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Anti-prostate cancer, and chemiluminescence activities of some selected Sudanese Medicinal plants

Montasir Ahmed Elnour[1]*, Mohamed Ahmed Mesaik[2] and Asaad Khalid[2]

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[1]Medicinal and Aromatic Plants Research Institute, National Center for Research, P.O.Box, 2404 Khartoum, Sudan.
[2]Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi - 75270, Pakistan.

ABSTRACT

Plants have been used in treating human diseases for thousands of years; Sudanese medicinal plants used locally in traditional medicine to treat wide range of diseases were investigated for their anticancer, antioxidant activities and Cytotoxicity. This work was carried out to investigate the anticancer, antioxidant and cytotoxicity activities of four Sudanese medicinal plants commonly used as anti-inflammatory and anti-tumor. *Hibiscus sabdariffa* L fruits, *Sonchus oleraceus* L.leaves, *Halexylon salicornietum* (MAB) whole plant, *Prosopis juliflora* (SW.) DC leaves. 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 Chemiluminescence 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 *Prosopis juliflora* (SW.) DC is showed very high against-PC3 and *Hibiscus sabdariffa* is showed high against-PC3 the extract of *Halexylon salicornietum* and *Sonchus oleraceus* L is showed none active anti- PC3 with IC_{50} values 30.1 , 94.7, > 100, and > 100 µg/ml respectively. All the extracts revealed cytotoxicity activity against Vero cell line except last concentration in extract of *Sonchus oleraceus* L, and the inhibition percentage with (90.56,87.12,86.24) (82.78,82.31,77.38) (75.21,59.49,41.24) (74.93,73.78,71.13) respectively. The extracs of *Hibiscus sabdariffa*, *Halexylon salicornietum* and *Sonchus oleraceus* L. are revealed low active against Chemiluminescence assay, *Prosopis juliflora* (SW.) DC is revealed high active against Chemiluminescence assay.with IC_{50} values 166.6, 189.5, > 176.2 and 75.4 µg/ml respectively.

Keywords: Anticancer, medicinal plants, cytotoxicity, PC3, chemiluminescence

*Corresponding author E-mail: montesrelnour@yahoo.com Tel. 00249123770969

INTRODUCTION

Cancer is a collective term used for a group of diseases that are characterized by the loss of control of the growth, division, and spread of a group of cells, leading to a primary tumor that invades and destroys adjacent tissues. It may also spread to other regions of the body through a process known as metastasis, which is the cause of 90% of cancer deaths. Cancer remains one of the most difficult diseases to treat and is responsible for about 13% of all deaths worldwide, and this incidence is increasing due to the ageing of population in most countries, but especially in the developed ones (Segota and Bukowski, 2004) Around half of the drugs currently in clinical use as anticancer drugs are of natural product origin, and it has been estimated that about 60% of new chemical entities introduced in the 1981–2002 period in this field were natural products or were derived from a natural lead compound. Despite this, pharmaceutical companies have recently neglected the development of potential natural drug candidates in favor of combinatorial chemistry and High-throughput synthesis of large compound libraries. The main reason of this reluctance to use natural products as drug candidates lies primarily in supply problems that make the development of synthetic routes necessary, which are often long and difficult to

Table 1. IC_{50} of the methanol extracts of the selected Sudanese medicinal plants for cytotoxicity against PC3 (Prostate cancer) cell line proliferation.

Scientific name	Part used	IC_{50}±SD
S.oleraceus L.	leaves	>100
H. salicornietum (MAB).	whole plant	>100
H. sabdariffa L.	fruits	94.7 ± 9.1
P.juliflora (SW.) DC.	leaves	30.1 ± 0.3

scale-up owing to their structural complexity. It is becoming increasingly apparent, however, that the unguided production of vast libraries of compounds is unlikely to result in the identification of new drugs (Cozzi et al., 2004) 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. 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 (Madhuri and Govind, 2009).

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. All of the native plant species discussed in detail in this work was used by native people in traditional medicine. The fact that hundreds of additional species were also used by First Nations Canadians (Arnason et al., 1981) suggested that many of these also have important pharmacological constituents that could be valuable in modern medicine. Up to the 18th century, the professions of doctor and botanist were closely linked. Indeed, the first modern botanic gardens, which were founded in 16th century Italy, in Pisa, Padova and Florence, were medicinal plant gardens attached to medical faculties or schools. The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines (Duke and Actchly, 1984). A 1997 survey showed that 23% of Canadians have used herbal medicines. In addition, as much as 25% of modern Pharmaceutical drugs contain plant ingredients (Duke, 1993).

Since most of the standard anticancer treatments are not selective and affect both tumor and normal cells, thereby causing systemic toxicity or increased risk of other cancers. Thus, there is a desire need for the development of safer alternatives for the treatment of cancer which are affordable, accessible, having less toxicity and minimum side effects. A change in the life style including healthy diet and exercise still remains a better preventive measure against cancer. Furthermore, in folklore system several herbal medicines or mixtures have been used to treat cancer by either boiling the plant material in water or soaking it in alcohol. These observations and claims have generated tremendous interest of the researchers to provide scientific basis of their anticancer activities. As consequence variety of molecules with diverse mechanism of action has emerged as inhibitors of cancer (Kinghorn et al., 2004)(6) and there are phenomenal number of research articles for comprehensive reviews such as Dorai and Agarwa (2004), Srivastava et al. (2005).

Table 1 presents some of the anticancer agents derived from plants and the list is growing due to revival and interest in alternative medicine, new technologies with greater chances of discovering novel anticancer agents. *Hibiscus sabdariffa* family is Malvaceae reported to be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant, resolvent, sedative, stomachic, and tonic, roselle is a folk remedy for abscesses, bilious conditions, cancer, cough, debility, dyspepsia, dysuria, fever, hangover, heart ailments, hypertension, neurosis, scurvy, and strangury. The drink made by placing, the calyx in water, is said to be a folk remedy for cancer. Medicinally, leaves are emollient, and are much used in Guinea as a diuretic, refrigerant, and sedative; fruits are antiscorbutic; leaves, seeds, and ripe calyces are diuretic and antiscorbutic; and the succulent calyx, boiled in water, is used as a drink in bilious attacks; flowers contain gossypetin, anthocyanin, and glucoside hibiscin, which may have diuretic and choleretic effects, decreasing the viscosity of the blood, reducing blood pressure and stimulating intestinal peristalsis. In Burma, the seeds are used for debility, the leaves as emollient. Taiwanese regard the seeds as diuretic, laxative, and tonic. Philippines use the bitter root as an aperitive and tonic (Perry, 1980). Angolans use the mucilaginous leaves as an emollient and as a soothing cough remedy. Central Africans poultice the leaves on abscesses. Alcoholics might consider one item: simulated ingestion of the plant extract decreased the rate of absorption of alcohol, lessening the intensity of alcohol effects in chickens (Watt and Breyer-Brandwijk, 1962).

Sonchus oleraceus study of SO extracts showed concentration-dependent antioxidant activity. The methanol extracts yielded the greatest the most phenolic and flavonoid contents. Cytotoxicity activity showed the ethanol extract had the best activity against the growth of stomach cancer cell (Zeinab et al., 2010). Antitumor study evaluated three types of extracts on AM-3 (Murine

Table 2. Screening of ethyl acetate and aqueous fractionations of Sudanese medicinal plants against PC3 (Prostate cancer) cell line proliferation.

Scientific name	Ethyl acetate extract	Aqueous extract
	IC_{50}±SD	IC_{50}±SD
H.salicornietum (MAB).	> 100	> 100
H. sabdariffa L.	55.5 ± 1.6	> 100

mammary adenocarcinoma). In the group treated with cold aqueous extract for a month, necrotic changes in cancer mass were noted. Results showed antitumor effects and, possibly, activation of the immune system (Zeinab et al., 2010).

Halexylon salicornietum Family Chenopodiaceae contains Gluids like Haloxine, Halosaline, Anabazine and oxiderene. this medicinal plants uses in folk medicine in the wounds, fever Rhumatizme, and antidiptic.

Porsopis juliflora the juice is used in folk remedies for that cancerous condition he terms "superfluous flesh." Reported to be cathartic, cyanogenetic, discutient, emetic, poison, stomachic, and vulnerary, mesquite is a folk remedy for catarrh, colds, diarrhea, dysentery, excrescences, eyes, flu, headcold, hoarseness, inflammation, itch, measles, pinkeye, stomachache, sore throat, and wounds, Pima Indians drank the hot tea for sore throat (Lewis and Elvin-Lewis, 1977). This work was carried out to investigate the anticancer, antioxidant and cytotoxicity activities of four Sudanese medicinal plants commonly used as anti-inflammatory and anti tumor.

MATERIAL AND METHODS

Collection of tested plant parts

Tested plant parts of the *Hibiscus sabdariffa* collected from the farm of Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan (MAPRI) and the Cajanus *cajan* were collected of Algezira state Plant material consisted of the fresh bulb part of *Hibiscus sabdariffa* , *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

100 g of each plant sample was art coarsely powdered using Mortar and pistil and extracted with 80% methanol for 18 h using shaker (Stuart scientific, flash shaker, S F 1, U K). The extract was filter and evaporated using rotary evaporator at 40°C (Buchi, 461, Switzerland).

Fractionations of methanolic extracts

Specific weight of each sample was dissolved in 250 ml distilled water and transferred to 500 ml capacity separating funnel. 100 ml of ethyl acetate was added, shacked gently and allowed to stand till two layers appeared clear. Ethyl acetate layer separated in conical flask and the aqueous was shacked tow times more with 100 ml of ethyl acetate in each time. Ethyl acetate layers combined together and evaporated under reduced pressure using rotary evaporator (Table 2). Aqueous layer was lyophilized using frees dryer apparatus and the yield percentages of both fractions was calculated Harborne, (1984).

Chemiluminescence assay

Luminol or lucigenin–enhanced chemiluminescence assay was performed as described by Helfand *et al*. (1982) and Haklar *et al*. (2001). Briefly, 25 µL diluted whole blood (1:50 dilution in sterile $HBSS^{++}$) or 25 µL of PMNCs (1×10^6) or MNCs (5×10^6) cells were incubated with 25 µL of serially diluted plant extract at concentration ranges between 6.25 and 100 µg/mL. Control wells received $HBSS^{++}$ and cells but no extract. Tests were performed in white 96 wells plates, which were incubated at 37°C for 30 min in the thermostated chamber of the luminometer. Opsonized zymosan-A or PMA 25 µL, followed by 25 µL luminol (7×10^5M) or lucigenin (0.5 mM) along with $HBSS^{++}$ were added to each well to obtain a 200 µL 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.

Culture media and human tumor cell lines

Human Cell lines

PC3 (prostate cancer cell line) were obtained frozen in liquid nitrogen (-180 ^{0}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_2 and were sub cultured twice a week.

Procedure

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 was 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% CO_2 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 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 min. 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 min. 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.

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.

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

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 10^4 -10^5 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 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).

Microculture tetrazolium (MTT) assay

MTT assay

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

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 2 µl 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 × 10^5/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% CO_2 incubator at 37°C for three-five days (72-120) h. 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. MTT suspension was vortexed and kept on magnetic stirrer until all MTT dissolved. 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 h in CO_2 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 min then read at 540 nm using micro plate reader.

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

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

Table 3. Effect of extracts on whole blood phagocytes. ROS production.

Scientific Name	Whole Blood IC_{50} (µg/mL)
S. oleraceus L.	176.2 ± 6.9
P. juliflora (SW.) DC.	75.4 ± 1.5
H.sabdariffa L.	166.6 ± 24.8
H.salicornietum (MAB).	189.5 ± 14.4

Table 4. Effect of fractionation on Whole blood phagocytes.ROS production.

Scientific name	Ethyl acetate extract	Aqueous extract
	IC_{50}±SD	IC_{50}±SD
H. salicornietum (MAB).	> 200	> 200
H. sabdariffa L.	> 200	> 200

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 .

RESULTS AND DISCUSSION

The most frequent types of cancer differ between men and women, about 30% of cancer deaths are due to the five leading behavioral and dietary risks: high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use and alcohol use, tobacco use is the most important risk factor for cancer causing 22% of global cancer deaths and 71% of global lung cancer deaths, cancer causing viral infections such as HBV/HCV and HPV are responsible for up to 20% of cancer deaths in low and middle-income countries, about 70% of all cancer deaths in 2008 occurred in low and middle-income countries, deaths from cancer worldwide are projected to continue rising, with an estimated 13.1 million deaths in 2030 (WHO, 2012). The antioxidants may prevent and cure cancer and other diseases by protecting the cells from damages caused by 'free radicals' the highly reactive oxygen compounds. Many naturally occurring substances present in the human diet have been identified as potential chemo-preventive agents; and consuming relatively large amounts of vegetables and fruits can prevent the development of cancer. Many plant-derived products have been reported to exhibit potent anti-tumors activity against several rodent and human cancer cell lines **(**Madhuri and Govind, 2009). So the main objective of this paper was to screen and fractionation of active plant in four Sudanese medicinal plants for their anticancer to find more medicinal plants potent anticancer activity to be the future plants can cure cancer and leads to isolation of active compounds (Table 1). *Hibiscus sabdariffa* belong to the family Malvaceae against PC3 showed high activity IC_{50} (94.6, µg/ml). The most common phytochemical groups in saponin the tested plants are the flavonoids and tannins, as shown in the (Tables 3 and 4) (Montasir et al., 2013).

Cytotoxicity in Vero cell line not toxic in all concentrations under this study (Montasir et al., 2013). In folk medicine reported to be antiseptic, refrigerant, resolvent, sedative, stomachic, and tonic, rosella is a folk remedy for abscesses, bilious conditions, cancer, cough, debility, dyspepsia, dysuria, fever, hangover, heart ailments, hypertension, neurosis, scurvy, and strangely. The drink made by placing, the calyx in water, is said to be a folk remedy for cancer. Antioxidant compound in hibiscus called phenolic acid protect the liver and helps prevent cancer cells, anthocyanins and poly phenol-rich extracts of hibiscus promoted early cell death in tissue cultures of leukemia and stomach cancer cell line (McCall et al.,2011), in these plants the fractionation activity is high activity in ethyl acetate fractionation IC_{50} (55.5 µg/ml).

Prosopis juilflora belong to the family Mimosaceae against PC3 showed very high activity IC_{50} 30.1 µg/ml show (Table 1), and cytotoxicity in Vero cell line not toxic in all concentrations under this study (Montasir et al., 2013). The previous study showed that *Prosopis juliflora* contain many secondary metabolites compounds for example the leaves contain tannins, acids, glycosides, flavonoids and alkaloids, (Sathiya and Muthuchelian, 2008). The methanol extracts of the plants *Halexylon salicornietum* which belong to the family Chenopodiaceae showed none anticancer activity against PC3 cell line And cytotoxicity in Vero cell line not toxic in all concentrations under this study (Montasir et al., 2013). On the other hand the anticancer activity of the methanol extracts of *Sonchus oleraceus belong* to the family Asteraceae against PC3 showed none activity And cytotoxicity in Vero cell line not toxic in all concentrations under this study.

Conclusion

In this study four Sudanese medicinal plants were investigated for their anticancer and antioxidant activity to discover some new medicinal plants that can be used for treatement of cancer diseases. Anticancer activity of these plants was determined using MTT colorometric assay for PC3 (prostat cancer) *P. juliflora* leaves, *H. sabdariffa* fruits with IC_{50} 30.1, 94.6 µg/ml respectively. The extracts of *Hibiscus sabdariffa*, *Halexylon*

salicornietum and *Sonchus oleraceus* L. showed low active against Chemiluminescence assay, *Prosopis juliflora* (SW.) DC are showed high active against Chemiluminescence assay with IC_{50} values 166.6, 189.5, > 176.2 and 75.4 µg/ml respectively. All the extract revealed cytotoxicity activity against Vero cell line except last concentration in extract of *Sonchus oleraceus*

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