV.

Plant species	Extracts (mg/ml)	MV Dilutions	Morphology of Hep-2 cell after incubation
C. argentea	5	10^{-5}	R
	10	10^{-5}	C
	15	10^{-5}	C
H. sabdariffa (green leaved)	5	10^{-5}	R
	10	10^{-5}	N
	15	10^{-5}	N
H. sabdariffa (red leaved)	5	10^{-5}	N
	10	10^{-5}	N
	15	10^{-5}	N

N: Cells were normal in shape (that is, extracts had inhibitory effect on viruses).
R: Cells were rounded (that is, extract had no inhibitory effect on virus).
C: Cell Contaminated with bacteria.

DISCUSSION

The antiviral activities exhibited by the ethanolic extracts of *H. sabdariffa* on MV at all concentrations used confirmed earlier work by Hoof et al. (1989). The inhibitory activities of *H. sabdariffa* may be due to the presence of gossypetin, anthocyanin and glucoside hibicin contained in the plant (Duke, 1987). *H. sabdariffa* also displayed antiviral activities when used before the adsorption of virus to the cell. This could be due to the fact that the herb was able to bind to certain receptors on the cell, thereby preventing the virus from adsorbing to the cell (Oxford and Thomas, 1990).

C. argentea exhibited the least antiviral effect on MV. This could be due to the structural components that resisted the effects of the extracts on MV (Yip et al., 1995). Contrary to the study by Singh et al. (1998), *C. argentea* had no antiviral activity when introduced into the cell prior to the introduction of the virus. The cytotoxicity assay showed that none of the extracts was toxic to Hep-2 cells at all the concentrations used revealing great prospect in the use of the herbs for the treatment of viral diseases.

The result of this research has suggested the antiviral activity of *H. sabdariffa* plant extracts. As at the moment, very little work has been done on the antiviral study of the test plant extracts. In vivo screening of the extracts would be done in order to confirm this in vitro finding. Once this is confirmed, these extracts could be recommended for both prophylactic and therapeutic control of MV. Furthermore, study to test the antiviral impact of these extracts on Yellow Fever Virus, Hepatitis viruses and HIV

would be carried out.

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