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Anti-Cancer, Anti-Oxidant Activity and Phytochemical Properties of *Acacia arabica*

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ABSTRACT

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 need for the development of safer alternatives for the treatment of cancer which are affordable, accessible, having less toxicity and minimum side effects. This paper is carried out to investigate the anticancer MCF7 (breast cancer), HeLa (cervical cancer) cell line, antioxidant (iron chelating properties and chemiluminescence assay), cytotoxicity activities and phytochemical investigation Sudanese medicinal plant *A. arabica* leaves and gum.

Plant parts were extracted using 80% methanol. The anticancer activity was examined by using MTT assay. And determine their antioxidant activities, 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 is showed high activity against chemiluminescence assay IC50 values 27.3 µg/ml and no activity in leaves >100 µg/ml, antioxidant (Iron chelating properties) no activity levels of inhibition % in *A. arabica* gum 11.92 µg/ml. The methanol extracts of the plant showed considerable activity against MCF7 (breast cancer) according to the inhibition percentage *A. arabica* gum. Also *A. arabica* leaves and gum against HeLa (cervical cancer) cell line no activity IC50 values>100 µg/ml.

Phytochemical screening of the extracts *A. arabica* leaves indicated presence of alkaloids, cumarin, triterpenes, saponins in trace amount and sterols in moderate amount and tannins, flavonoids in high amount and anthraqunones were not detected. *A. arabica* gum indicated presence of alkaloids, cumarin in trace amount and triterpenes in moderate amount and tannins, flavonoids, sterols, saponins in high amount and anthraqunones were not detected.

Keywords: Anticancer, Chemiluminescence, Phytochemical, HeLa

INTRODUCTION

Two classes of use of medicinal preparations are commonly recognized, often under the titles herbal and pharmaceutical. Pharmaceuticals, discussed below, are refined or synthesized drugs. The World Health Organization has defined medicinal herbals as follows [1]. Finished, labeled medicinal products that contain as active ingredients aerial or underground parts of plants or other plant material or combinations thereof, whether in the crude state or as plant preparations. Plant material includes juices, gums, fatty oils, essential oils and any other substances of this nature. Herbal medicines may contain excipients [inert additives such as starch used to improve adhesive quality in order to prepare pills or tablets in addition to the active ingredients. Medicines containing plant material combined with chemically defined active substances, including chemically defined, isolated constituents of plants, are not considered to be herbal medicines. Exceptionally, in some countries herbal medicines may also contain, by tradition, natural organic or inorganic active ingredients which are not of plant origin [1].

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Botanical description

First described in Africa by the Swedish botanist Carl Linnaeus in 1773. They are pod-bearing, with sap and leaves typically bearing large amounts of tannins and condensed tannins that historically found use as pharmaceuticals and preservatives. The genus Acacia previously contained roughly 1300 species, about 960 of them native to Australia, with the remainder spread around the tropical to warm-temperate regions of both hemispheres, including Europe, Africa, southern Asia and the Americas. However, in 2005 the genus was divided into five separate genera under the tribe "Acacieae". The genus Acacia was retained for the majority of the Australian species and a few in tropical Asia, Madagascar and Pacific islands. Most of the species outside Australia and a small number of Australian species were reclassified into vachellia and senegalia. The two final genera, Acaciella and Mariosousa, each contain about a dozen species from the America.

Biological activity

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 [2]. An astringent medicine high in tannins, called catechuor cutch, is procured from several species, but more especially from Acacia catechu.by boiling down the wood and evaporating the solution so as to get an extract [3]. The catechu extract from *Acacia catechu* figures in the history of chemistry in giving its name to the catechin, catechol and catecholamine chemical families ultimately derived from it [2].

PHYTOCHEMICAL PROPERTIES

Acacias contain a number of organic compounds that defend them from pests and grazing animals [4]. Many of these compounds are psychoactive in humans. The alkaloids found in Acacias include dimethyltryptamine (DMT), 5-methoxy-dimethyltryptamine (5-MeO-DMT) and N-methyltryptamine (NMT). The plant leaves, stems and/or roots are sometimes made into a brew together with some MAOI-containing plant and consumed orally for healing, ceremonial or religious uses. Nineteen different species of Acacia in the Americas contain cynogenic glycosides, which, if exposed to an enzyme which specifically splits glycosides, can release hydrogen cyanid (HCN) in the acacia "leaves". If fresh plant material spontaneously produces 200 ppm or more HCN, then it is potentially toxic. This corresponds to about 7.5 µmol HCN/g of fresh plant material. 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 [4].

MATERIALS AND METHODS

Collection of tested plant parts

Tested plant parts of the *Acacia arabica* leaves and gum were collected from Elsunut Forest, Khartoum State, collected of 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 extracted for 18 h with by 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).

Culture media and human tumor cell lines human cell lines

MCF7 (breast cancer), HeLa (cervical 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_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 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% 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 hemocytometer. Viable cells were counted and the following equation was used to calculate the cell count/ml of cell suspension.

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

The cells were then diluted to give the concentration of single cell suspension required for each experiment. The cell count was adjusted to 1×10^4-10^5 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).

Micro culture 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 walls of the plate were filled with 250 µl of incomplete 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 2-3 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.

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

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.

Antioxidant assay

Metal chelating activity assay: The iron chelating ability was determined according to the modified method of Dinis et al. [5]. The Fe^{+2} 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 $FeSO_4$. The reaction was initiated by adding 5 mM ferrozine. The mixture was shaken and left at room temperature for 10 min. The absorbance was measured at 562 nm. 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. Briefly, 25 µL diluted whole blood (1:50 dilution in sterile HBSS++ (Hanks Balance Salt Solution with Ca and Mg) or 25 µL of PMNCs (polymorph nuclear cells) (1×10^6) or MNCs (mono nuclear cells) (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 (7×10^5 M) 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 s intervals and one second points measuring time.

Phytochemical screening

General phytochemical screening to detect the chemical groups was carried out for all extracts using the methods described by various researchers [6-9] with few modifications.

Identification of tannins

0.2 g of each extract was dissolved in 10 ml of hot saline solution and divided in two test tubes. In the first tube 2-3 drops of ferric chloride were added and 2-3 drops of gelatin salt reagent were added to the other tube. The occurrence of a blackish blue color in the first test tube and turbidity in the second one indicates the presence of tannins.

Test of sterols and triterpenes

0.2 g of each extract was dissolved in 10 ml of chloroform, to 50 ml of the solution 0.5 ml acetic anhydride was added and then 3 drops of conc. Sulphuric acid at the bottom of the test tube. At the contact zone of the two liquids a gradual appearance of green, blue pink to purple color is indicates the presence of sterols (green to blue) and or triterpenes (pink to purple) in the sample.

Test for alkaloids

0.5 g of each extract was dissolved in 2 ml of 2 N HCl in water bath and stirred while heating for 10 min, then cooled filtered and divided into two test tubes. To the first test tube a few drops of Mayer's reagent were added while to the other tube a few drops of Valser's reagent were added. A slight turbidity or heavy precipitate in either of the two test tubes indicates the presence of alkaloids.

Tests for flavonoids

0.5 g of each extract was dissolved in 30 ml of 80% ethanol and filtered. The filtrate was used for following tests:

(i) To 3 ml of the filtrate in a test tube 1 ml of 1% aluminum chloride solution was in methanol was added. Formation of a yellow color indicated the presence of flavonoids. Flavones and or chalcone.

(ii) To 3 ml of the filtrate in a test tube 1 ml of 1% potassium hydroxide solution was added. A dark yellow color indicated the presence of flavonoids compounds (flavones or flavonones) chalcone and/or flavonols.

(iii) To 2 ml of the filtrate 0.5 ml of magnesium turnings were added. Production of a faint pink or red color was taken as presumptive evidence that flavonones are present in the plant sample.

Test for saponins

0.3 g of each extract was placed in a clean test tube. 10 ml of distilled water were added, the tube stoppered and vigorously shaken for about 30 s. The tube was then allowed to stand and observed for the formation of foam, which persisted for at least 1 h, was taken as evidence for presence of saponins.

Test for cumarins

0.2 g of each extract dissolved in 10 ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5 N KOH put on it. Then the filter paper was inspected under UV light, the presence of coumarins was indicated if the spot was found to have adsorbed the UV light.

Test for anthraquinone glycosides

0.2 g of each extract was boiled with 10 ml of 0.5 N KOH containing 1 ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5 ml of the benzene solution was shaken with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones was indicated if the alkaline layer was found to have assumed pink or red color.

Test for cyanogenic glycosides

0.2 g of extract fraction was placed in Erlenmeyer flask and sufficient amount of water was added to moisten the sample, followed by 1 ml of chloroform (to enhance every activity). A piece of freshly prepared sodium picrate paper was carefully inserted between a split cork which was used to stopper the flask, a change in color of the sodium picrate paper from yellow to various shades of red was taken as an indication of the presence of cyanogenic glycoside.

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.

RESULTS AND DISCUSSION

The importance of medicinal plants, and the contribution of phytomedicine to the well-being of a significant number of the world's population, has attracted interest from a variety of disciplines. The isolated polyphenols from strawberry including kaempferol, quercetin, anthocyanins, coumaric acid and ellagic acid were shown to inhibit the growth of human cancer cell lines originated from breast (MCF-7), oral (KB, CAL-27), colon (HT-29, HCT-116) and prostata (LNCaP, DU-145) [10].

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 [11].

So the main objective of this paper was to screen Sudanese medicinal plant parts *A. arabica* for their anticancer and antioxidant activity to find medicinal plants having potent anticancer activity.

In the present work the first experiment was determined the anticancer activity against MCF7 (Breast cancer) and HeLa (cervical cancer) used traditionally to treat various diseases followed by two methods determine the antioxidant activity of the crude extracts which were Iron chelating properties and chemiluminescence assay.

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 [12]. 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 [11].

Very high activity against PC3 cell lines (IC_{50} 39.4 µg/ml) the antioxidant activity using the DPPH assay of the gum was very high (IC_{50} 1.009 µg/ml) whereas the leaves showed high activity with (IC_{50} 12.8 µg/ml) [13]. Moreover both tested parts (leaves and gum) were not toxic on normal cell lines (IC_{50}>100 µg/ml) [13]. Phytochemical screening of the extracts *A. arabica* leaves indicated presence of alkaloids, coumarin, triterpenes, saponins in trace amount and sterols in moderate amount and tannins, flavonoids in high amount and anthraquenones were not detected. Phytochemical, screening of the extracts *A. arabica* gum indicated presence of alkaloids, coumarin in trace amount and triterpenes in moderate amount and tannins, flavonoids, sterols, saponins in high amount and anthraqunones were not detected. Gum of Acacia for their oxidative burst high activity against whole blood (IC_{50} 27.3 µg/ml). In previous study Acacia species have possible uses in folk medicine. A 19th century Ethiopian medical text describes a potion made from the gum and leaves and mixed with the roots of the tacha, then boiled and used as a cure for rabies [2]. An astringent medicine high in tannins, called catechuor cutch, is procured from several species, but more especially from *Acacia catechu* by boiling down the wood and evaporating the solution so as to get an extract [3]. The catechu extract from *Acacia catechu* figures in the history of chemistry in giving its name to the catechin, catechol and catecholamine chemical families ultimately derived from it [2]. Acacia contain a number of organic compounds that defend them from pests and grazing animals [4]. Many of these compounds are psychoactive in humans. The alkaloids found in Acacia include dimethyltryptamine (DMT), 5-methoxy-dimethyltryptamine (5-MeO-DMT) and N-methyltryptamine (NMT). The plant leaves, stems and/or roots are sometimes made into a brew together with some MAOI-containing plant and consumed orally for healing, ceremonial or religious uses. Nineteen different species of Acacia in the Americas contain cynogenic glycosides, which, if exposed to an enzyme which specifically splits glycosides, can release hydrogen cyanid (HCN) in the Acacia "leaves". If fresh plant material spontaneously produces 200 ppm or more HCN, then it is potentially toxic. This corresponds to about 7.5 µmol HCN/g of fresh plant material. 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 [4].

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