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IN VITRO ANTICANCER ACTIVITY OF *PAPPEA CAPENSIS* MEDICINAL PLANT FROM MOKOPANE

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Abstract

The present study reports on the anticancer activity of *Pappea capensis*, used by traditional healers in the Limpopo province of South Africa for the treatment of most prominent diseases in humans including cancer. More than 80% of the world population rely on natural products due the economic state of not affording western medicine. Even though this medicinal plant is easily accessible, cheap and found everywhere its efficacy is an issue that scientists should assess. MCF7 (breast cancer); HCT116 (colon cancer), and PC30 (prostate cancer) cells used in-vitro Sulforhodamine B (SRB) assay. The anticancer activities of *Pappea capensis* extracts dichloromethane/methanol (1:1) and aqueous extracts were tested for their growth inhibitory effects *in vitro* against three human cancer cell lines. Parthenolide was used as a standard. Data analysis was performed using GraphPad Prism software. Fifty per cent (50%) of cell growth inhibition (IC_{50}) was determined by non-linear regression and absorbance measured at 540nm. Extracts were classified into four categories based on their total growth inhibition of the cell lines. Extracts which exhibited a total growth inhibition (TGI) of less than 6.25 µg/mL were regarded as potent. Dichloromethane/methanol extract of *Pappea capensis* exhibited pronounced activity especially against the PC30 cell line. A relatively low Dichloromethane/methanol extract concentration was necessary to reach 50% proliferation inhibition of the PC-3 cell line and moderate for MCF-7 cell line and weak for HCT-116 cell line. The Dichloromethane/methanol extract was classified as weakly active. So, *Pappea capensis* extracts are promising as possible drugs for the treatment of prostate cancer. Further tests such as genotoxicity, antioxidant determination, phenolic and active compounds should be done scientifically to establish compounds responsible for growth inhibition towards prostate PC3 cell lines.

Keywords: *Anticancer activity, cancer, Limpopo*

Introduction

Cancer is a major global public health problem (1). It is presented when abnormalities are observed within the cells of an individual's body and can as a result lead to death (2). It is possible that these cells grow due to imbalance in the body, therefore by correcting this imbalance cancer may be treated (2, 3). Cancer accounts for 3500 million deaths worldwide, contributing 2-3% of the world deaths recorded annually (4; 5). Cancer is the second leading cause of death in America with breast cancer being the most common in women and prostate cancer in men worldwide (2, 6, 7, 8). Among South African women one out of every 31 is likely to develop breast cancer at some time in her life time (7). A lot of money has been spent on cancer research but it is still not fully understood what causes cancer (7). Despite the progress of medicinal research in the past decades cancer treatment remains vague.

Several chemotherapeutic agents are used in the treatment of cancer, but they cause toxicity that prevents their usage leading to the possible use of plants for cancer treatment (5). Chemotherapy being used for the control of advanced stage of malignancies and a prophylactic against possible metastasis, exhibit severe toxicity to normal tissues (6). Treatments like chemotherapy, radiotherapy and surgery are less accessible in the developing countries (1). According to World Health Organization, more that 80% of population in these countries rely on traditional medicine for some aspects of primary health care (9, 10). Other studies have shown that more than 60% of cancer patients use vitamins and herbs as therapy (11, 12). Plants have played an important role as an effective anticancer agent, and recently over 60% of the presently used anticancer agents are derived from natural sources including plants (13, 14, 15; 16). The increased incidence of cancer and the lack of anticancer drugs have forced researchers to study the pharmacological and chemical investigations in the area of medicinal plants to search for the discovery of new possible anticancer agents (8).

Recently there has been great scientific interest in the discovery of new anti-cancer drugs from natural products sources (2, 17; 18). Research has developed great interest towards understanding the role of free radical reactions in biology, suggesting that such reactions are important for several metabolic reactions and could also be potentially harmful to health. Free radicals such as reactive oxygen species (ROS) have been reported to play an important role in the development of tissue damage in living organisms. There is an increase in evidence relating the occurrence of cancer due to the oxidative damage to DNA, proteins and lipids in the body caused by radicals and other carcinogens (19). Although medicinal plants are not used as antioxidants in traditional medicine, studies have reported plants containing several phytochemicals which possess strong antioxidants. There is a possibility that these antioxidants may prevent and cure cancer, as well as other diseases by protecting the cells from damage caused by free radicals (20, 21).

Considering the importance of this untapped research area of the medicinal plant *Pappea capensis*, the aim of the study was to evaluate the anticancer activities of this medicinal plant used in Limpopo for the treatment of cancer. The ultimate objective of this research is to reveal the anticancer inhibitory effect of this plant.

Methods

Plant material

The plant was collected from the south of Limpopo province; 209 km from the town Naboomspruit using R101 to Mokopane in April 2014. A voucher specimen was supplied to the Free State Museum and the Free State Botanical Gardens. The plant was positively identified by Dr Zietsman at the Free State Museum and verified by the National Botanical Gardens in Pretoria.

Extraction of plant material

Finely ground plant mixture (100g) was separately extracted using water and dichloromethane/methanol (1:1) at room temperature for 24 hours. Organic extracts were filtered and concentrated by rotary vacuum at 50-60°C; the aqueous extracts were concentrated to dryness using a freeze-dryer. The plant extracts generated was stored in a cold room at -20°C until further use. Five times two-fold serial dilution of the

plant extract and 10 times two fold serial dilutions of the control (parthenolide) were used with concentration of 100-6.25 ug/ml and 100-0.20 ug/ml respectively.

Determination of inhibitory effect on three cell lines

For the estimation of the growth inhibitory effects, the extract was tested in the 3-cell line panels consisting of MCF7 (breast cancer), HCT116 (colon cancer) and PC3 (prostate cancer) cells by Sulforhodamine B (SRB) assay. The SRB assay was developed by Skehan and colleagues to measure drug-induced cytotoxicity and cell proliferation. Its principle is based on the ability of the protein dye sulforhodamine B (Acid Red 52) to bind electrostatically in a pH-dependent manner to protein basic amino acid residues of trichloroacetic acid-fixed cells. Under mild acidic conditions it binds to the fixed cellular protein, while under mild basic conditions it can be extracted from cells and solubilized for measurement. The SRB Assay was performed at Council for Scientific and Industrial Research (CSIR) of South Africa in accordance with the protocol of the Drug Evaluation Branch, USA National Cancer Institute (NCI), and the assay has been adopted for this screen.

The human cancer cell lines MCF7 and PC3 were obtained from NCI in a collaborative research program between CSIR and NCI. The HCT-116 cell line was obtained from European Collection of Cell Culture (ECACC). Cell lines were routinely maintained as a monolayer cell culture at 37°C, 5% CO2, 95% air and 100% relative humidity in RPMI containing 5% fetal bovine serum, 2 mM L-glutamine and 50µg/ml gentamicin.

For the screening experiment, the cells (3-19 passages) were inoculated in a 96-well microtiter plates at plating densities of 7-10 000 cells/well and were incubated for 24 hours. After 24 hours the cells were treated with the experimental drug which were previously dissolved in DMSO and diluted in medium to produce 5 concentrations. Cells without drug served as control. The blank contained complete medium without cells and Parthenolide was used as a standard.

The plates were incubated for 48 hours after addition of the plant extract. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed, and protein-bound dye was extracted with 10mM Tris base for optical density determination at the wavelength 540 nm using a multi well spectrophotometer.

Data analysis was performed using GraphPad Prism software. Fifty per cent (50%) of cell growth inhibition (IC_{50}) was determined by non-linear regression and absorbance measured at 540nm.

Results

The results (Table 1 and Figure 1) indicated that the proliferation activities of PC-3, HTC-116 and MCF-7 cell lines were inversely related to the increased levels of parthenolide concentrations. This proliferation inhibition is acceptable for parthenolide as it indicates the accuracy of the assay, a significant dose response manner was shown (Z=0.9). A relatively low parthenolide concentration was necessary to reach 50% proliferation inhibition of the three cell lines.

The results (Table 2 and Figure 2) indicated that the proliferation activity of PC-3, HTC-116 and MCF-7cells lines were inversely related to the increased levels of the plant extract concentration. This proliferation inhibition effect is acceptable for plant extract, a significant dose response manner was shown (Z=0.9). A relatively low plant extract concentration was necessary to reach 50% proliferation inhibition of the PC-3 and moderate for MCF-7 and HCT-116 cell lines.

The CSIR interpretation criterion (Table 4) was used since as it describes the sample is considered inactive if parameter IC_{50} for two or three cell lines is higher than 100 µg/ml. Table 3 reports the IC_{50} values for the investigated *in vitro* anticancer activity of the plant extract. The extract showed a potent anti-cancer activity against PC-3 and moderate activity against MCF-7 and HTC-116.

Discussion

Recently, relevant attention has been focused on the screening for the best possible plants and plant's parts that have anticancer activity for the treatment of cancer. The results indeed indicated that the plant *Pappea capensis* have high anticancer activity against PC-3 and can be a suitable source for the treatment of prostate cancer. These results correlated to what have

been published by previous studies indicating the importance of traditional medicine and plants as natural sources for cancer treatment (15, 13, 14, 12, 8, 16, 11). The present screen justifies the use of this plant by the traditional healer as a prime source of the drugs for the treatment of cancers. Based on the obtained data the results suggested that this plant could be a new potential source of anticancer agent. No data relative to cytotoxic components had been reported, however bio-guided fractionation and isolation to identify compounds responsible for the observed activity is in progress

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Table1: The survival rate of the three cell lines exposed to different parthenolide (control) concentrations

Conc. (µg/ml)	Log Conc.	%Viability MCF-7	SD	%Viability HCT-116	SD	%Viability PC-3	SD
100	2.0	16.13	0.20	6.86	0.92	5.82	0.61
50	1.7	16.56	0.38	5.91	1.21	4.07	0.90
25	1.4	15.55	1.07	5.42	0.40	3.35	0.79
12.5	1.1	13.89	0.21	6.57	0.51	3.87	0.61
6.25	0.8	12.10	0.17	8.94	0.97	5.98	1.01
3.13	0.5	11.54	0.17	21.27	2.13	12.24	2.51
1.56	0.2	31.35	1.01	84.10	2.38	40.80	1.16
0.78	-0.1	57.15	4.13	98.87	0.98	66.60	0.19
0.39	-	85.28	1.99	99.63	0.80	82.52	4.09
0.20	-	96.70	2.90	100.59	0.27	93.16	2.29

Z' factor: 0.9

Table2: The survival rate of the three cell lines exposed to different plant extract (*Pappea capensis*) concentrations

Z' factor: 0.9

Conc. (µg/ml)	Log Conc.	%Viability MCF-7	SD	%Viability HCT-116	SD	%Viability PC-3	SD
100	2.0	19.46	1.90	29.88	0.83	19.58	5.14
50	1.7	27.05	1.82	33.40	0.59	24.83	0.65
25	1.4	30.04	1.30	37.26	1.33	26.84	2.62
12.5	1.1	36.86	1.35	48.49	0.35	30.56	2.61
6.25	0.8	60.84	0.81	61.63	1.37	41.78	0.16

Table3. Results summary

No	Compound	IC50 for MCF-7, µg/ml	IC50 for HCT-116,µg/ml	IC50 for PC-3,µg/ml
1	*Pappea capensis*	8.528	12.52	<6.25
	Parthenolide	**0.81**	**2.22**	**1.25**

Table4: CSIR standard criteria for IC50 interpretation

IC50,µg/ml	Status
> 100 µg/ml	Inactive
< 100 µg/ml >15 µg/ml	Weak Activity
< 15 µg/ml > 6.25 µg/ml	Moderate Activity
< 6.25 µg/ml	Potent Activity

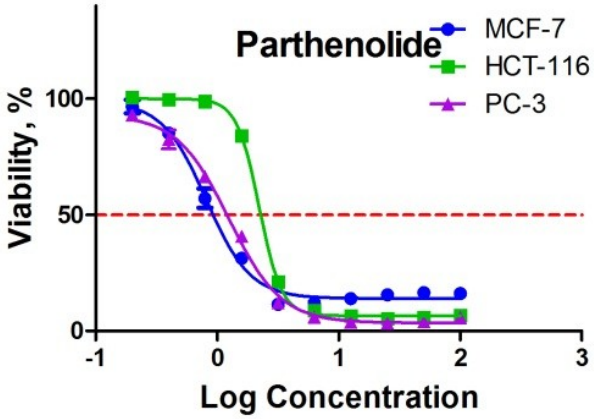

Figure 1: A viability curve of the three cell lines exposed to Parthenolide (control)

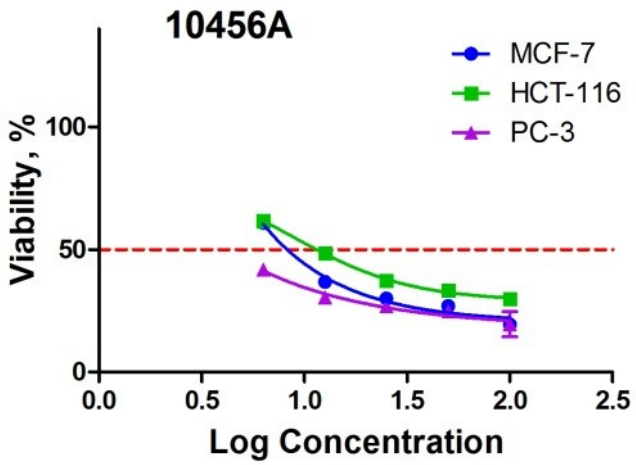

Figure 2: A viability curve of the three cell lines exposed to plant extract (*Pappea capensis* (CSIR Lab no 10456A)