

ANTICANCER POTENTIAL OF PLANT EXTRACTS FROM RIYADH (SAUDI ARABIA) ON MDA-MB-231 BREAST CANCER CELLS

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Abstract

Background: Medicinal plants have been used in traditional medicine for the treatment of numerous diseases worldwide. There is a dire need for new anticancer agents and plants used in traditional medicine are a particularly useful source.

Materials and methods: In this study, extracts of five different plants that grow in the desert of Saudi Arabia were evaluated to assess their cytotoxicity against the MDA-MB-231 breast cancer cell line. Soxhlet extraction was used for the leaves and stems, using different solvents. The cytotoxicity of these extracts against MDA-MB-231 breast cancer cells was assessed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays. The apoptotic cellular morphological changes were observed using inverted and fluorescence microscopes.

Results: Our results showed that two of the five different medicinal plants (*Rumex vesicarius* and *Malva parviflora*) exhibited strong anticancer activity against the breast cancer cells. Specifically, 2 of the 40 extracts (from the five studied plants) showed promising activity. The chloroform extract of the stem of *R. vesicarius* (RSV CHCL₃) exhibited moderate anticancer activity with a half-maximal inhibitory concentration (IC₅₀) of 230 µg/mL while that of the hexane extract of *M. parviflora* stems (MPS Hex) was 248 µg/mL. Loss of cell integrity, shrinkage of the cytoplasm, and cell detachment were observed in the extract-treated MDA-MB-231 cells.

Conclusion: *R. vesicarius* and *M. parviflora* chloroform and n-hexane stem extracts showed significant cytotoxicity against MDA-MB-231 human breast carcinoma cells.

Keywords: Anticancer, *Malva parviflora*, *Rumex vesicarius*, Hoechst 33342

Introduction

The Kingdom of Saudi Arabia has a rich flora with numerous herbs, shrubs, and trees with great medicinal diversity. It is estimated that there are more than 1200 species currently used medicinally (Rahman et al., 2004). The harsh climatic conditions of drought, low nutrient soils, intense solar radiation, extreme temperatures, and water scarcity in Saudi Arabia are considered as positive factors for medicinal plants because they are conferred with more chemical defences than those of plants growing under favourable conditions (Harlev et al., 2012). Such attributes make locally grown plants a good resource to study for potential biological activity. However, very few studies have investigated the potential anticancer activity of indigenous plants. The most common cancer in women is breast cancer, which affects 1.5 million women annually and causes 16% of cancer-related deaths (Dellaire et al., 2013). In 2010, the Saudi Cancer Registry (SCR) reported breast cancer as the first encountered cancer among Saudis of all ages (15%) and ranked it first in newly diagnosed female patients (27.4%) according to a 2010 report (Al-Eid and Quindo, 2010). Currently, the use of natural plant products is one of the best choices of patients, particularly female patients with breast cancer. A recent survey revealed that 80% of female patients with breast cancer currently use plant products (Roberts, 2010; Tautz et al., 2012). In addition, 60% of anticancer chemotherapeutic agents were originally isolated from natural sources such as plants and microorganisms (Harvey et al., 2015; Juárez, 2014).

This study aimed to evaluate the anticancer potential of the extracts of some plants grown in Saudi Arabia against the MDA-MB-231 breast cancer cell line. In this study, we investigated the comparative biological activity of the selected

plant parts of the studied plants and various solvents with different polarities were used to prepare the extracts using a Soxhlet apparatus, which is a novel feature of this study.

Material and Methods

Collection of Plant Material

Five different plants were collected from Raudhat Khuraim and Raudhat Alkhafs, Riyadh, Saudi Arabia from January to April 2014. The plants were taxonomically identified by the Department of Botany and Microbiology, College of Science, King Saud University. Within 24 h, the plants were rinsed with tap water, dried, and ground into powder using an electric grinder. Table 1 shows the local or traditional medicinal use of the plants investigated in this study.

Table 1: List of medicinal plants from Rawdat Khuraim and Raudhat Alkhafs and their medicinal uses

Voucher specimen	Taxon	Family	Use of the plant in folk medicine	Ref.
FAN22 (KSUH)	<i>Cakile arabica Velen & Bormm</i>	Brassicaceae	It used in Saudi folk medicine in treatment of gastric disorder.	(Awaad et al., 2016)
FAN18 (KSUH)	<i>Echium horridum</i>	Boraginaceae	Antibacterial	(Mahmoud and Gairola, 2013)
FAN5 (KSUH)	<i>Emex Spinosa (L) Camod</i>	Polygonaceae	It has a purgative and diuretic effect and the boiled leaf is used by African tribes for the cure of dyspepsia and biliousness, and to stimulate appetite.	(Rahman et al., 2004)
FAN15 (KSUH)	<i>Malva parviflora</i>	Malvaceae	It uses in the treatment of cough, throat infection and other bronchial problems as well as stomach and intestine irritations. It also used for the treatment of headache, fever, sores, and various digestive complaints.	(Akbar et al., 2014)
FAN21 (KSUH)	<i>Rumex vesicarius</i>	Polygonaceae	It has been used in treatment of hepatic diseases, bad digestion, constipation, pains, and diseases of spleen.	(Rahman et al., 2004)

3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) proliferation assay

MDA-MB-231 breast cancer cells were grown in a 24-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum for in vitro antiproliferative activity. Then, 1 mL of the cell suspension (5×10^4 cells/mL) was seeded in each well, incubated at 37°C for 24 h in a 5% CO₂ atmosphere, and then treated with different concentrations (10, 100, 500, and 1000 µg/mL) of the plant extracts to determine the cytotoxicity using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay. The last row of wells in the plate was used as the control and was treated with the vehicle. The plates were read at 540 nm using a microtiter plate reader (Biochrom, England). For each extract tested, the half maximal inhibitory concentration (IC₅₀, the concentration of tested compound needed to inhibit cell growth by 50%) was generated from the dose-response curves.

Lactate dehydrogenase (LDH) cytotoxicity assay

The lactate dehydrogenase (LDH) cytotoxicity assay measures cell membrane integrity by detecting LDH enzyme release into the culture medium induced by cell membrane injury. Briefly, MDA-MB-231 cells (5×10^4 cells) were seeded into a 24-well plate, pre-cultured for 24 h, and then they were treated with the IC₅₀ of the extracts. The enzyme activity was measured using an LDH kit (Sigma-Aldrich Corp., Inc., USA), according to the manufacturer's protocol in the user's manual.

Apoptosis-induced morphological changes of MDA-MB-231 cells using light and fluorescent microscopy

MDA-MB-231 cells were grown in 12-well plates and treated with the crude extracts at their IC₅₀ for 48 h. The morphological changes were examined under an inverted light microscope attached to a Leica MC-170 HD camera (Leica, Germany). For Hoechst 33258 fluorescence staining, the cells were incubated with the extracts, washed twice with phosphate-buffered saline (PBS) at 20°C, fixed with 4% paraformaldehyde, permeabilized with cold methanol,

and then stained with 10 mg/mL Hoechst 33258 (Sigma-Aldrich diluted in PBS (final concentration 0.1 µg/mL) (Yang et al., 2013). Then, the cells were observed for nuclear changes (i.e. chromatin condensation and nuclear fragmentation) and characteristics of apoptosis under a fluorescence microscope attached to an Axiocam 506 colour camera (Zeiss, Germany).

Fourier transform infrared (FTIR) analysis of bioactive fractions

The active extracts were analysed using Fourier transform infrared (FTIR) spectroscopy using potassium bromide (KBr) plates (JASCO FTIR model 420, Japan).

Statistical analysis: The results are presented as the means ± standard deviation (SD) of three independent experiments and the statistical analysis was performed using the Student's *t*-test. $P < 0.05$ and $P < 0.01$ were considered statistically significant.

Results

The development of new anticancer agents from traditional medicinal plants is a promising alternative to synthetic agents. In our continued research to identify cytotoxic plants from plants used in traditional medicine, we evaluated the effects of 40 extracts of five different plants against MDA-MB-231 breast cancer cells using the MTT assay. The IC₅₀ values (µg/mL) of these extracts are summarised in Table 2. The screening results revealed that 11 extracts showed moderate or low activity while the other 29 exhibited very weak activity (Table 2). Among the promising extracts, the stems of *R. vesicarius* and *M. parviflora* (Figure 1) extracted with two different solvents showed cytotoxicity.

Table 2: Half-maximal inhibitory concentrations (IC₅₀) of medicinal plant extracts in this study

Plant Species	Part Used	Cytotoxic activity (IC ₅₀) (µg/ mL)			
		n-Hexane (Hex)	Chloroform (CHCl ₃)	Ethyl acetate (EtOAc)	Methanol (MeOH)
<i>Cakile arabica Velen & Bornm</i>	Leaves	500	>500	467	>500
	Stem	500	>500	>500	>500
<i>Echium horridum</i>	Leaves	>500	478	>500	>500
	Stem	480	>500	>500	>500
<i>Emex Spinosa (L) Camod</i>	Leaves	>500	>500	>500	>500
	Stem	340	>500	>500	>500
<i>Malva parviflora</i>	Leaves	>500	>500	>500	>500
	Stem	248	>500	>500	>500
<i>Rumex vesicarius</i>	Leaves	>500	>500	>500	>500
	Stem	497	230	>500	>500



Figure 1: *Malva parviflora* (left) and *Rumex vesicarius* (right) are widely used in traditional folk medicine in Saudi Arabia.

Regarding potency, the chloroform stem extract of *R. vesicarius* (RVS CHCL₃) was the most potent with an IC₅₀ value of 230 µg/mL (Figure 2), whereas the n-hexane stem extract of *M. parviflora* (MPS Hex) showed an IC₅₀ value of 240 µg/mL. The dose-response curve of these two extracts on MDA-MB- 231 are presented in Figure 2.

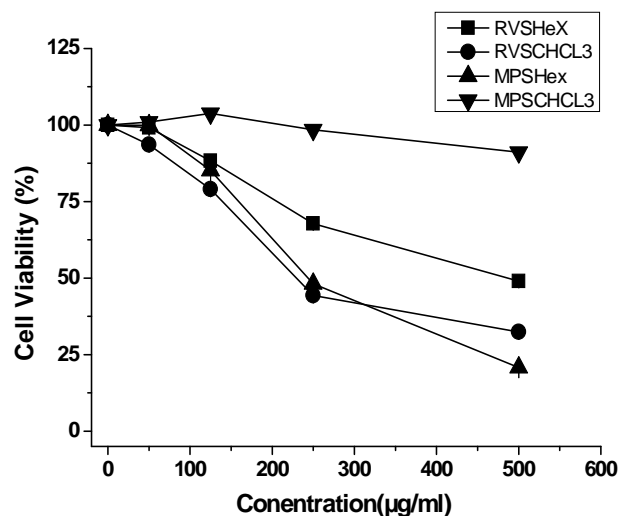


Figure 2: Dose-dependent cytotoxicity of n-hexane and chloroform extracts of *R. vesicarius* stem (RVShEx and RVSchCl₃) and n-hexane and chloroform stem extracts of *M. parviflora* (MPSHex and MPSchCl₃) on MDA-MB-231 breast cancer cell line. Values are mean ± standard deviation (SD, n = 3).

The damage to MDA-MB-231 cell membranes after treatment with the different extracts was determined by measuring the release of LDH. The RVS CHCL₃- and MPS Hex-treated cells showed a more significant ($P < 0.05$ and $P < 0.01$) increase in LDH release than the control MeOH-treated cells did (Figure 3) However, the highest accumulation of LDH in the cell culture medium was induced by the RVS CHCl₃ and MPS Hex.

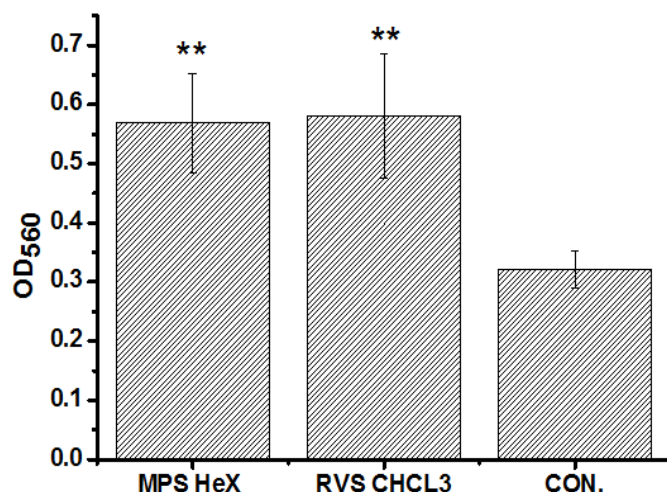
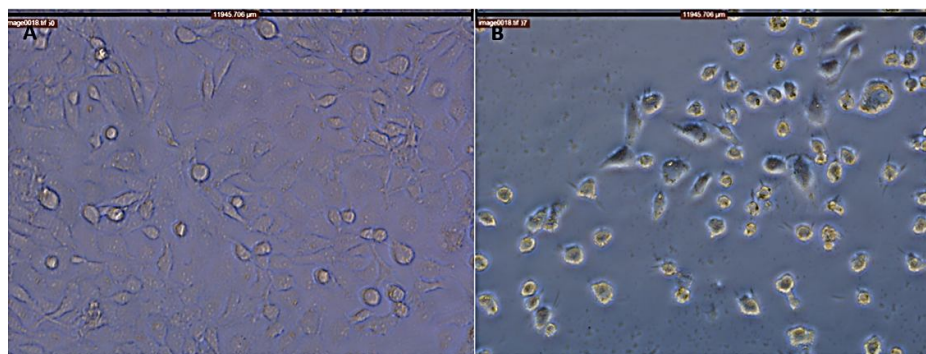
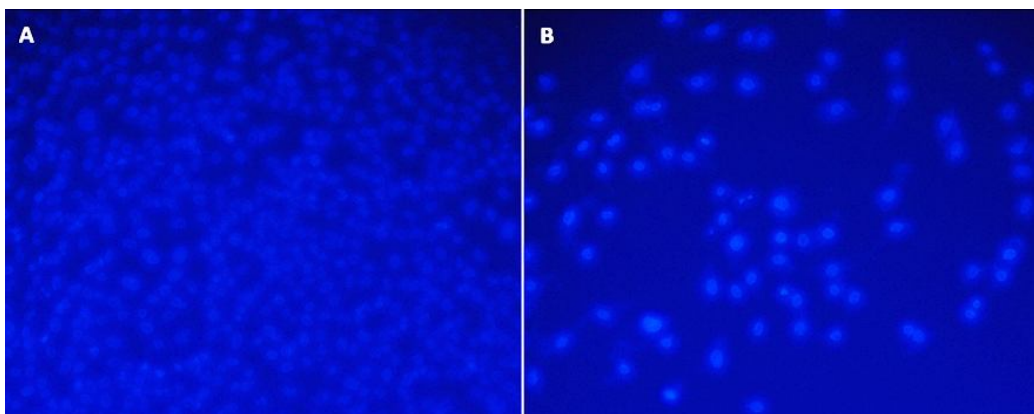


Figure 3: Effects of stem chloroform extract of *Rumex vesicarius* (RVS CHCL₃) and stem hexane extract of *Malva parviflora* (MPS HeX) on MDA-MB-231 using the lactate dehydrogenase (LDH) assay in control and treated cells for 48 h. Cell death was calculated as the OD of LDH release. Data are reported as mean ± standard deviation (SD) of three independent experiments. Statistical analysis was performed using the Student *t*-test with **P* < 0.05, ***P* < 0.01, and ****P* < 0.001, compared to control.

The normal morphology of the MDA-MB-231 cells was altered after treatment with the *R. vesicarius*, and *M. parviflora* crude extracts. The control (untreated) cells retained their normal cellular morphology, whereas loss of cell integrity, shrinkage of the cytoplasm, and cell detachment were observed in the extract-treated MDA-MB-231 cells (Figure 4 A and B).



The effects of *M. parviflora* and *R. vesicarius* crude extracts on the cell morphology of the cancer cell line were also examined using DNA-binding dye (Hoechst 33258). As shown in Figure 5A, the control cells appeared to be uniformly stained, oval in shape, and intact, whereas the extract-treated cells showed chromatin structural changes such as multiple fragmentation, chromatin condensation, segregated bodies, cell shrinkage, apoptotic body formation, uniformly fluorescent fragmented chromatin, and cell decrement (Figure 5B).



The IR spectra are presented in (Figure 6). The IR analysis of the extract revealed a peak between 1150–1270 cm^{-1} , corresponding to the carbonyl C-O or O-H and absorption over the range of 1300–1450 cm^{-1} , corresponding to the C-O (amide) and C-C (phenyl groups). Furthermore, the absorption at 1600–1760 cm^{-1} corresponds to N-H (amino acids) and C=O (aldehydes and acetones, esters). In addition, the signals between 28000–2900 cm^{-1} correspond to the C-H specific to CH_3 and CH_2 from lipids, C-H (aldehydes), and double bonds. While the absorption between 3350–3600 cm^{-1} corresponds to OH groups from alcohols, phenols, and carbohydrates.

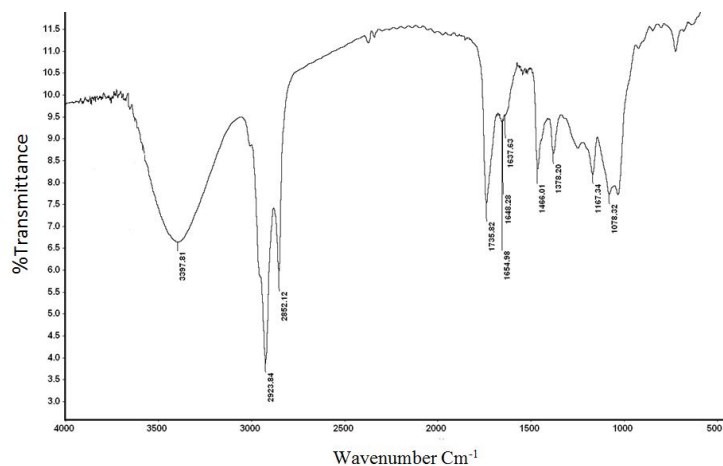


Figure 6: Infrared spectrum of chloroform stem extract of *Rumex vesicarius* (RVS CHCL_3).

Discussion

Plant parts such as the stem, bark, and leaf contain different molecules with diverse functional groups and, so, the comparative study of the biological activity of all these plant parts would be useful, but is not normally performed. We studied the extracts of various parts of plants grown in Saudi Arabia to determine the plant part with suitable biological activity. Moreover, not all molecules are soluble in a particular solvent, and hence, it is very important to use solvents of different polarities to extract all biologically active molecules. Solvents with different polarity isolate different components from crude plant material (Sasidharan et al., 2011). Hexane and dichloromethane, for example, extract waxes, fats, fixed and volatile oils, alkaloids, and aglycone. On the other hand, methanol extracts sugars, amino acids, and glycosides. To isolate maximum amounts and diverse biologically active phytochemicals, we extracted the selected plants using the solvents (hexane, dichloromethane, ethyl acetate, and methanol) with varying polarity to

prepare the crude extract. The antiproliferative activity of the hexane and chloroform extracts evaluated in this study could also be easily attributed to their ability to isolate apolar and moderately polar compounds.

The *M. parviflora* and *R. vesicarius* extracts were cytotoxic against the MDA-MB-231 cell line in a dose-dependent manner. The LDH assay is a simple and fast cytotoxicity assay that measures the LDH released into the extracellular medium following cell membrane damage (Fotakis and Timbrell, 2006). The intracellular LDH released into the medium is a measure of irreversible cell death due to cell membrane damage, whereas Xia et al., (2007) reported the direct involvement of LDH upregulation and subsequent induction of apoptosis.

The results revealed morphological changes in MDA-MB-231 cells following incubation with RVS CHCL₃ and MPS Hex, which is a sign of cell apoptosis. The morphological changes in the apoptotic cells were also apparent in the Hoechst 33258 staining, which facilitated the detection of cell death caused by apoptosis. Induction of apoptosis has been considered as a hallmark for the identification of anticancer drugs (Suh et al., 2009). Chemotherapeutic drugs act mainly by inducing cell death through apoptosis (Johnstone et al., 2002). One of the noteworthy findings of this study was that both plants induced apoptosis. Recently, FTIR spectroscopy has gained approval in plant sciences, which requires a considerable high-throughput screening to classify the numerous samples according to their overall chemical profile. The IR spectrum reflects the panorama of the chemical profile in the crude extract, thereby validating traditional and herbal medicine (Yadav and Dixit, 2008; Raaman, 2006).

Conclusion

In conclusion, RVS CHCL₃ and MPS Hex extracts, among the 40 extracts from five different plants varieties, showed significant cytotoxicity against human breast carcinoma cells (MDA-MB-231 cells). An investigation is currently ongoing to study the antiangiogenic potential of these two extracts in a zebrafish model, which would further facilitate the elucidation of mechanisms of action and therapeutic potential as an anticancer agent. Based on the present investigation, these plant species could be further investigated for pharmaceutical applications and the development of novel anticancer compounds.

Conflict of Interest: The authors declare that they have no conflict of interest.

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