



## GROWTH INHIBITORY EVALUATIONS OF FOUR NIGERIAN MEDICINAL PLANTS AGAINST CANCER CELLS, WITH ACTIVE CYTOTOXIC FRACTIONS FROM THE LEAVES OF *PARQUETINA NIGRESCENS*

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### ABSTRACT

Based on ethnomedicinal applications in treating tumour related ailments, effects of the methanol extracts of *Brachystegia eurycoma* (leaf and stem bark), *Parquetina nigrescens* and *Struchium sparganophora* (leaves) were evaluated on lung cancer cells at concentrations of 1-250 µg/ml. Hexane, dichloromethane and aqueous fractions of *P. nigrescens* were tested on the cells at concentrations of 1-100 µg/ml. While the extracts of *B. eurycoma* (leaf) and *S. sparganophora* did not have effect on the cells at the highest concentration tested, the other plant produced remarkably inhibited growth of the cancer cells at concentration of 100 µg/ml. Fractionation of the methanol extract of *P. nigrescens* produced an enhanced activity over the crude extract as the dichloromethane fraction exhibited cytotoxic effects at concentration of 50 µg/ml with GI<sub>50</sub> of 14 ± 4.0, TGI of 30 ± 2.0 and LC<sub>50</sub> of 45 ± 1.0 µg/ml. The overall results showed that the methanol extracts of *B. eurycoma* (stem bark) and *Parquetina nigrescens* can be used to prevent the proliferation of lung cancer cells and hence justify the ethnomedicinal uses of the plants in treating tumour related ailments.

**Keywords:** *Brachystegia eurycoma*, *Parquetina nigrescens*, *Struchium sparganophora*, cytotoxicity, growth inhibitory.

### INTRODUCTION

Medicinal plants are used in the treatment of many ailments and diseased conditions. These applications are not limited to particular group of ailments but cut across physical or dermatological infections to enteric or chronic ailments including life threatening ones like cancer.

Cancer is regarded as the most dreaded non communicable disease in developing countries (Kolawole, 2011). When diagnosed, its treatment involves radiotherapy, chemotherapy and even surgery and the costs of all these drastically deplete or totally consume the income and savings of many patients and their relations. As the treatments are also accompanied by unbearable side effects, it is appropriate to beam the searchlight of research into medicinal plants used in folkloric medicine in the treatment of tumour ailments. Information obtained from traditional medical practitioners in the South Western part of Nigeria revealed that drinking decoctions of *Parquetina nigrescens* (Periplocaceae) (Soladoye *et al.*, 2010) leaf, *Struchium sparganophora* Ktze (Asteraceae) leaf or the decoction of the stem bark or leaf of

*Brachystegia eurycoma* Harms (Fabaceae) could significantly arrest the progression of tumour proliferation in patients. *S. sparganophora* is a vegetable that is cultivated in water logged areas in the South Western part of Nigeria (Oboh, 2006) where it is also used for its medicinal properties as a cure for cutaneous and sub cutaneous parasitic infections, diarrhea and dysentery (Burkill, 1985). Its use as a oxytotic crude drug has been reported (Ayinde *et al.*, 2012). *Parquetina nigrescens* is a perennial twinner usually found in the forest and often planted around houses by traditional herbal practitioners in the South Western part of Nigeria probably for its numerous medicinal applications. Gill (1992) noted that the plant is used in the treatment of diarrhea, skin infections and gonorrhoea. It is also used as a cardiac tonic. Commonly called *Ako* among the Yoruba in the South Western part of Nigeria, *Brachystegia eurycoma* is a tree of about 35 m tall with a bole of about 2m diameter. It is found in river banks of the forest zone in southern Nigeria and Cameroon (Burkill, 1985). The leaf, bark and root of the plant are used in ethno medicines in combination with other plant part in the treatment of various diseases including malaria, diabetes, rheumatism, hypertension, and in bone setting (Adikwu *et al.*, 2007). In the eastern part of Nigeria, its seeds are used as thickener in

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preparation of local soups particularly egusi and ogbono soup (Keay *et al.*, 1964), while its wound healing and inflammatory properties have been mentioned in literature (Uhegbu, 2009), the ethanol and aqueous extracts of the bark have been reported to have antimicrobial activities particularly against fungi (Kunle, 2000). This work aimed at verifying the ethnomedical claim of the use of these plant extracts and fractions (where applicable) in treating tumour related ailments using human lung cancer cell line.

## MATERIALS AND METHODS

### *Collection and preparation of plant materials*

The medicinally useful parts of *B. eurycoma* (leaves and stem bark), *P. nigrescens* (leaves) and *S. sparganophora* (leaves) were collected in South West part of Nigeria. The identity of each plant was authenticated by Dr. Olufemi Shasanya, Plant Taxonomist, Forest Research Institute of Nigeria (F.R.I.N.). Herbarium specimen of each plant was deposited at the Institute with number FHI 108436, 109515, and 108438 respectively. Each of the plant materials was air dried accordingly for 5 days followed by drying in the oven at 40°C for the leaves and 50°C for the stem bark after which they were separately reduced to powder form using an electric milling machine (Christy Norris, England). The powdered samples were kept in air tight containers until needed.

### *Extraction of the plant materials*

About 1.2 kg of each powdered material was extracted with 7.5 l methanol using continuous hot extraction with Soxhlet apparatus till the extracting solvent became clear. The extracts obtained were separately concentrated under pressure using rotary evaporator maintained at 40°C.

### **Evaluation of the cytotoxic activities of the extracts against Lung cancer cells.**

#### *Reagents, Chemicals and Equipment*

These include Luminol (Research organics), Hank's Balanced Salt Solution (HBSS; Sigma Aldrich, Germany); Zymosan A (*Saccharomyces cerevisiae* origin) (Fluka Biochemika); Dimethylsulphoxide (DMSO) (Merck Chemicals, Darmstadt, Germany); Luminoskan RS Microplate reader.

Safety cabinet Class II, CO<sub>2</sub> incubator, inverted microscope, and 96-well plate reader were used to perform the anti cancer assay (lung cancer cell line NCI-H460).

Anticancer activities of the medicinal plant extracts and fractions on human lung cancer cell line NCI-H460

Anti-cancer sulphorhodamine -B assay (SRB assay)

For the preparations of the stock solution, plant extracts and fractions (40 mg/ml) were prepared in DMSO (100%) while doxorubicin (20 mM) as prepared in sterile water (D/W). The stock solutions were diluted with plain RPMI medium to get the desired concentrations with final concentration of DMSO not exceeding 0.5% (v/v).

The growth inhibitory effect of the methanol extracts of *P. nigrescens* (leaves), *B. eurycoma* (leaves and stem bark), *S. sparganophora* (leaves) were examined against human lung cancer cell line NCI-H460 (large cell lung cancer) using sulphorhodamine -B assay. Lung cancer cells were plated in 96-well (7500 cells/well) microplate and incubated at 37°C for 24 h in a humidified 5% CO<sub>2</sub> incubator. The plant extracts (1-250 µg/ml), fractions (1-100 µg/ml) and standard drug (0.1-10 µM) were added into appropriate well and incubated at 37°C for 48 h in a humidified 5% CO<sub>2</sub> incubator.

In order to fix the reaction, 50 µl of 50% cold trichloroacetic acid (TCA) was added and left for 30 min. at room temperature. This was followed by washing with distilled water and dried overnight. To stain the fixed protein, 100 µl of sulphorhodamine-B (SRB solution) (0.4% wt/vol. in 1% acetic acid) was added. After 30 min, the unbound SRB was removed by washing with 1 % acetic acid, and air dried at room temperature. The protein bound stain was solubilized with 10mM Tris base (pH 10.2) and plates were shaken for 5 min. using a plate shaker. Absorbance was measured at 515 nm using a microplate reader. The absorbance of the appropriate blanks, including sample blanks, and control (without sample), was used to calculate the percentage net growth, and the cytotoxicity of the extracts and fractions. The extracts and fractions which showed higher percentage net growth inhibition at the screening stage were further studied for concentration response. Various dilutions of the extracts (1, 10, 50, 100 and 250 µg/ml) and fractions (1, 5, 10, 50, 100 µg/ml) were used. Doxorubicin, an anticancer drug was used as the standard. The growth inhibition and the cytotoxicity of the extracts, fractions and the isolated compounds are presented as GI<sub>50</sub>, TGI and LC<sub>50</sub> (µg/ml) values.

### **Fractionation of methanol extract of *P. nigrescens*.**

55g of the methanol extract was dissolved in aqueous methanol (1:1) and partitioned with hexane, dichloromethane (DCM) 250 ml x 2 each in succession. These and the residual aqueous fraction obtained were separately concentrated and subjected to growth inhibitory or cytotoxicity evaluations on lung cancer cells.

## RESULTS AND DISCUSSION

The methanol extracts of two of the four medicinal plants evaluated for anticancer activities against the lung cancer cells were observed to produce growth inhibitory effects

against the cells to varying degrees. Extracts of *P. nigrescens* and the stem bark of *B. eurycoma* increasingly inhibited the growth of the cells with increase in concentration up to 250 µg/ml whereas the extracts of the leaves of *B. eurycoma* and *S. sparganophora* did not show any significant activities even at the highest concentration tested. At a concentration of 100 µg/ml, the extract of *B. eurycoma* (stem bark) had  $+94 \pm 3.5$  % growth inhibition whereas the crude extract of *P. nigrescens* attained  $+85 \pm 5.5$  % inhibition at the concentration 250 µg/ml. While the *B. eurycoma* produced a  $GI_{50}$  of  $34 \pm 1$  µg/ml, that of *P. nigrescens* was found to be  $154 \pm 6$  µg/ml. TGI and  $LC_{50}$  for both extracts were noted to be greater than 250 µg/ml (Table 1). Out of the three fractions obtained from the partitioning of the methanol extract of *P. nigrescens*, only the DCM fraction showed highly significant activities against the cancer cells. The fraction produced growth inhibitory effects up to the concentration of 10 µg/ml, whereas the activities became cytotoxic as from 50 µg/ml which produced  $-63$

$\pm 8.0$  % . The fraction induced  $GI_{50}$ , TGI,  $LC_{50}$  of  $14 \pm 4$ ,  $30 \pm 2$  and  $45 \pm 1$  µg/ml, respectively (Table 2).

Lung cancer has been reported to be one of the most prevalent form of cancers found particularly in men (WHO, 2008). The infection has been found to be the leading cause of death from cancer in many western countries and the mortality is more than the death rate from combinations of breast, prostate, and colon cancers (Mannino 1998, Schiller, 2002). The causes of the ailment are associated with modifiable factors like smoking, occupational and indoor exposure to polluted air in addition to non modifiable factors like genetic susceptibility and age (Fajerstetzn *et al.*, 2013; Jonsson *et al.*, 2004). Its treatment is being achieved by combination of chemotherapy and radiotherapy. However, in a country like Nigeria with less than 100 oncologists with about 100 pathologists and four radiotherapy centers (Durosinmi, 2004), research into medicinal plants with probable anti tumour effects may be

Table 1. Sensitivities of the lung cancer cells to the methanol extracts of the medicinal plants.

Crude extracts	Concentrations (µg/ml)	% Growth Inhibition / Cytotoxicity	$GI_{50}$	TGI	$LC_{50}$
			(µg/ml)		
<i>P. nigrescens</i>	1.0	$+0.0 \pm 0.0$	$154 \pm 6$	>250	>250
	10	$+0.0 \pm 0.0$			
	50	$+3.0 \pm 1.0$			
	100	$+29 \pm 1.6$			
	250	$+85 \pm 5.5$			
<i>B. eurycoma</i> leaf	250	< 50	>250	>250	>250
<i>B. eurycoma</i> stem bark	1.0	$+0.0 \pm 0.0$	$34 \pm 1.0$	>250	>250
	10	$+0.0 \pm 0.0$			
	50	$+84 \pm 3.0$			
	100	$+94 \pm 3.5$			
	250	$+85 \pm 4.0$			
<i>S. sparganophora</i>	250	< 50	>250	>250	>250

Control absorbance (520 nm): NCI-H460 ( $2.9 \pm 0.4$ )

Each value represents % mean  $\pm$  SEM of three independent experiments as compared to control.

Growth inhibition = + and cytotoxicity = -

$GI_{50}$  and TGI = Concentration of drug causing 50% and 100 % growth inhibition of cells.

$LC_{50}$  = Lethal concentration of drug that killed 50% cells.

Table 2. Growth inhibitory and cytotoxic effects of organic solvent fractions of *P. nigrescens* against large lung cancer (NCI-H460) cell line.

Fractions	Concentrations µg/ml	% Growth Inhibition / Cytotoxicity	$GI_{50}$	TGI	$LC_{50}$
			(µg/ml)		
Hexane	100	< 50	>100	>100	>100
Dichloro-Methane	1.0	$+2.0 \pm 1.0$	$14 \pm 4.0$	$30 \pm 2.0$	$45 \pm 1.0$
	5.0	$+4.0 \pm 2.0$			
	10	$+32 \pm 18$			
	50	$-63 \pm 8.0$			
	100	$-65 \pm 4.5$			
Aqueous	100	0	>100	>100	>100

encouraged for treatment particularly at the early stage of infection. The results here showed the variations that exist in the anticancer activities of the plants which to a large extent are direct reflections of the constituents each of them contains. Also, the results have further established the antiproliferative and cytotoxic effects of plant extracts to guinea corn radicle and tadpoles respectively to predict the probable antiproliferative and cytotoxic effects of extracts to cancer cells. Except for *P. nigrescens*, other plants have been subjected to such predictive bioassays (Sogbaïke *et al.*, 2002; Ayinde and Agbakwuru, 2010; Ayinde and Abada, 2010) Out of all the medicinal plants examined, only the methanol extracts of *P. nigrescens* and *B. eurycoma* stem bark were observed to remarkably inhibit the growth of the cancer cells. In both plants, the increase in the concentrations was matched with corresponding increase in the growth inhibitory activities. However, the stem bark of *B. eurycoma* can be noticed to be more active than *P. nigrescens*. At a concentration of 50 µg/ml, the former showed growth inhibition of  $+84 \pm 3.0$  µg/ml whereas, the latter had  $+3.0 \pm 1.0$ . In addition to this, the stem bark produced a GI<sub>50</sub> of  $34 \pm 1.0$  µg/ml while the leaf extract of *P. nigrescens* produced GI<sub>50</sub> of  $154 \pm 6$  µg/ml. It is important to note that organic partitioning of the crude extract of *P. nigrescens* resulted in enhanced activity over the crude extract with the dichloromethane fraction producing cytotoxicity of  $-63 \pm 8.0$  % at concentration of 50 µg/ml. This fact was further established with a GI<sub>50</sub> of  $14 \pm 4.0$  µg/ml obtained for the fraction. While the extracts could be said to inhibit the growth of the cells probably by interfering negatively with the molecular processes involve in the G2/M phase, it could be that the fraction induced apoptosis in the cells through any or combinations of various mechanisms like mitochondrial transmembrane depolarization, increased cytochrome-c release, caspase-3 and caspase-7 activation, and increased poly ADP-ribose polymerase degradation as noted by Badaboina *et al.* (2013). The extract of *S. sparganophora* was here observed to have no effect on the lungs cancer cells. However, the methanol extract of the leaf and its organic solvent fractions have been reported to have cytotoxic effects on the melanoma and ovarian cancer cell lines (Kasim *et al.*, 2011). This report suggests probable variations in the sensitivity and specificity of cancer cells to constituents of plant extracts. It can also explain the variations that exist in chemical composition of anticancer drugs used in orthodox medical practice. The results of this work validate the use of *P. nigrescens* leaves and *B. eurycoma* stem bark in treating tumour related ailments in ethnomedical practice. More works are being carried out to ascertain the nature of the constituents responsible for the activities.

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