



RESEARCH ARTICLE

Anti Prostate Cancer, Anti Oxidant Activity and Cytotoxicity of Some Sudanese Medicinal Plants

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Abstract

Cancer is a class of diseases characterized by out of control cell growth and behavior; Plants have been used in treating human diseases for thousands of years. Since prehistoric times, shamans or medicine men and women of European, Africa, Asia and the Americas acquired a tremendous knowledge of medicinal plants. This paper is carried out to investigate the prostate cancer, antioxidant and Cytotoxicity activities of some Sudanese medicinal plants. *A. Arabica* leaves and gum, *A. mellifera* leaves, *C. Cajan* leaves *T. Foenum graecum* L. Seeds. All the plant parts were extracted using 80% methanol. The anticancer activity was examined by using MTT assay against PC3 (prostate cancer) cell lines and determine their antioxidant activities by testing DPPH activity, screened for their cytotoxicity using - (4, 5-Dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), filter and kept in dark, prepared freshly. The extract *A. Arabica* gum and leaves has shown very high activity against-PC3 and the extract *C.cajan* leaves and *T. Foenum graecum* L and *A. mellifera* leaves is shown none active anti-PC3 with IC50 values 39.4, 64.9, > 100, > 100 and > 100 µg/ml respectively. All the extract revealed Cytotoxicity activity against Vero cell line were found of no toxic and the inhibition percentage with (87.3, 71.7, 66.6) (-48.03, -46.2, 3.7) respectively. The extract shown very high activity against DPPH (above 80%) *A. mellifera* leaves 87.64 µg/ml.

Keywords: PC3; Cytotoxicity; Anticancer; Medicinal Plants; DPPH

Introduction

Cancer is a generic term for a large group of diseases that can affect any part of the body. Other terms used are malignant tumors and neoplasm. Medicinal plants are plants that have at least one of their parts (leaves, stem, barks, or roots) used for therapeutic purposes [1]. Recently, medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria, anemia [2]. Acacia species have possible uses in folk medicine. A 19th-century Ethiopian medical text describes a potion made from an Ethiopian mixed with the roots of the tacha, then boiled, as a cure for rabies [3]. The catechu extract from *Acacia catechu* in the history of chemistry in giving its name to the catechin, catechol and catecholamine chemical families ultimately derived from it [3]. Antitumor study evaluated three types of extracts on AM-3 (Murine mammary adenocarcinoma). The bioactive constituents of fenugreek seeds include mucilage, volatile oils, and alkaloids such as choline and trigonelline. Studies have shown that fenugreek is a potent stimulator of breast milk production and its use was associated with increases in milk production [4]. It can be found in capsule form in many health food stores. Several human intervention trials demonstrated that the anti-diabetic effects of fenugreek seeds ameliorate most metabolic symptoms associated with type-1 and type-2 diabetes in both humans and relevant animal

models by reducing serum glucose and improving glucose tolerance [5]. Study at the Australian Centre for Integrative Clinical and Molecular Medicine in June 2011 found that men aged 25 to 52 who took a fenugreek extract twice daily for six weeks scored 25% higher on tests gauging libido levels than those who took a placebo [6,7]. Pigeon pea is best known as a human food. Short duration shrubby varieties such as ICPL 87 can yield 5-8 t/ha of grain when grown as sole crops [8]. In India, decorticated, split dried peas (dam) are an important protein source. Dahl is 25% protein and has a good balance of all amino acids except methionine and cystine, which are slightly deficient for the human diet [9]. Some anti-nutritional factors are present, but are destroyed by cooking. In the Caribbean and East Africa, pigeon peas are eaten green as a vegetable and are commercial grown and canned in the West Indies. Vitamin A (470 mg/100g) and C (25 mg/100g) contents of vegetable pigeon peas are five times those of green peas [9].

Material and Methods

Collection of tested plant parts

Tested plant parts of the *A. mellifera* leaves collected from

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the MAPRI farm (Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan) *T. Foenum graecum* L., Seeds. In the local market in Khartoum and the *Cajanus cajan* were collected of Algezira state Plant material consisted of the fresh bulb part of *A. arabica* leaves and gum, *Cajanus cajan* during the period June and July 2010 and identified of taxonomist team of MABRI (Medicinal and Aromatic Plants Research Institute, National Center of Research), Khartoum, Sudan and herbarium voucher deposit at herbarium medicinal plants in the MAPRI.

Preparation of crude plant extract

One hundred grams of each plant sample was art coarsely powdered using Mortar and pestle and extracted with 80% methanol for 18 hours using shaker (Stuart scientific, flash shaker, SF 1, UK). The extract was filtered and evaporated using rotary evaporator at 40°C (Buchi, 461, Switzerland).

Fractionations of methanolic extracts

Specific weight of each sample methanolic extract was dissolved in 250 ml distilled water and transferred to 500 ml capacity separating funnel. 100 ml of ethyl acetate was added, shaken gently and allowed to stand till two layers appeared clear. Ethyl acetate layer separated in conical flask and the aqueous was shaken two times more with 100 ml of ethyl acetate in each time. Ethyl acetate layers combined together and evaporated under reduced pressure using rotary evaporator. Aqueous layer was lyophilized using freeze dryer apparatus and the yield percentages of both fractions was calculated.

DPPH free radical scavenging activity method

The DPPH radical scavenging was determined according to the method of Shimada *et al.*, [10], with whose modification. In 96-wells plate, the test samples were allowed to react with 2,2-Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

$$\text{Percent of DPPH inhibition} = \frac{(AB-AA)}{AB} \times 100$$

Where; AA and AB are the absorbance values of the test and of the blank sample, respectively. A percent inhibition versus concentration curve was plotted.

1. 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.

2. 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% CO₂ and were sub cultured twice a week.

3. Procedure

3.1. Maintenance of the human cancer cell lines in the laboratory

A cry tube containing frozen cells was taken out of the liquid nitrogen container and then thawed in a water bath at 37°C. The cry tube was opened under strict aseptic conditions and its content were supplied by 5 ml complete media (RPMI- 1640 in 10% fetal bovine serum) drop by drop in a 50 ml disposable sterile falcon tubes and were centrifuged at 1200 rpm for 10 min to discard the preserving solution. The supernatant was discarded and the cell pellet was seeded in 5 ml complete media in T25 Nunclon sterile tissue culture flasks. The cell suspension was incubated at 37°C in a humidified atmosphere with 5% CO₂ and followed up daily with changing the supplemented medium every 2-3 days.

3.2. Collection of cells by trypsinization

The media was discarded. The cell monolayer was washed twice with 5 ml phosphate buffered saline and all the adherent cells were dispersed from their monolayer by the addition of 1 ml 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 5 ml of the complete media. The trypsin content was discarded by centrifugation at 1200 rpm 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 5 ml complete media in T25 Nunclon sterile tissue culture flasks.

3.3. Determination and counting of viable cells

50 µl of fresh culture media was added to 50 µl 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.

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

The cells were then diluted to give the concentration of single cell suspension required for each experiment. The cell count was adjusted to 1 x 10⁴ -10⁵ cells/ml using medium containing 10% fetal bovine serum.

3.4. 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 cry tubes. The cry tubes were racked in appropriately labeled polystyrene boxes gradually cooled till reaching -80 °C. Then the cry tubes were transferred to a liquid nitrogen (-196°C).

4. Microculture tetrazolium (MTT) assay

4.1. MTT assay

In order to evaluate the Cytotoxicity effect of the extracts and compounds, the following procedure of the MTT was used.

4.2. MTT procedure

Serial dilutions of extract were prepared in a 96 well flat bottomed plate (Nalge Nunc, Inter.). The outer wells of the plate were filled with 250 µl of in-complete culture medium except the last row 6 middle wells (B - G), which were used for the negative control receiving 50 µl of culture medium and 2µl of sterile 0.5% Triton x. To the rest of the plate, 50 µl/wells (CCM) were added and 30 µl more were added to second column wells (B – G) that were used as first extract dilution wells. To the first dilution wells in the row, 500 µg of c suspension extract were added to the 80 µl. extract were then serially diluted by two-fold dilution from well B3 till B11 by transferring 250 µl to the next well after proper mixing. From the last dilution wells (B-11), 50 µl were discarded. Each compound was tested in triplicate. Cell suspension in a complete culture medium containing 2.5 X 10⁵/ml was properly mixed, and 150 µl of it were transferred into each well of the plate. The plate was covered and placed in 5% CO₂ incubator at 37 °C for three-five days (72 hours-120 hours). On the third/fifth day, the supernatant was removed from each well without detaching cells. MTT stock (5 mg/ml) was prepared earlier in 100 ml PBS. The clear suspension was filter sterilized with 0.2 µ Millipore filter and stored at 4 °C or –20 until use. MTT was diluted (1:3.5) in a culture medium and brought to room temperature. To each well of the 96 well plates, 50 µl of diluted MTT were added. The plate was incubated further at 37° C for 2 to 3 hours in CO₂ incubator. MTT was removed carefully without detaching cells, and 200 µl of DMSO were added to each well. The plate was agitated at room temperature for 15 minutes then read at 540 nm using micro plate reader.

$$\% \text{ Inhibition} = [(A \text{ Control} - A \text{ Sample}) / A \text{ Control}] \times 100$$

Where A Control is the absorbance of the negative control and A Sample the absorbance of tested samples or standard.

All data are an average of triplicate analyses.

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

Modern pharmacology, however, relies on refined chemicals - either obtained from plants, or synthesized. The first pure medicinal substance derived from plants was morphine, extracted from the opium poppy at the turn of the 19th century. Often; chemicals extracted from plants are altered to produce drugs. For example, diosgenin is obtained from various yam (*Dioscorea*) species of South America, and is converted to progesterone, the basis of the oral contraceptive pill. Aspirin-like chemicals were once obtained from willows (*Salix* species) and European meadowsweet (*Filipendula ulmaria*), but aspirin is now synthesized in the laboratory. Higher plants have given rise to about 120 commercial drugs and 10-25% of all prescription drugs contain at least one active compound from a higher plant [11,12]. A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). It has also been used to quantify antioxidants in complex biological systems in recent years. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm when the odd electron of DPPH radical becomes paired with hydrogen the resulting decolonization is stoichiometric with respect to number of electrons captured. So the main objective of this paper was to screen and fractionation of active plant in four Sudanese medicinal plants for their anticancer and antioxidant to find more medicinal plants potent anticancer activity to be the future plants can cure cancer and leads to isolation of active compounds.

A. mellifera leaves belong to the family Fabaceae against PC3 showed none activity show table 1,2, but fractionations Aqueous extract is good anti prostate cancer activity IC₅₀ 81 show table 3 and Cytotoxicity in Vero cell line not toxic in all concentrations under this study show table 4,5. Many of these

Traditional medicine	Yield (%)	Part used	Family	Scientific name
cancer, cough, dysuria, fever	31.381	leaves	Fabaceae	<i>Acacia arabica</i> (LAM.) Wild.
	44.850	Gum		
cough, dysuria, fever	21.861	leaves		<i>Acacia mellifera</i> (Vahl) Benth.
Cancer, antibacterial, antiplasmodic.	18.581	leaves		<i>Cajanus cajan</i> (L.) Millsp.
Asthma, arthritis, gastrointestinal ailments, bronchitis, metabolism disorders, male impotency, skin problems.	13.4	seeds		<i>Trigonella foenum-graecum</i> L.

Table 1: Indicate the Yield% of methanol (80%) extract and traditional use of selected Sudanese medicinal plants investigates of their anti cancer and antioxidant activities.

Name of plant	weighting	Ethyl acetate extract		Aqueous extract	
		Weight	Yield (%)	Weight	Yield (%)
<i>A. mellifera</i> (Vahl) Benth.	100	3.572	3.5	3.612	3.6

Table 2: Yield percentage of water and ethyl acetate fractions.

Scientific name	Plant parts	Inhibition % \pm SD
<i>A. arabica</i> (LAM.) Wild.	leaves	64.9 \pm 3.9
	Gum	39.4 \pm 1.6
<i>A. mellifera</i> (Vahl) Benth.	leaves	>100
<i>C. cajan</i> (L.) Millsp.	Leaves	> 100
<i>T. foenum graecum</i> L.	seeds	>100
<i>C. cajan</i> (L.) Millsp.	Leaves	> 100
<i>T. foenum graecum</i> L.	seeds	>100

Table 3: Screening of selected Sudanese medicinal plants with methanol extracts for Cytotoxicity against PC3 (prostate cancer) cell line.

Scientific name	Ethyl acetate extract	Aqueous extract
	IC ₅₀ \pm SD	IC ₅₀ \pm SD
<i>A. mellifera</i> (Vahl) Benth.	> 100	81 \pm 2.6

Table 4: Screening of ethyl acetate and aqueous fractionations of *A. mellifera* plants against PC3 (Prostate cancer) cell line proliferation.

Scientific name	Part used	Concentration μ g/mL	Inhibition % \pm SD	IC ₅₀ (μ g/mL)
<i>A. mellifera</i> (Vahl) Benth.	leaves	500	76.24602 \pm 0.06	In active
		250	72.95151 \pm 0.05	
		125	55.31019 \pm 0.01	
<i>C. cajan</i> (L.) Millsp.	leaves	500	87.34495 \pm 0.03	In active
		250	71.78946 \pm 0.06	
		125	66.69819 \pm 0.10	
<i>T. foenum graecum</i> L.	seeds	500	- 48.03 \pm 30.2	429.4
		250	- 46.22 \pm 18.3	
		125	3.76 \pm 21.5	

Table 5: This table indicates the MTT Cytotoxicity of 80% methanol extract of some selected Sudanese medicinal plants the reading in triplicate for different concentration from 125-500 μ g/mL.

compounds are psychoactive in humans. The alkaloids found in Acacias include dimethyltryptamine (DMT), 5-methoxydimethyltryptamine (5-MeO-DMT) and N-methyltryptamine (NMT). Nineteen different species of Acacia in the Americas contain cynogenic glycosides, which, if exposed to an enzyme which specifically splits glycosides, can release hydrogen cyanide (HCN) in the acacia leaves. It turns out that, if acacia "leaves" lack the specific glycoside-splitting enzyme, then they may be less toxic than otherwise, even those containing significant quantities of cyanic glycosides [13]. The methanol extracts of the plants *Cajanus cajan* which belong to the family Fabaceae showed none anticancer activity against PC3 cell line and Cytotoxicity in Vero cell line none toxic in all concentrations under this study show table 5, and also moderate activity against antioxidant DPPH the inhibition percentage (41.47), Chemical studies reveal 2' -2' methyl cajanone, 2' -hydroxygenistein, isoflavones, cajanin, cahanones etc., which impart antioxidant properties [11]. Presence of cajanuslactone, a coumarin imparts antibacterial activity. Presence of cajanin stilbene acid, pinostrobin, vitexinandorientin is responsible for antiplasmodic activity [11].

On the other hand the anticancer activity of the methanol extracts of Fenugreek seeds belong to the family Fabaceae against PC3 showed none activity show table 3 And Cytotoxicity in Vero cell line not toxic in all concentrations under this study show table 5, moderate activity against antioxidant DPPH the inhibition percentage (59.13). Fenugreek seeds are a rich source of the polysaccharide galactomannan. They are also a source of saponins such as diosgenin, yamogenin, gitogenin, tigogenin, and neotigogens. Other bioactive constituents of fenugreek include mucilage, volatile oils, and alkaloids such as choline and trigonelline. Studies have shown that fenugreek is a potent stimulator of breast milk production and its use was associated with increases in milk production [4]. In India and China, Fenugreek seeds are recommended treating asthma, arthritis, gastrointestinal ailments, bronchitis, metabolism disorders, male impotency, skin problems (open wounds, hives and boils), sore throat, and acid reflux [5].

Conclusion

Numerous medicines in use today are extracted from plants. About 50 to 60% of pharmaceutical drugs are either of natural origin or obtained through use of natural products as starting points in their synthesis [14-16]. In this important study some Sudanese medicinal plants were investigated for their anticancer, antioxidant and Cytotoxicity to discover some new medicinal plants and compound that can be used for discovery drug to treatment of cancer diseases. The extract of *A. arabica* gum belong to the family Fabaceae against PC3 shown very high activity IC₅₀ 39.4 μ g/ml MTT Cytotoxicity shown activity IC₅₀ 296.2 μ g/ml and *A. Arabica* leaves against PC3 shown high activity IC₅₀ 64.9 μ g/ml. and MTT Cytotoxicity none toxic.

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