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In vitro cytotoxic effect of aqueous and hydroethanolic *leaf extracts of Stachytarpheta cayennensis* on the human pancreatic cell line (ASPC-1)

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ABSTRACT: There is an urgent need for agents with improved efficacy against pancreatic cancer, as only 20% of pancreatic cancer patients undergoing chemotherapy respond to treatment. This study investigated the antiproliferative attributes of hydroethanolic and aqueous leaf extracts of *Stachytarpheta cayennensis* on human pancreatic carcinoma cell lines (AsPC-1). Human pancreatic (AsPC-1) cells were seeded (1.07×10^5 cells/well) into 96 well microtitre plate, allowed to stabilize, then exposed for 24hrs to graded (0 – 10000 µg ml^{-1}) concentrations of crude aqueous or hydroethanolic (60%) extract of pulverised air dried leaves. There after cytotoxicity was evaluated using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay**.** Crude aqueous and hydroethanolic leaf extracts of *Stachytarpheta cayennensis* displayed significantly ($p < 0.05$) increased cell cytotoxicity (CC) in a dose-dependent manner and the CC_{50} values were 1190 µg ml^{-1} ($R^2 = 0.92$) and 2537 µg ml^{-1}($R^2 = 0.96$) respectively.These results showed the possible application of aqueous as well as hydroethanolic leaf extracts of *Stachytarpheta cayennensis*leaves as a source of bioactive compounds, potent as antiproliferative and cytotoxic agents that could be utilized pharmaceutically.

Keywords: Medicinal plant, AsPC-1, Cytotoxicity, MTT.

Introduction

Medicinal plants play a crucial role in health care needs of people around the world especially in developing countries where it was estimated that between 60-90% of their populations use traditional and botanical medicines almost exclusively and consider them to be a normal part of primary healthcare (WHO, 2002; Rao *et al*., 2004; Bekalo *et al.,* 2009). The application of ethnobotany, ethnopharmacology and complimentary alternative medicine in combating current health issues like Cancer is economical, sustainable and beneficial (Cochrane *et al*., 2008). Cancer is one of the major human diseases which causes considerable suffering and economic loss worldwide. Numerous studies have evaluated the biological activities of various phytochemicals produced by plants, particularly the anti-proliferative and cell cycle regulatory effects, in relation to cancer prevention (Chu *et al*., 2002; Zhang *et al*., 2005; Cheng *et al*., 2011).

Pancreatic cancer is the only major cancer with a five-year relative survival rate in the single digits and unlike many other cancers, the survival rate for the disease has not improved substantially since passage of the *National Cancer Act* over 40 years ago in the United State (American Cancer Society, 2013). Of all the racial/ethnic groups in the United States, African-Americans have the highest incidence rate of pancreatic cancer, between 32 percent and 66 percent higher than other groups (SEER, 2009). A recent report issued by the

Pancreatic Cancer Action Network, indicated that by 2020, pancreatic cancer is expected to become the second leading cause of cancer death in the United States (Matrisian *et al*., 2012). The principal methods of cancer treatment include radiotherapy, surgery and chemotherapy (Fan *et al*., 1998; Martinsson *et al*., 2001; American Cancer Society, 2013). Chemotherapeutics enhance serious side effects or long term complication (Jonsson *et al*., 1997; Martinsson *et al*., 2001; American Cancer Society, 2013).

There are currently no curative treatments for pancreatic cancer, hence, research in the area of pancreatic cancer treatment is desperately needed (American Cancer Society, 2013). Plants are the chief source of natural products that are used in medicine, hence the application of ethnopharmacology and complimentary alternative medicine in combating current health issues (Zhang *et al*., 2005; Tan *et al*., 2012). Ethnobotanically, *Stachytarpheta cayennensis* is used in traditional medicine for its analgesic, antacid, anti-inflammatory, antipyretic, antispasmodic, anti-ulcerogenic, diuretic, gastroprotective, hepatoprotective, hypoglycemic, hypotensive, diabetes, sedative and tonic properties, and for the management of oedema (Schwontkowski, 1993; Burkil, 1966; Comerford 1996; Melita *et al*, 1996; Schapoval *et al*., 1998; Mesia-Vela *et al*., 2004; Akanmu *et al*., 2005; Penido *et al*., 2006; Siju *et al*., 2012).

A number of researchers have verified the importance of *Stachytarpheta cayennensis* and its high ethnopharmacological values; but little or none has stated or verified its antiproliferative activities and currently, only 20% of pancreatic cancer patients undergoing chemotherapy respond to treatment (Shah *et al*., 2007). Clearly, there is an urgentneed for agents with improved efficacy against pancreatic cancer. This study therefore investigated the antiproliferative effects of hydroethanolic and aqueous extract of *Stachytarpheta cayennensis*on on human pancreatic carcinoma cell lines (AsPC-1).

Materials and Methods

Plant collection and preparation of extracts

Fresh plants of *Stachytarpheta cayennensis* (Verbenaceae) were collected in University of Lagos, Akoka (6.51667N/3.38611E). Plants was identified and authenticated (LUH6018) at the University of Lagos Herbarium, Akoka, Lagos, Nigeria.

The leaves of the plant samples were hand-picked, washed, dried in shade and pulverized to coarse powder in a mechanical grinder. The ethanol and water extracts were prepared by soaking 93.65 g and 120 g of dried powdered plants 1000 in mL of 60 % ethanol solution prepared with distilled water and 100 ml of distilled waterrespectively, in a mechanical shaker at room temperature for 48 hrs. The crude extracts were filtered using filter paper and the filtrate was evaporated to dryness at 40 ± 2 °C using a rotary evaporator. The concentrated extract was stored in a sealed container at 4 °C until use. The percentage yield of ethanol and aqueous extracts were found to be 0.67 % and 0.75 % respectively.

The ethanol / aqueous extract stock solution (100 mg/ml) was prepared by dissolving extract in 30ul sterile DMSO (dimethyl sulfoxide) (Saetung *et al*., 2005), the volume was made up to 10ml with PBS (phosphate buffered saline) and 0.22 micron filter sterilized. The filterates where stored at 4 ^{o}C until use.

Cell Culture

The human pancreatic carcinoma (AsPC-1) cell line used in this study,was obtained from Cell line Services (Germany). This cell line was originally obtained from a patient with pancreatic ductal adenocarcinoma. The cells were grown as a monolayer in RPMI 1640 containing, glutamine, 10 % fetal bovine serum (inactivated at 55 °C for 30 min), penicillin (100 μg ml^{-1}), streptomycin (100 μg/ml) and 0.2 mM sodium pyruvate. The cells were incubated to confluence (80-90 %) in a humidified incubator with 5 % CO_2 (95 % air) in polystyrene culture flasks at 37^{o}C.

MTT Cell Viability Assay

Confluent monolayer cells were washed thrice with phosphate buffer saline (PBS), trypsinized and suspended in fresh medium. Harvested cells were seeded in a flat 96-well plate (Becton-Dickinson, US) at a concentration of 1.07×10^{5} cells/well and allowed to stabilize. The cultured cells were then treated in triplicate with *Stachytarpheta cayennensis* extracts at concentrations between 0 and 10000 μg ml^{-1} for 24 hours, control wells had cells and medium only while blank wells contained 100 μl of medium only. After 24 hours, 10 μl of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (Science Cell, USA) was added to all wells and the plates were further incubated for another 4hrs. Lastly, 100 μlof DMSO was added to all wells to dissolve the formazan crystals. The absorbance was measured at 570 nm (reference wavelength: 690 nm). Cell viability was calculated using the following formula:

Percentage of cell viability= $\frac{optical density of treated cells}{optical density of control cells} * 100\%$

% Cytotoxicity = 1- (% Viability)

A dose-response curve was plotted and the half maximal cytotoxic concentration (CC_{50}) was determined.

Statistical Analysis

All the assays were performed in triplicates. Data expressed as mean ± SD and analyzed using one-way analysis of variance (ANOVA) followed by Tukey Post Test, using GraphPad Prism computer software package (GraphPad Prism Inc. U.S.A). Differences at $P<0.05$ were considered significant.

Results

Both extracts inhibited cell proliferation (Fig.1) in a dose dependent manner with CC_{50} values of 1190 µgml^{-1} (R^2 = 0.92) and 2537 µgml^{-1} (R^2 = 0.96) for Crude aqueous and hydroethanolic leaf extracts respectively. Concentrations above 300.00 µgml^{-1} of crude aqueous extract and 1200.00 µgml^{-1} of crude hydroethanolic extract were significantly cytotoxic to human pancreatic carcinoma (AsPC-1) cells.

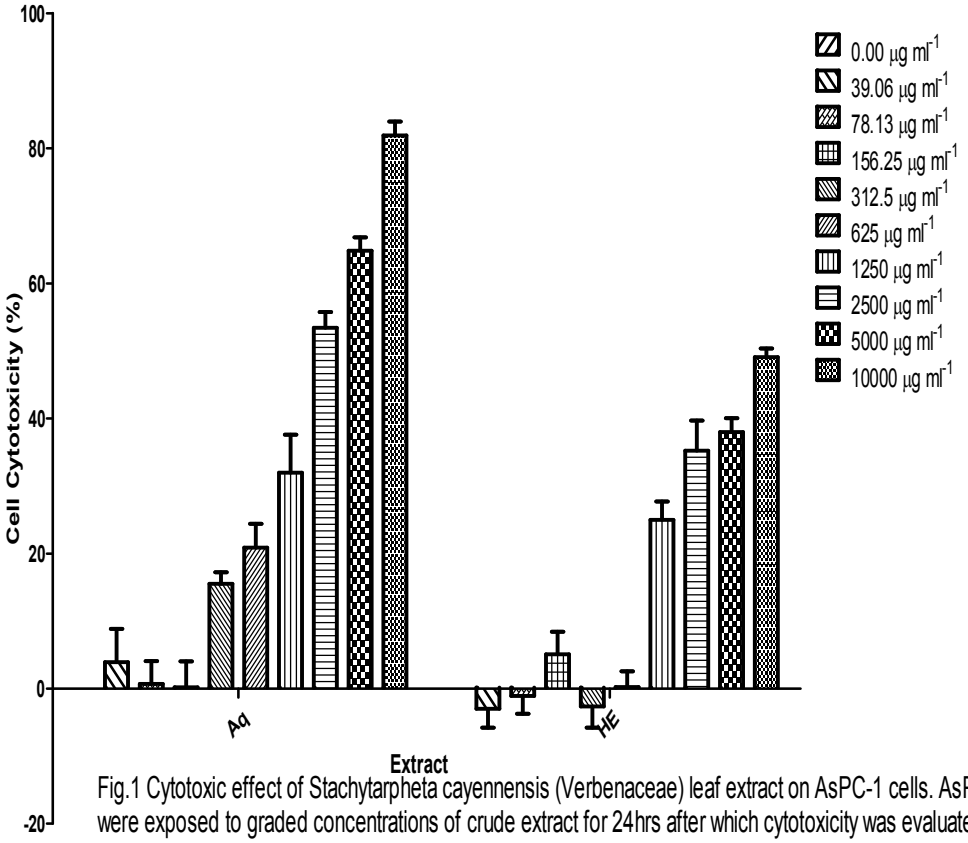

Fig.1 Cytotoxic effect of Stachytarpheta cayennensis (Verbenaceae) leaf extract on AsPC-1 cells. AsPC-1 cells were exposed to graded concentrations of crude extract for 24hrs after which cytotoxicity was evaluated using the MTT assay. Data expressed as mean±sem of three replicates;ANOVA ($P\angle 0.05$)

Discussion

In sub-Saharan Africa, little is known about the prevalence of pancreatic cancermainly because the condition is mostly underreported as well as the difficulty encountered in making the diagnosis (Alatise *et al*., 2009).

Abdulkareem *et al.* (2009) found that pancreatic cancer is the fourth most common gastrointestinal cancer seen in Lagos, Nigeria.

The differences in the toxicity of different extracts could be attributed to the presence of the active principle(s) or compound(s) in the plant material or even different active principle(s) that may be influenced by several factors such as age of plant, method of extraction and type of extracting solvent (Caamal-Fuentesa *et al*., 2011). The greater effectiveness of aqueous extract as compared with the ethanol extract may be due to differences in constituent of the bioactive constituent(s). The aqueous and hydroethanolic extracts of *Stachytarpheta cayennensi*s possess varying levels of anticancer activity *in vitro*. This was evident by the concentration dependent manner of reduction in the final number of cancer cells as a consequence of treatment. Aqueous extract of *Stachytarpheta cayennensis* (CC_{50}:1190 μgml^{-1}) appeared more potent than ethanol extract (CC_{50}:2537 μgml^{-1}), suggesting that the potency of the aqueous extract may be as a result of the ability of this solvent to isolate more active ingredient compared to the hydroethanolic extract from the plant.

Plant-derived products contain a great diversity of phytochemicals such as phenolic acids, flavonoids, tannins, lignin, and other small compounds (Cowan, 1999). Presence of alkaloids, iridoids, and phenylpropanoidshas been indicated via chemical studies of the genus Stachytarpheta (Alice *et al*., 1991; Schapoval *et al*., 1998; Edeoga *et al*., 2005; Sahoo *et al*., 2014). These biologically active compounds possess numerous beneficial health-related effects, and possibly new sources of new drugs for infectious diseases (Meenakshi *et al*., 2001). This plant is interesting for further phytochemical studies to isolate cytotoxic compounds.

Conclusion

These results indicated the possible application of aqueous as well as hydroethanolic leaf extracts of *Stachytarpheta cayennensis*leaves as a source of bioactive compounds, potent as antiproliferative and cytotoxic agents that could be utilized pharmaceutically.

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