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Anticancer Activity of *Bauhinia rufescens* (Lam) leaf Extracts on MCF-7 Human Breast Cancer Cells

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Abstract

Cancer is clearly worldwide problem. The incidence and mortality rates for various cancers are similar, but not identical, among developed countries. The present study was designed to investigate the cytotoxic activities of *Bauhinia rufescens* extracts against MCF-7 cell line. MCF-7 cells were cultured in MEM medium and incubated with different concentrations 31.25, 62.50, 125, 250 and 500 µg/ml of *Bauhinia rufescens* extracts for 72 h. Post-treatment, percent cell mortality was studied by (MTT) assays. The results showed that petroleum ether and methanol extracts significantly reduced cell mortality on MCF-7 cells in a concentration dependent manner. Concentrations of 31.25 µg/ml and above of petroleum ether and 500 µg/ml of methanol extract were found to be cytotoxic in MCF-7 cells. Cell mortality at 31.25, 62.50, 125, 250 and 500 µg/ml of petroleum ether extract was recorded as 61.69%, 64.12%, 78.39%, 82.34% and 87.35% respectively, whereas at 31.25, 62.50, 125, 250 and 500 µg/ml of methanol extract values were 56.97%, 59.35%, 66.86%, 71.26% and 74.22% respectively by MTT assay. The data revealed that the treatment with petroleum ether and methanol of *Bauhinia rufescens* leaf extract induced cell death in MCF-7 cells. However, further work is running on to identify the compound(s) responsible for anticancer activity in each extract.

Keywords: *Bauhinia rufescens*, MCF-7, Anticancer, Cytotoxicity.

1. Introduction

Cancer is the major cause of mortality in the world and it claims more than 6 million lives each year (Chermahini *et al.*, 2010) [5]. Methods commonly used for the treatment of cancer although possess some benefits but still there is a significant need to improve current cancer therapies and search for novel compounds (Chermahini *et al.*, 2010) [5]. Breast cancer is one of the most common causes of the cancer in females in whole world (WCR, 2008) [25]. It has been observed that breast cancer accounts for 23% of all newly occurring cancers in women worldwide and represents 13.7% of all cancer deaths due to the breast cancer in male and female (Ferlay *et al.*, 2000) [7].

It is the most frequent cancer in both developed and developing regions, but the rate of human breast cancer is higher in developing countries in compared to developed nations (Ferlay *et al.*, 2000) [7]. Over the past several decades, there has been a particular interest in the role of medicinal plant extracts in cancer prevention. Plants are rich sources of chemically diverse compounds, many with beneficial properties to human health. Consequently, about 50% of the anticancer therapeutic agents known are derived from plants (Balunas and Kinghorn, 2005) [3]. For example, compounds such as Taxol and vinca alkaloids act to destabilize the microtubules of cancer cells, preventing the rapid proliferation of tumors (Prasain and Barnus, 2007) [17]. Plants have always a great importance in many cultures. Humans use them for their basic needs: feeding, clothing, sheltering, hunting and nursing. As source of medicines, plants have formed the basis for sophisticated traditional systems and continue providing mankind with new remedies. In recent years, the interest in folk medicine has highly increased. It is a fact that 25% of all medical prescriptions are based on substances derived from plants or plant-derived synthetic analogues (Sara *et al.*, 2009) [20].

The plant *Bauhinia rufescens* Lam is a scandent shrub or small tree belonging to the giant family Leguminosae, subfamily Leguminosae-caesalpiniodeae; usually 1-3 m high, sometimes reaching 8 m; often scraggy, stunted and multi-stemmed. Bark ash-grey, smooth, very fibrous and scaly when old, slash pink, twigs arranged in 1 plane like a fishbone, with thornlike

lignified, lateral shoots, 10 cm long. The leaflets are very small, bilobate almost to base, with semi-circular lobes, glabrous, with long petioles, greyish-green, less than 3 cm long. Flowers are greenish-yellow to white and pale pink, petals 5, spatulate, 15-20 mm long; stamens 10, filaments hairy at the base. Fruits aggregated, long, narrow pods, twisted, up to 10 cm long, glabrous, obliquely constricted, shining dark red-brown, with 4-10 seeds each (Burkill, 1995) [4]. The plant is deciduous in the drier area and evergreen in the wetter area, often found in the dry Savannah region, especially near streams or river banks; occurring throughout West Africa and extends across Africa up to Sudan. It has wide array of medicinal and socio-cultural uses.

Several *Bauhinia* species are utilized as folk medicines worldwide, including Africa, Asia, South America and Central America. An extract of the root is used as an astringent or antipyretic in local medicine. Leaves and fruit are applied for the treatment of diarrhoea, dysentery and ophthalmic diseases. The bark of the roots and trunk is used to cure chest complaints, syphilis and other venereal diseases, leprosy, diarrhea and dysentery and to reduce fever (Ayensu, 1978) [2].

Bauhinia rufescens was selected to evaluate the activity of petroleum ether and methanol crude extracts against human breast cancer MCF-7 cell line.

Materials and Methods

Plant materials

Bauhinia rufescens in this study was collected from, West Sudan, on February 2012. The taxonomic identification of the plant was carried out at Medicinal and Aromatic Plants and Traditional Medicine Research Institute, National Center for Research by Dr. Hider Abdelgadir. A voucher specimen was deposited at the herbarium of the institute. The (Leaflets) were air-dried at room temperature (28-30 °C) for three weeks and coarsely ground to powder by a mechanical grinder.

Preparation of crude extracts

30 grams of the coarsely ground material of the leaf were successively extracted for by soxhlet apparatus using petroleum ether and methanol. The extracts were then filtered and evaporated under reduced pressure using rotatory evaporator apparatus.

Cell lines

The cell lines MCF-7 (human breast adenocarcinoma) were provided from Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), National Centre for Research Khartoum. Sudan. Cell line was cultured in minimal essential medium (MEM) to obtain the desired growth and the growth curve of each cell line was plotted.

Cytotoxicity Screening

Microculture tetrazolium MTT assay was utilized to evaluate the cytotoxicity of the studied plants.

Microculture Tetrazolium (MTT) Assay

Principle of MTT assay

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells (Patel *et al.*, 2009) [16].

Preparation of *Bauhinia rufescens* extracts for MTT assay

Using a sensitive balance 5 mg of each extracts were weighed and put in eppendorf tubes. 50 µl of DMSO were added to the extract and the volume was completed to 1 ml with distilled water obtaining a concentration of 5 mg/ml. The mixture was vortexed and stirred by magnetic stirrer to obtain a homogenous solution.

Cell Line and Culturing Medium

MCF-7 (human Breast Cancer) cells were cultured in a culturing flask containing a complete medium consisting of 10% fetal bovine serum and 90% minimal essential medium (MEM) and then incubated at 37°C. The cells were sub cultured twice a week.

Cell counting

Cell counts were done using the improved Neubauer chamber. The cover slip and chamber were cleaned with detergent, rinsed thoroughly with distilled water and swapped with 70% ethanol, then dried. An aliquot of cell suspension was mixed with equal volume of 0.4% trypan blue in a small tube. The chamber was charged with cell suspension. After cells had settled, the chamber was placed under light microscope. Using 40 X objective, cells in the 4 large corner squares (each containing 16 small squares) were counted. The following formula was used for calculating cells:

$$\text{(Cells/ml) N} = \frac{\text{Number of cells counted} \times \text{Dilution factor} \times 10^4}{4}$$

MTT procedure

The monolayer cell culture formed in the culturing flasks was trypsinized and the cells were put in centrifuging tube and centrifuged for 5 minutes separating the cells from the supernatant that flicked out. 1 ml complete medium was added to the cells and all the cell suspension was contained in a basin. In a 96- well microtitre plate, serial dilutions of each extracts were prepared. 3 duplicated concentrations for each extracts i.e. 5 wells for each of 2 extracts. All wells in rows A, B and C were used in addition to first 4 wells from each rows D, E and F. The first 2 wells of row G were used for the negative control and the first 2 wells of row H were used for the positive control Triton X. 20 µl complete medium pipetted in all wells in rows B, C and mentioned wells of rows E and F. Then 20 µl from each extracts were pipetted in rows A and B and first 4 wells of rows E and F. 20 µl taken from row B were pipetted and mixed well in row C from which 20 µl were taken and flicked out. The same was done from E to F. After that 80 µl complete medium were added to all used wells. Then adjusting the cell account to 3000 cell/well, 100 µl of cell suspension were added completing all wells to the volume 200 µl. Now, we have duplicated five concentrations 500, 250, 125, 62.50 and 31.25µg/ml for each extract. Then the plate was covered and incubated at 37 °C for 72 hours.

On the fourth day, the supernatant was removed from each well without detaching the cells. MTT suspension stock (5 mg/ml) prepared earlier in 100 ml phosphate buffer solution (PBS) was diluted (1:3.5) in a culture medium. To each well of the 96-well plate, 50 µl of diluted MTT were added. The plate was incubated for further 4 hours at 37°C. MTT was removed carefully without detaching cells, and 100 µl of DMSO were added to each well. The plate was agitated at room temperature for 10 minutes then read at 540 nm using microplate reader. The percentage growth inhibition was calculated using the formula below:

$$\% \text{ cell inhibition} = 100 - \left\{ \frac{(\text{Ac}-\text{At})}{\text{Ac}} \right\} \times 100$$

Where, **At** = Absorbance value of test compound;
Ac = Absorbance value of control.

Statistical analysis

All data were presented as means \pm S.D. Statistical analysis for all the assays results were done using Microsoft Excel program.

Results

Identification of medicinal plants with significant cytotoxic potential useful for the development of cancer therapeutics has gained increasing importance in the last decade, and research in this field is still expanding.

In the present study, the yield percentage of *Bauhinia rufescens* leaf petroleum ether and methanol extract was 2.3 and 22.1 % respectively, the cytotoxic effect of *Bauhinia rufescens* extracts against human cancer cell lines MCF-7, was determined using the MTT assay.

The results have been summarized in tables 1 and 2.

Table 1: *Bauhinia rufescens* investigated against MCF-7.

Scientific Name	Family name	Part Used	Yield percentage	
			Petroleum ether	Methanol
<i>Bauhinia rufescens</i>	Leguminosae	Leaf	2.3	22.1

This table indicates the scientific names, families, parts used, yield percentage based on the dried weight of methanol and petroleum ether extracts of *Bauhinia rufescens*.

The extracts from *Bauhinia rufescens* showed significant anticancerous activity against MCF-7 cell line in all concentrations.

Anticancer activity of *Bauhinia rufescens* petroleum ether extract against breast cancer cell line MCF-7 through MTT assay reveals that maximum inhibition of 87.35%, 82.34%, 78.30%, 64.12 and 61.69% was found at 500 ppm, 250 ppm, 125 ppm, 62.5 ppm and 31.25 ppm respectively with IC_{50} 23.77 μ g/ml.

Meanwhile the methanol extract showed 74.22%, 71.26%, 66.86%, 59.35% and 56.97% at 500 ppm, 250 ppm, 125 ppm, 62.5 ppm and 31.25 ppm respectively with IC_{50} 14.75 μ g/ml.

The results of cytotoxicity evaluation of extract was ranging from (500 to 31.25) μ g/ml as shown in Fig (1) and table (2)

Table 2: Inhibition percentage and IC_{50} of *Bauhinia rufescens* MTT assay against MCF-7 cell line.

Name of plant	Concentration (μ g/ml)	Petroleum ether		Methanol	
		Inhibition (%) \pm SD	IC_{50} (μ g/ml)	Inhibition (%) \pm SD	IC_{50} (μ g/ml)
<i>Bauhinia rufescens</i>	500	87.35 \pm 0.13	23.77	74.22 \pm 0.04	14.75
	250	82.34 \pm 0.20		71.26 \pm 0.04	
	125	78.30 \pm 0.08		66.86 \pm 0.02	
	62.5	64.12 \pm 0.12		59.35 \pm 0.05	
	31.25	61.69 \pm 0.11		56.97 \pm 0.01	
*Control		98.3 \pm 0.09		98.3 \pm 0.01	

Key: *Control = Triton-x100 was used as the control positive at 0.2 μ g/mL.

The maximum concentration used was 500 μ g/mL. When this concentration produced less than 50% inhibition, the IC_{50} cannot be calculated.

This table indicates the % inhibition of MCF-7 cell line growth *in vitro* by Petroleum ether extract and methanolic extract of the *Bauhinia rufescens*. MTT colorimetric assay was used. Reading in triplicate for different concentrations 500-31.25 μ g/mL.

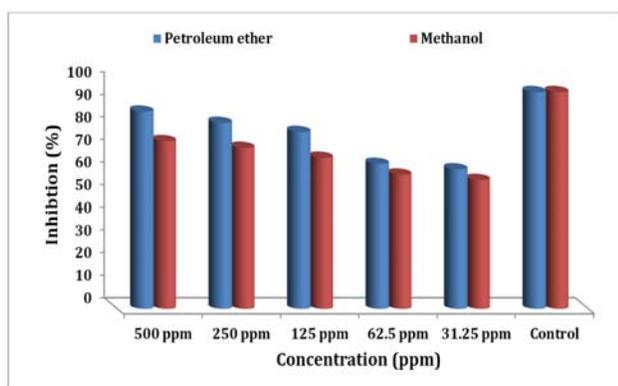


Fig 1: Inhibition percentage of MTT reduction cytotoxic assay for evaluation of *Bauhinia rufescens* extracts against MCF-7 cell lines.

Discussion

World Health Organization investigation shows that 80% of world populations relies on traditional medicines (WHO, 1993) [24]. From these, at least 30% utilized medicinal plants

from clinical indication (Martins *et al.*, 1992) [14]. Available literatures on medicinal plants indicate that promising photochemicals can be developed for many health problems (Gupta, 1994; Rodeiro *et al.*, 2008; Farshori *et al.*, 2013) [8, 19, 6]. Plant extracts as a traditional remedies are already being used to treat a variety of diseases including cancer (Zheng *et al.*, 1992; Svejda *et al.*, 2010; Khan *et al.*, 2011; Randhawa and Alghamdi, 2011; Sharma *et al.*, 2011) [26, 22, 12, 18, 21]. The utilization of medicinal plants is more common in underdeveloped countries (Heck *et al.*, 2000) [11] and experimental studies showed that the extracts of the various plants can also protect against breast cancer cells (Farshori *et al.*, 2013) [6].

Bauhinia rufescens leaf extracts were tested for their 72 hours effect on MCF-7 human breast cancer cell lines using the MTT bioassay and the results are presented in Tabel 2. Petroleum ether extracts showed a high inhibition against the MCF-7 cell lines proliferation with the IC_{50} of 23.77 μ g/ml methanolic extracts had IC_{50} values of 14.75 μ g/ml, with the high prevalence of cancer cases, searching for naturally occurring agents that may inhibit cancer development is becoming an important objective for scientists.

In the last few decades, human cancer cell lines have aggregated an accessible, easily usable set of biological models to examine cancer biology (Green, 2003) [10]. The utility of cell lines acquired from tumor allows the investigation of tumor cells in a simplified and controlled environment (Arya *et al.*, 2011) [1]. MTT cytotoxicity assay used to measure the cytotoxic effect of *Bauhinia rufescens* on breast carcinoma (MCF-7) cells.

Primates, anatomically and physiologically similar with human, are a potential source of new drugs or lead compounds for chemoprevention or chemotherapy of human diseases.

So, the search for anticancer agents on the basis of follow-up of primate uses of plants is a new approach that is highly possible to get new anticancer drugs or lead compounds of plant origin.

In this study, we showed the extract of *Bauhinia rufescens* ingested by primates inhibited the growth of MCF-7 breast cancer cell lines and had strong cytotoxicity in a concentration-dependent manner.

The preliminary phytochemical studies of the partitioned portions showed the presence of aloes, anthraquinones derivatives, cardenolides and cardiac glycosides, flavonoids, resins, saponins and tannins (Usman *et al.*, 2009) [23]. Flavonoids and isoflavonoids are widely present in edible plants. Chemically, they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β -unsaturated carbonyl system. Among the flavonoids, glycosides are an interesting target class of compounds which are extensively investigated due to their broad spectrum of biological activities, including anti-inflammatory (Nowakowska, 2007) [15], anti-invasive (Go *et al.*, 2005) and antitumor properties. They are regarded as promising anticancer agents against most human cancers (Kumar *et al.*, 2011) [13].

As shown in Table 2 the tested extracts showed a variety of IC₅₀ values in inhibiting MCF-7 cancer cells proliferation. These values indicated the cytotoxicity level of the extracts, the lower IC₅₀ values the higher toxicity. So, based on the IC₅₀ values, the cytotoxicity level of the extracts might be divided into strong (<100 $\mu\text{g/ml}$), moderate (101-200 $\mu\text{g/ml}$), and weak (>200 $\mu\text{g/ml}$). The extracts of *Bauhinia rufescens* leaves which showed a high inhibition against the MCF-7 cell lines. In addition, the *Bauhinia rufescens* extracts were toxic to the MCF-7 cell lines.

Conclusion

In conclusion *Bauhinia rufescens* can be a better candidate for isolation of cytotoxic and anticancer compounds specially petroleum ether, extract and fraction of *Bauhinia rufescens*. On the basis of present investigation this plant species can be further investigated for pharmaceutical applications and achievement of novel anticancer compounds.

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