



Antiproliferative and Pro-apoptotic activities of the stem bark of *Persea Americana* (Lauraceae) in Human Breast Adenocarcinoma Cell Line

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ABSTRACT: *Persea americana* (Lauraceae) have been used in traditional medicine for a wide range of illness and some of these uses have been proven scientifically. The aim of this present study is to screen for the phytochemical content, determine the proximate parameter and determine the antiproliferative and apoptotic effects of the stem bark of *Persea americana* in MCF-7 cell line by flow cytometer. Result of the phytochemical screening showed the presence of carbohydrate, reducing sugar, tannins, saponin and alkaloid. Proximate analysis gave moisture content of 13.82 ± 0.24 %, acid insoluble ash of 1.17 ± 0.15 %, alcohol soluble extract 2.13 ± 0.14 % and water soluble extractive 1.67 ± 0.07 %. It lacks both antiproliferative and apoptotic activities in MCF-7 cell line but significantly reduced the percentage of cells in G2 phase of the cell cycle ($P < 0.1$). These results showed that the stem barks of *Persea americana* contained compounds that may be responsible for its activity. @JASEM

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Cancer is a leading cause of death in both developed and developing countries, it accounted for 7.6 million deaths (13% of all deaths) in 2008. While in under developed and medium income countries record about 70 % death as a result of cancer and it is predicted that by the year 2030, over 13.1 million deaths will be recorded annually (Globocan, 2008). The causes of cancer vary, recent studies have shown microbes to be implicated (Cover and Blaser, 1995). Microorganisms produce effects which range from causing infections, spoilage of food items, etc (Azu and Onyeagba, 2007), (Blackwell, 2006). However, some of these microbes have produced compounds of great benefit to man including chemotherapeutic agents (Schatz and Waksman, 1944). These lead compounds need to be purified and isolated using various methods of separation (Dinan *et al.*, 2001). The use of plants in medicine either in its powdered form or as lead source for the discovery of new compounds with better therapeutic potentials is an area that continues to enjoy patronage by researchers. Plants are known to possess compounds through which they exert actions that may be of importance to man, animal and its environment (Nguyen, 1999). Lauraceae is the laurel family, with 3000 species of

flowering plants in over 50 genera worldwide. They are native to warm temperate and tropical regions, especially Southeast Asia and South America. Most are aromatic evergreen trees or shrubs, usually 9 m to 18 m or more, trunk 30-60 cm in diameter, and can be greater in very old trees. Its fruits are pear-shaped, which may be 7.5-33 cm long and up to 15 cm wide. The colour of the skin varies from yellow to purple, which may be smooth or leathery and up to 6 mm thick. Immediately under the skin there is a thin layer of soft fruit, buttery and bland in flavour. It produces single seeds of different shapes, 5-6.4 cm long, some fruits are seedless because of lack of pollination or other factors (Burkill, 1995). Common names of *P. americana* include alligator pear, avocado, avocado-pear, butter pear, vegetable pear and butter fruit (Yasir *et al.*, 2010), (Lans, 2006). It is used in folk medicine for the treatment of tumor, hypertension, diabetes, inflammation, sore throat, haemorrhage, vermifuge, dysentery and as aphrodisiac (Lans, 2006), (Bartholomew, 2007), (Gill, 1992), (Ayinde *et al.*, 2011), (Adeyemi *et al.*, 2002), (Edem *et al.*, 2009), (Yasir *et al.*, 2010). Scientifically, some of these activities have been evaluated, for example the antihypertensive, anticonvulsant, analgesic and anti-

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inflammatory effects. (Gill, 1992), (Yasir *et al.*, 2010), (Edem *et al.*, 2009), (Ojewole *et al.*, 2007), (Ojewole and Amabeoku, 2006), (Owolabi *et al.*, 2005). This present study was designed to screen the phytochemical content, determine the proximate parameters, evaluate the antimicrobial, antiproliferative and apoptotic activities of the stem bark of *P. americana* in MCF-7 cell line and also to partially characterize the petroleum ether fraction using chromatographic analyses.

MATERIALS AND METHOD

COLLECTION OF PLANT MATERIAL: The stem bark of *P. americana* was collected within University of Benin, staff quarters B17 in September, 2011 from Ovia North-East local government area in Edo State, Nigeria by Mr. Kingsley Ugwu. The plant was identified and authenticated by Prof. MacDonald Idu of the department of Plant Biology and Biotechnology.

The fresh stem bark were carefully washed with water to remove earthy material and air-dried for a period of one week, after which they were placed in the oven for thirty minutes at a temperature of 40°C, before they were reduced to fine powder with the aid of an electric milling machine. The powdered sample was stored in an air tight container until used.

Extraction of Powdered root Bark: Powdered sample (700g) was macerated at room temperature with 2.5 litres of methanol at room temperature for 72 hrs (Brain and Turner, 1975). The filtrate was concentrated with a rotary evaporator at 40°C under reduced pressure. The total yield obtained was 19.53 g (2.79 %). The concentrated extract was stored in a refrigerator at 4°C until used. The dried extract (15 g) was partitioned into Petroleum ether (40-60°C) (PET), chloroform (CH), and ethylacetate (ET) respectively.

Phytochemical Screening: Chemical tests were carried out on the powdered drug for the qualitative determination of phytochemical constituents as described by (Harborne, 1973), (Trease and Evans, 1989) and (Sofowora, 1993). This involves test for carbohydrate, reducing sugar, saponins, alkaloids, tannins and flavonoids.

Proximate Analysis of the Powdered Drug: The following quantitative parameters were carried using standard methods (African Pharmacopoeia, 1986; British Pharmacopoeia, 1988 ; AOAC, 2003); moisture content, acid insoluble ash, alcohol soluble extractive and water soluble extractive.

Cell Culture: All cell line were obtained from the American Type Culture Collection (Manassas, VA, USA) and maintained at 37°C and in 5 % CO₂ atmosphere in a monolayer. Confluent cells will be passaged by treating them with 0.05 % trypsin-0.02% EDTA.

Treatment with Plant Extract: For all experiments 0.5 x 10⁶ cells were seeded in a 6-well plate in regular culture medium for 24 hours. Subsequently, cells line were washed with phosphate buffer saline (PBS) and adapted to phenol-red-free Dulbecco's modified Eagle's medium for 48 hours to avoid unspecific stimulation of endogenous hormones in the serum. Treatment with plant extracts (final concentration 10 ug/ml) and DMSO (0.1%) was done.

Flow Cytometric Measurement Of Cell Proliferation: The extent of cell cycle progression and apoptosis was estimated by flow cytometric analysis (Nadja *et al.*, 2011). For statistical evaluation, the S-phase and G2/M-phase cells were defined as proliferative cells.

Statistical Analysis: Every experiment was replicated three times; data sets were expressed as mean± standard deviations (SD). Statistical significance was determine by unpaired t-test (***P < 0.001, **P < 0.01, *P < 0.1).

RESULT AND DISCUSSION

The powdered stem bark of *P. americana* gave a yield of 4.21 % after extraction with methanol (Table 1). This low yield could be attributable to the method use for extraction which in this case is maceration and this could be due to the inability of methanol to extract sufficient amount of relevant material. Also, it will be observed that fractionation of the crude extract with solvents of increasing polarity have resulted in the production of fractions with decrease quantity as the polarity of the solvents increase, indicating that the crude extract possesses high level of non polar content. The phytochemical constituent of powdered stem bark indicates the presence of carbohydrates, reducing sugar, saponins, alkaloids and tannins while flavonoid was absent (Table 2). This result does not agree with previous work, which showed the presence of flavonoid and absence of alkaloid in the stem bark of *P. americana* (Ayinde, 2011). However this observation may be due to differences in the location of the plant since the presence of phytochemicals can be influence by environment in which the plant is grown (Folkers *et al.*, 2008; Shen *et al.* 2008). The proximate analysis carried out on the stem bark of *P. americana* gave a moisture content of 13.82 ± 0.24 % , acid insoluble

ash 1.17 ± 0.15 %, alcohol soluble extractive 2.13 ± 0.14 and water soluble extractive 1.67 ± 0.07 % (Table 3). It will be observed that the moisture content is high and thus is a strong indication that the powdered stem bark is susceptible to microbial attack. Implying that long time storage of the powdered stem bark may lead to its degradation. The acid insoluble ash value of the stem bark is low showing that the stem bark contains low level of inorganic constituents, like sand. The extractive value is used to determine the best solvents for the extraction of the chemical constituents from the plant samples. The alcohol soluble extractive value is higher than the water soluble extractive values for the stem bark, showing that alcohol will extract more of the chemical constituents present in the stem bark than water. Cell cycle analysis (Figure 1), shows the percentage number of cells in the G2 phase after treatment with the crude extract of *P. americana* (SBPA) to be 14.80 %, while the control (DMSO) which was used to dissolve the crude extract produced 20.74 %. This shows a significant decrease of 5.94 % in the percentage number of cells ($P < 0.1$). The S phase also show the percentage number of cell to be 12.10 % for SBPA treated cells and 11.37 % for DMSO treated cells, giving 0.73 % increase in percentage number of cells which is not significant. G1 phase show percentage number of MCF-7 cells after treatment with SBPA to be 73.10 %, this show a decrease of 4.09 % when it was compared with the control (68.11 %). MCF-7 cell cycle analysis post treatment with SBPA shows the percentage of

proliferative cells to be 26.90 % (Figure 2), while DMSO treated cells gave 31.88 %. This shows a decrease in the percentage of proliferative cells of 4.98 %, this was not significant. Extent of apoptosis was also evaluated (Figure 3) and it was observed that the SBPA treated MCF-7 cells produced 6.47 % apoptosis while DMSO treated cells show 6.26 %. This shows 0.21 % increase in apoptotic cells, though not significant. These results show that SBPA lacks both antiproliferative and apoptotic activity even though it may have affected the cells in the G2 phase of the cell cycle. It is may have produced this effect by activating G2 checkpoint which may be responsible for correcting the damage the cells may have encountered in the process of moving through the cycle.

Conclusion: The stem bark of *P. americana* is rich in phytochemical constituents that lacks antiproliferative and apoptotic effects in MCF-7 cell line. Chromatographic analysis confirmed the presence of a number of compounds in the stem bark extract.

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Table 1: Percentage yield of the powdered stem bark of *P. americana*

Extact/Fractions	Percentage yield (%)
Methanol extract	4.21
Petroleum ether	19.51
Chloroform	13.7
Ethyl acetate	3.62

Table 2: Phytochemical screening of the powder stem bark of *P. americana*

Carbohydrate	+
Reducing sugar	+
Tannin	+
Saponin	+
Alkaloid	+
Flavonoids	-

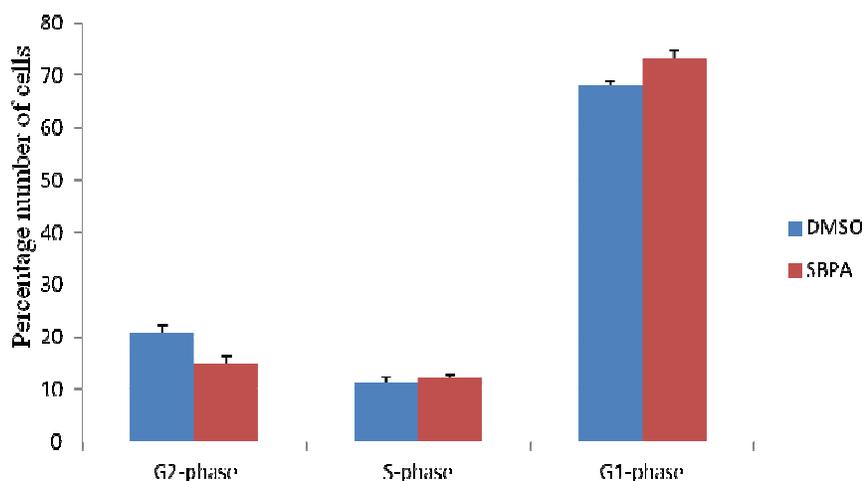
+ = Indicates presence of components

- = Indicates absence of components

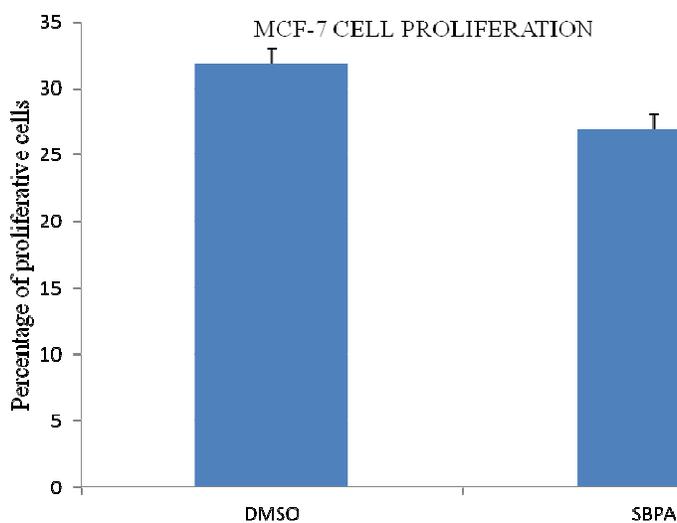
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Table 3: Percentage (%) values of proximate analysis of the stem bark of *P. americana*

Parameter	Values \pm SEM (%)
Moisture content	13.82 \pm 0.24
Acid insoluble ash	1.17 \pm 0.15
Alcohol soluble extractive	2.13 \pm 0.14
Water soluble extractive	1.67 \pm 0.07

**Fig1:** percentage of human breast adenocarcinoma cell line (MCF-7) after treatment with the crude extract of *P. americana* and DMSO.

DMSO = Dimethylsulphoxide
 SBPA = Stem bark of *P. Americana*

**Fig 2:** percentage of proliferation of human breast adenocarcinoma cell line (MCF-7) after treatment with the crude extract of *P. americana* and DMSO.

DMSO = Dimethylsulphoxide
 SBPA = Stem bark of *P. americana*

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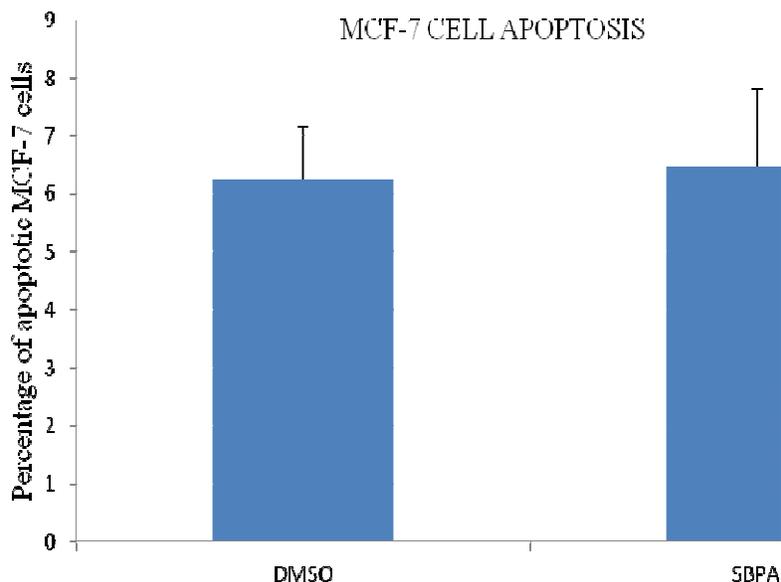


Fig 3: percentage of apoptosis of human breast adenocarcinoma cell line (MCF-7) after treatment with the

crude extract of *P. americana* and DMSO.

DMSO = Dimethylsulphoxide

SBPA = Stem bark of *P. americana*

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