



# Combretum Adenogonium Induce Anticancer and Antioxidant Effects in Prostate Cancer Cell Line

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## Abstract

Plants have been used for treating various diseases of human beings and animals since time immemorial. They maintain the health and vitality of individuals, and also cure diseases, including cancer without causing toxicity. This work investigated the anticancer, antioxidant activities of *C. adenogonium* roots, leaves, and stems commonly used as anti-inflammatory and anti-tumor. All the plant parts were extracted using 80% methanol, the anticancer activity was examined by using MTT assay against PC3 (Prostate Cancer) and determine their antioxidant activities by testing Effect of Plant Methanol Extracts on suppressing whole blood Phagocytes and ROS Production and Metal chelating activities, filter and kept in dark, prepared freshly. The extract *C. adenogonium* roots, DC has shown very high activity against PC3 (Prostate Cancer) IC<sub>50</sub> values 24 µg/ml. The extract *C. adenogonium* leaves, and stems has shown none active anti against PC3 (Prostate Cancer) IC<sub>50</sub> values >100, and >100 µg/ml respectively. All the extracts Effect of Plant Methanol Extracts on suppressing whole blood Phagocytes and ROS Production has shown none active in all plants parts under study *C. adenogonium* roots, leaves, and stems IC<sub>50</sub> values >200. All the examined plant methanol extracts showed different levels of inhibition % for their metal chelating activity at concentration 50 µg/mL. *C. adenogonium* stem 50.87 µg/m, *C. adenogonium* leaves 49.85 µg/m, *C. adenogonium* roots 25.99 µg/m.

**Keywords:** PC3; Chemiluminescence; Anticancer; Medicinal plants

## Introduction

The high costs of western pharmaceuticals put modern health care services out of reach of most of the world's population, which relies on traditional medicine and medicinal plants to meet their primary health care needs. Even where modern medical care is available and affordable, many people prefer more traditional practices. This is particularly true for First Nations and immigrant populations, who have tended to retain ethnic medical practices. In the last decade, there has been considerable interest in resurrecting medicinal plants in western medicine, and integrating their use into modern medical systems. The reasons for this interest are varied, and include low cost herbals are relatively inexpensive and the cost of pharmaceuticals to governments and individuals is rising, drug resistance the need for alternative treatments for drug-resistant pathogens, limitations of medicine: the existence of ailments without an effective pharmaceutical treatment, medicinal value laboratory and clinical corroboration of safety and efficacy for a growing number of medicinal plants, Cultural exchange expanding contact and growing respect for foreign cultures, including alternative systems of medicine and commercial value growing appreciation of trade and other commercial economic opportunities represented by medicinal plants [1]. However, the pace of re-adopting the use of traditional medicinal plants is by no means uniform in western medicine [2,3]. In parts of Europe, especially in Germany, herbal medicine or (phyto-medicine) is much more popular than North

America. About 67,000 different herbal products are available in Germany (Foster, 1995a). The already well-established medicinal plant trade of Europe is increasing at an annual rate of about 10%. In Canada, and the US, the regulatory climate has been much less receptive to herbal medicines [4]. This is because lack of proper scientific evaluation, limited regulation, absence of quality control, limited education of many herbal practitioners, and the presence of "snake-oil salesmen" have all combined to give herbal medicine a bad reputation. However, in response to public demand for "alternative" or "complementary" medicine, this situation is changing. At least 20% of Canadians have used some form of alternative therapy, such as herbalism, naturopathy, acupuncture, and homeopathy [5].

Herbs are the fastest-growing part of the pharmacy industry of North America, with an annual growth variously estimated as 15 to 20%, and thousands of herbal products are now available to Canadians [4]. Herbal remedies have been estimated to have a current value of between two and ten billion dollars in North America, depending on how comprehensively the category of medicinal herbs is interpreted [6] (Foster, 1995).

## Material and Methods

Collection of tested plant parts of the *C. adenogonium* Collected from the Farm of Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan (MAPRI) during the period of June

and July 2010 and identified of taxonomist team of Medicinal and Aromatic Plants Research Institute, National Center of Research, Khartoum, Sudan. And herbarium voucher was deposited at herbarium medicinal plants in the MAPRI.

### Preparation of the crude extracts

The plant parts were shade dried. The powdered materials were accurately weighed and extracted by shaker SF1 from (Stuart Scientific, made in Great Britain) of room temperature by using 80% methanol. After that the filtered extracts were concentrated on rotatory BUCHI 461 evaporator. The extracts, were dried and concentrated by using hood extracts and the yield percentage was then calculated.

### Culture media and human tumor cell lines

**Human cell lines:** PC3 (prostate cancer cell line) were obtained frozen in liquid nitrogen (-180 °C), the tumor cell lines were maintained in the Institute of ICCB, University of Karachi Pakistan.

**Culture media:** RPMI -1640 medium was used for culturing and maintenance of the human tumor cell lines. The medium was supplied in a soluble form. Before using the medium it was warm at 37°C in a water bath and supplemented with penicillin/streptomycin and Fetal bovine serum (FBS) with 10% concentration. The cells were maintained at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> and were sub cultured twice a week.

### Procedure

#### Maintenance of the human cancer cell lines in the laboratory

A cryo tube containing frozen cells was taken out of the liquid nitrogen container and then thawed in a water bath at 37°C. The cryo tube was opened under strict aseptic conditions and its content was supplied by 5ml complete media (RPMI-1640 with 10% fetal bovine serum) drop by drop in a 50ml disposable sterile falcon tubes and were centrifuged at 1200rpm for 10min to discard the preserving solution. The supernatant was discarded and the cell pellet was seeded in 5ml complete media in T25 Nunclon sterile tissue culture flasks. The cell suspension was incubated at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> and followed up daily with changing the supplemented medium every 2-3 days. Incubation was continued until a confluent growth was achieved and the cells were freshly sub cultured before each experiment.

#### Collection of cells by trypsinization

The media was discarded. The cell monolayer was washed twice with 5ml phosphate buffered saline and all the adherent cells were dispersed from their monolayer by the addition of 1ml trypsin solution (0.025 % trypsin w/v) for 2 minutes. The flask was left in the incubator till complete detachment of all the cells and checked with the inverted microscope (Olympus). Trypsin was inactivated by the addition of 5ml of the complete media. The trypsin content was discarded by centrifugation at 1200rpm for 10 minutes. The supernatant was discarded and the cells were separated into single cell suspension by gentle dispersion several times, then suspended and seeded in 5ml complete media in T25 Nunclon sterile tissue culture flasks.

### Determination and counting of viable cells

50µl of fresh culture media was added to 50µl of the single cell suspension. The cells were examined under the inverted microscope using the haemocytometer. Viable cells were counted and the following equation was used to calculate the cell count/ml of cell suspension.

$$\text{Viable cells / ml} = \frac{\text{number of cells in 4 quarters} \times 2 (\text{dilution factor}) \times 10^4}{4}$$

The cells were then diluted to give the concentration of single cell suspension required for each experiment. The cell count was adjusted to 1x10<sup>4</sup>-10<sup>5</sup> cells/ml using medium containing 10% fetal bovine serum.

### Cryopreservation of cells

To avoid the loss of the cell line, excess cells were preserved in liquid nitrogen as follows: Equal parts of the cell suspension and freezing medium (10% DMSO in complete media) were dispersed to cryo tubes. The cryo tubes were racked in appropriately labeled polystyrene boxes gradually cooled till reaching -80 °C. Then the cryo tubes were transferred to a liquid nitrogen (-196°C).

### Antioxidant Assay

#### Metal chelating activity assay

The iron chelating ability was determined according to the modified method of Pratt et al.[7]. The Fe<sup>2+</sup> were monitored by measuring the formation of ferrous ion-ferrozine complex. The experiment was carried out in 96 micro titer plates. The plant extracts were mixed with FeSO<sub>4</sub>. The reaction was initiated by adding 5mM ferrozine. The mixture was shaken and left at room temperature for 10min. The absorbance was measured at 562nm. EDTA was used as standard, and DMSO as control. All tests and analysis were run in triplicate.

#### Chemiluminescence assay

Luminol or lucigenin-enhanced chemiluminescence assay was performed as described by Helfand 1982 and Haklar 2001. Briefly, 25µL diluted whole blood (1:50 dilution in sterile HBSS<sup>++</sup> (Hanks Balance Salt Solution with Ca and Mg) or 25µL of PMNCs (polymorph nuclear cells) (1x10<sup>6</sup>) or MNCs (mono nuclear cells) (5x10<sup>6</sup>) cells were incubated with 25µL of serially diluted plant extract at concentration ranges between 6.25 and 100µg/mL. Control wells received HBSS<sup>++</sup> and cells but no extract. Tests were performed in white 96 wells plates, which were incubated at 37 °C for 30minutes in the thermostated chamber of the lumino meter. Opsonized zymosan-A or PMA 25µL, followed by 25µL luminol (7x10<sup>5</sup>M) or lucigenin (0.5mM) along with HBSS<sup>++</sup> were added to each well to obtain a 200µL volume/well. The luminometer results were monitored as chemiluminescence RLU (reading luminometer unit) with peak and total integral values set with repeated scans at 30 seconds intervals and one second points measuring time.

### Statistical Analysis

All data are presented as mean±standard deviation of the mean-statistical analysis for all the assays result were done using students t-test significance was tribute to probability values P<0.05 or P< 0.01 in some cases.

## Result and Discussion

More than 50% of all modern drugs in clinical use are of natural products, many of which have the ability to control cancer cells. According to the estimates of the WHO, more than 80% of people in developing countries depend on traditional medicine for their primary health needs. A recent survey shows that more than 60% of cancer patients use vitamins or herbs as therapy [8].

**Table 1:** IC<sub>50</sub> of the methanol extracts of the selected Sudanese medicinal plants for cytotoxicity against PC3 (Prostate cancer) cell line proliferation.

Scientific Name	Part Used	IC <sub>50</sub> ±SD
<i>C. adenogonium</i> ium. steud. exA. RICH	roots	24±0.2
	leaves	>100
	stem	>100

**Table 2:** Effect of plant methanolic extracts on suppressing whole blood Phagocytes and ROS Production.

Scientific Name	Whole Blood IC50 (µg/mL)
<i>C. adenogonium</i> ium. steud. exA. RICH (root)	>200
<i>C. adenogonium</i> ium. steud. exA. RICH (leave)	>200
<i>C. adenogonium</i> ium. steud. exA. RICH (stem)	>200

In the family *Combretaceae*, the extract *C. adenogonium* roots, DC has shown very high activity against PC3 (Prostate Cancer) IC<sub>50</sub> values 24µg/ml. The extract *C. adenogonium* leaves, and stems has shown none active anti against PC3 (Prostate Cancer) IC<sub>50</sub> values >100, and >100µg/ml respectively. All the extracts Effect of Plant Methanol Extracts on suppressing whole blood Phagocytes and ROS Production has shown none active in all plants parts under study *C. adenogonium* roots, leaves, and stems IC<sub>50</sub> values >200. All the examined plant methanol extracts showed different levels of inhibition % for their metal chelating activity at concentration 50µg/mL. *C. adenogonium* stem 50.87µg/m, *C. adenogonium* leaves 49.85µg/m, *C. adenogonium* roots 25.99µg/m, *Combretaceae* is used in traditional medicine systems of several tribes in Tanzania an antibacterial activity at least one of the test bacteria with MIC-

values ranging from 0.31-5.0mg/ml. Two extracts, namely, stem bark and leaf extracts showed mild toxicity with LC<sub>50</sub> values of 65.768µg/ml and 76.965µg/ml, respectively, whereas roots were relatively non-toxic (LC<sub>50</sub> 110.042µg/ml), anti-HIV-1 protease activity, toxicity properties roots and stem bark exhibited anti-HIV-1 PR activity with IC<sub>50</sub> values of 24.7 and 26.5µg/ml, respectively [9]. Phytochemicals in extracts from *C. adenogonium* Steud. Ex A. Rich (*Combretaceae*) indicated presence of flavonoids, terpenoids, alkaloids, tannins, glycosides and saponins. Phytochemicals can be defined, in the strictest sense, as chemicals produced by plants. However, the term is generally used to describe chemicals from plants that may affect health, but are not essential nutrients. There is ample evidence to support the health benefits of diets rich in fruits, vegetables, legumes, whole grains and nuts. Because plant-based foods are complex mixtures of bioactive compounds, information on the potential health effects of individual phytochemicals is linked to information on the health effects of foods [5].

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