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PHYTOCHEMICAL AND ANTICANCER STUDIES ON TEN MEDICINAL PLANTS USED IN NIGERIA

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ABSTRACT
Ten medicinal plants used in Nigerian ethno medicine were subjected to phytochemical and anticancer studies using established standard procedures.
The result of the study revealed the presence of phytochemical constituents such as alkaloids, saponins, tannins and flavonoids. Anona muricata, Andrographis paniculata and Garcinia kola were active against the lung cancer cell lines at different concentrations. The study justifies the use of the plant in traditional medicine as remedy against cancer.

Keywords: cytotoxic, anticancer, plant extracts

INTRODUCTION

Traditional medicine in many areas of the world relies on the use of a wide variety of plant species. In Africa, phytotherapy still plays an important role in the management of diseases, mainly among population with very low income (Geoffrey and Kirby 1996).

Cancer is a dreadful disease caused by abnormal and uncontrolled cell division. About 6 million new incidences of cancer are reported yearly worldwide. Nature has given man a variety of useful sources of remedies to cure a number of diseases. Natural products have played a significant role in drug discovery and development, especially agents active against cancer and infectious diseases (Butler, 2008). More than 70 per cent of all cancer deaths occurred in low- and middle-income countries. The WHO noted that tobacco use, alcohol use, low fruit and vegetable intake, and chronic infections from hepatitis B virus (HBV), hepatitis C virus (HCV) and some types of human papillomavirus (HPV) are leading risk factors for cancer in low- and middle-income countries. Deaths from cancer worldwide are projected to continue rising with an estimated 12 million deaths by 2030 (Wang et al, 2007). The most frequent types of cancer worldwide in order of the number of global deaths are; among men – lung, stomach, liver, colorectal, oesophagus and prostate; and among women – breast, lung, stomach, colorectal and cervical. Ultrasound, the term used to describe sounds ranging from 20 kHz to 1 GHz, is usually generated by a transducer which converts mechanical or electrical energy into high frequency vibrations. Vinatoru (2001) and Romdhane and Gourdon (2002) reported that the enhancement of extraction efficiency of organic compounds using ultrasound is attributed to a phenomenon called 'cavitation' which is produced in the solvent by the passage of an ultrasonic wave. They found that cavitation bubbles are produced and compressed during the application of ultrasound, allowing higher penetration of the solvent into the raw plant materials and causing intracellular products to be released by disrupting the cell walls. Albu *et al* (2004) and Rostagno *et al* (2003) reported that ultrasound has been shown to aid extraction in a number of plant materials by significantly reducing extraction time and increasing extraction yield, respectively. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Goldfrank, 1982). This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient and expensive, coupled with the adverse side effects of synthetic drugs. However, there is little or no literature on the anticancer properties of the selected medicinal plants used in ethnomedicine in Nigeria. Hence, the need to scientifically validate the claimed biological activity of the plants against lung cancer cell line.

MATERIALS AND METHODS

Plant Material

The fresh plant samples were collected from different locations in Nigeria, in March 2009, identified by Mr. Simon Peters and authenticated by Prof. M. Idu of the Department of Botany, Faculty of Life Science, University of Benin, Benin City, Nigeria. Voucher specimens of samples were deposited in the same department.

Phytochemical screening

The crude powdered plant samples were qualitatively tested for the presence of secondary metabolites using standard established methods (Trease and Evans, 1989; Harbone, 1973; Sofowora, 1993).

Test for reducing sugars (Fehling's test)
The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution
(A and B) in a test tube. The solution was observed for a colour reaction.

Test for flavonoids
Three methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with ml of dilute ammonia solution. A yellow colouration indicates the presence of flavonoids.

Test for saponins
To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigourously after which it was observed for the formation of an emulsion.

Test for tannins
About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few
drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black
colouration.

Test for alkaloids
0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test)
To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing
one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Ultrasonic extraction
The powdered sample (10g each) was mixed with 100ml of methanol and extracted using ultrasonic cleaner (SB-5200DTD, 40 kHz; Xinzhi Biotech Co., Ningbo, China) according to the method of Yang *et al* (2006). The extraction was held for 30 min at 30^{o}C and then filtered through a Whatman no. 1 filter paper. The filtrate was concentrated with a rotary evaporator at 25^{o}C under vacuum. The dry extracts were stored at 4^{o}C pending further analysis.

Extraction yield
The extract was analyzed by the method of Zhang *et al* (2007) as follows. The filtrate obtained was concentrated to dryness by a rotary evaporator at reduced pressure and temperature (40^{o}C).

BIOLOGICAL SCREENING
Cell line and culture
A-549 (human lung adenocarcinoma epithelial cell line) cell culture was obtained from Biomedicine Research and Development Centre of Jinan University (Guangzhou, China). The cell lines were cultured in growth medium (RPMI-1640 medium, pH 7.4), supplemented with 10 % fetal bovine serum (FBS) and antibiotics [penicillin (100 units/ml) and streptomycin sulfate (100 µg/ml)]. The cell lines were grown in 50 cm^2 tissue culture flasks (Corning, NY, USA) and used for cytotoxicity assay.

Cytotoxicity assay
The cytotoxicity assay of the samples was determined according to the method of Zhao et al (2007) by MTT (3-(4,5-dimethyl thiazole-2yl)-2,5-diphenyl tetrazolium bromide) assay. In brief, human cancer cells were plated at 2×10^4 cells per well in 96 well microtiter plates (Costar 3599, Corning, NY, USA) with 100 µl RPMI-1640 growth medium and incubated for 24 h at 37°C, with 5 % CO_2 in a humidified atmosphere (Incu-Safe, Sanyo, Japan), during which period a partial monolayer was formed. Later, the medium was removed and fresh growth medium containing different concentrations (100, 50, 25, 12.5, 6.5, and 3.125 µg/ml) of the tested compound was added. After 2 days of incubation at 37°C, with 5 % CO_2, the growth medium was removed and MTT reagent (0.1 mg/ml) was added. After incubating at 37 ºC for 4 h, the MTT reagent was removed and dimethylsuphoxide, DMSO (100 µl) was added to each well and then shaken for another 15 min. The absorbance was then determined by an ELISA reader (Bio-Rad, USA) at a wavelength of 492 nm. Control wells received only the media without the tested compound. The conventional anticancer drug, cisplatin, was used as a positive control in this study. The inhibition of cellular growth by the tested sample was calculated as the percent inhibitory activity and expressed as the IC_{50} value (concentration of the tested sample to inhibit 50 % growth of the cells).

Statistical analysis
Data were expressed as means ± standard deviations (SD) of three replicate determinations and then analyzed by SPSS V.13 (SPSS Inc., Chicago, USA). One way analysis of variance (ANOVA) and the Duncan's New Multiple-range test were used to determine the differences among these means. *P* values < 0.05 were regarded to be significant.

RESULTS AND DISCUSSION

The results of the phytochemical screening are presented in Table 1. The presence of secondary metabolites such as alkaloids, tannins, flavonoids and saponins were observed in the various plant preparations. The presence of these secondary metabolites could be responsible for the biological activity of the plants. Some of these chemical constituents are present in trace amounts while others are secreted in large quantities as found in some flavonoids (responsible for the pigmentation of the plant). Plants have been linked to reduce the risks of major chronic diseases and cancers. Phytochemicals in common fruits and vegetables can have complementary and overlapping mechanisms of action, including of gene expression in cell proliferation, cell differentiation, oncogenes and tumour suppressor genes; induction of cell-cycle arrest and apoptosis; modulation of enzyme activities in detoxification, oxidation and reduction; stimulation of the immune.

Numerous plants have investigated and shown to have cytotoxic activity in cancer cell lines. They include those of *Solanum lyratum* tested on human colon adenocarcinoma cell line (colo 205)13, *Annona glabra* on human leukemia cell lines-CEM/VLB (Cochrane *et al,* 2008*).* , *Gynostemma pentaphyllum* on human lung cancer cell line -A549 (Lu *et al*., 2008) and *Blumea balsamifera* on rat and human hepatocellular carcinoma cells (McA-RH7777 and HepG2) (Norikura *et al.,* 2008*).*The results of the anticancer screening of the plant preparations are shown in Table 2. As can be seen from Table 2 cell type cyto-toxic is observed in some plant extracts. Among the ten extracts tested, *Anona muricata* extract had greater anticancer potential, followed by *Andrographis paniculata* extract while the other extracts showed less activity. The result of the experiment revealed significant anticancer property of *Anona muricata* (AM) and *Andrographis paniculata* (AP) at concentrations 6.25, 12.5 25, 50, 100 and 200 µg/ml. *Anona muricata* was active against the lung cancel lines at IC_{50} value of 7.29 while that of *A.paniculata* was 10.60. This shows that the extract of *A. muricata* and *A. paniculata* were active against the lung cancer cell line. AM and AP contained copious amount of phytoestrogens such as flavonoids which could be responsible for the anticancer activity. Some groups of flavonoids are known for their anti-inflammatory and anti-allergic, anti-thrombotic, vasoprotective and antitumour activities (Anne *et al*., 2007). The extract of *G. kola* demonstrated a marked inhibition of the cancer cell line with IC_{50} value of 59. 76. *G. kola* is also known to be rich in flavonoids and other secondary metabolites which probably are responsible for the anticancer activity.

To the best of our knowledge, there is no previous reported work on the antitumor activity of the plant extracts except that of *Anona muricata* and *Andrographis paniculata*.

Table 1: Phytochemical screening of the crude plant extracts

	Alkaloids	**Saponins**	**Tannins**	**Flavonoids**
Bush mango	-	+	+	++
J. gossipyfolia	-	+	-	_
K. grandifoliola	-	+	-	++
N. latifolia	+	+	-	+
P. staudtii	+	+	+	+
A. precatorious(BS)	++	++	+	+
A. paniculata	_	-	-	+
G.kola	+	+	-	++
A. muricata	+	+	+	+
Orange peels	-	++	++	++

+, presence of component, -; absence of component; ++, copious amount of component

Table 2: Growth Inhibition Assay (Human lung cancer cell line A549)

Sample Code	**Concentration (µg/mL)**						**IC_{50} (µg/mL)**
	6.25	12.5	25	50	100	200	
1 (BM)	2.46	6.01	7.43	10.91	26.10	34.43	424.09
2 (JG)	5.80	6.58	5.38	4.28	6.72	11.89	1.15E+09
3 (KG)	4.63	-13.84	-16.22	-4.75	13.10	23.13	---
4 (NL)	22.23	23.38	24.19	29.30	35.29	39.06	1416.23
5 (PS)	4.41	-9.94	5.79	9.04	0.87	19.35	---
6 (BS)	12.93	26.46	34.07	35.93	47.14	59.63	108.35
7 (AP)	38.49	56.33	56.72	89.88	92.75	91.60	10. 60
8 (GK)	-3.04	-0.66	23.24	92.00	92.40	90.23	59.76
9 (AM)	21.88	60.52	93.85	93.34	92.75	90.37	7.29
10 (OP)	0.43	1.73	4.60	15.00	20.45	60.89	171.89

CONCLUSION

The study has shown the anticancer activity of some of the plant samples and thus showed a strong validation for the use of the extracts of the plant for the treatment of cancer. Further studies will involve the isolation and identification of the active secondary metabolites and elucidation of possible mechanizm of anticancer activity.

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