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In vitro Anticancer Activity of Selected Medicinal Plants from Oman

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAS designed the study, wrote the protocols, performed laboratory and statistical analyses and wrote the first draft of the manuscript. Author YT wrote the protocol, performed statistical analysis and managed cytotoxic analysis. Authors MSA and AMW designed the study, collected the plant materials and managed laboratory analysis. Authors SSAK and QAAR designed the study. All authors read and approved the final manuscript.

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ABSTRACT

This paper reports *in vitro* anticancer activity of hexane, chloroform, ethyl acetate, butanol and water extracts obtained from twelve (12) medicinal plants including *Aloe dhufarensis*, *Calotropis procera*, *Juniperus servaschanica*, *Lawsonia inermis*, *Maytenus dhofarensis*, *Moringa peregrina*, *Polygala senensis*, *Punica granatum*, *Rhazya stricta*, *Solanum incanum*, *Teucrium mascatense*. and *Zataria multiflora* collected from Oman. Crude ethanol extracts prepared by maceration of plant materials in ethanol were Kupchan partitioned to give hexane, chloroform, ethyl acetate, butanol and aqueous fractions. Cytotoxicities of the prepared fractions were measured by Alamar blue assay against ovarian cancer cell line (MCAS) and breast cancer cell line (MDA MB231). Thirteen extracts from five plants *C. procera*, *J. servaschanica*, *M. dhofarensis*, *S. incanum* and

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T. mascatense were found active against MCAS; hexane extract from *J. servaschanica* was the most active followed by chloroform extract from leaves of *S. incanum* (IC_{50} = 8.50 and 10.90 µg/ml, respectively). Furthermore, nine extracts from these plants except *C. procera* inhibited the growth of MDA MB321; hexane extract from *J. servaschanica* was again the most active followed by butanol extract of *S. incanum*. (IC_{50} = 11.4 and 19.44 µg/ml, respectively). Ethnobotanical and ethnopharmacological information on flora growing in this intemperate, hot climate region could provide new chemical entities for development of new and more potent cancer chemotherapeutics.

Keywords: In vitro anticancer; medicinal plants; Oman; breast cancer; ovarian cancer.

1. INTRODUCTION

Cancer is among the highest global health burden. This problem is exacerbated by continued growth and ageing of the world's population [1]. In 2012, the worldwide burden of cancer rose to an estimated 14 million new cases per year, and the figure is expected to rise to 22 million annually within the next two decades. Furthermore, the cancer deaths are estimated to rise from 8.2 million annually to 13 million per year over the same period [1]. The rapid increase in the cancer burden represents a crisis for public health and healthcare systems worldwide and is demanding identification of new and more effective measures for cancer control. Chemotherapy is among the main pillars of cancer therapy. Natural products from terrestrial medicinal plants are considered as one of the major sources of new antineoplastic agents [2-4].

Although large area of the Gulf region is covered by desert and rocky hills and mountains, a considerable amount of terrestrial plants exist in this region. For instance, there are a total of 1204 terrestrial plants in Oman alone [5]. Furthermore, a large number of these plants are endemic and are reported to be of medicinal value [6-8]. Herbal medicine occupies a significant part of this region's heritage and until recently functioned as the main health care system [9]. Ethnobotanical and ethnopharmacological knowledge present at this region could provide new potent candidates for development for new chemotherapeutics including anticancer agents. This paper reports *in vitro* anticancer property of some medicinal plants collected from Oman against Ovarian cancer cell line (MCAS) and breast cancer cell line (MDA MB231).

2. MATERIALS AND METHODS

2.1 Plant Material

A dozen of medicinal plants were collected from various locations in Oman. Selection of these plants was based on their ethnomedical history and their anti-cancer properties reported in the literature. Medicinal plants that were included are *Aloe dhufarensis* Lavranos, *Calotropis procera* (Ait.) Ait. *Juniperus servaschanica* Kom., *Lawsonia inermis* L., *Maytenus dhofarensis* Sebsebe, *Moringa peregrina* (Forssk.) Fiori, *Polygala senensis* Klotzsch var. *senensis, Punica granatum,* L. *Rhazya stricta* Decne, *Solanum incanum* L, *Teucrium mascatense* Boiss. and *Zataria multiflora* Boiss. All plants were identified by taxonomist Amina AlFarsy of the Life Science Unit, Sultan Qaboos University (SQU), Oman. A specimen of each sample has been deposited at the Herbarium of SQU and voucher numbers as well as the part of the plant used in this study, the date and the site of collection are presented in Table 1.

2.2 Preparation of Extracts

Dried plant samples were homogenized and macerated twice in ethanol. Each soaking lasted for 72 hours. The solvent was evaporated and the resulting extract was suspended in 10% aqueous methanol and extracted with hexane. The aqueous layer was then concentrated to remove methanol and diluted with distilled water. The diluted aqueous solution was later consecutively extracted with chloroform, ethyl acetate and butanol. All solvents were then removed at low pressure using a rotary evaporator to give corresponding extracts.

2.3 *In vitro* Anticancer Assay

Alamar blue assay [10] was used to test the cytotoxic activity of the extracts against Ovarian (MCAS) and breast (MDA MB231) cancer cell lines. Each extract was initially tested at 11 concentrations including 50, and 100 – 1000 ug/ml to determine their IC_{50} values. Extracts having IC_{50} values below 100 ug/ml were further assayed on a normal cell line (skin fibroblast), to observe their toxicity on normal cells.

Table 1. Calculated IC_{50} values of tested extracts against MCAS and MDA MB231

Species/(Family)	Date Collected/ (site)	Voucher no.	Part used	Extract	IC_{50} (µg/ml)	
					MCAS	MDA MB 231
Aloe dhufarensis Lavranos (Xanthorrhoeaceae)	29-09-2014 (Dhofar)	*Al-Farsi*, A. 574	Aerial part	Hexane	n.a.	n.a.
				Chloroform	n.a.	n.a.
				Ethyl actetate	n.a.	n.a.
				Butanol	n.a.	n.a.
				Water	n.a.	n.a.
Calotropis procera (Ait.) Ait. (Apocynaceae - subf. Asclepiadoideae)	12-05-2015 (Jebel Akhdar)	*Al-Farsi, A.* 596	Leaves	Hexane	26.19±2.29	n.a.
				Chloroform	21.17±1.33	n.a.
				Ethyl actetate	n.a.	n.a.
				Water	n.a.	n.a.
Juniperus servaschanica Kom. (Cupressaceae)	12-05-2015 (Jebel Akhdar)	*Al-Farsi, A.* 599	Leaves	Hexane	8.50±0.92	11.40±1.06
				Chloroform	n.a.	34.05±1.51
				Ethyl actetate	n.a.	n.a.
				Water	n.a.	n.a.
Lawsonia inermis L. (Lythraceae)	30-11-2015 (SQU)	*Al-Farsi, A.* 606	Leaves	Hexane	n.a.	n.a.
				Chloroform	n.a.	n.a.
				Ethyl actetate	n.a.	n.a.
				Water	n.a.	n.a.
Maytenus dhofarensis Sebsebe (Celastraceae)	28-09-2014 (Dhofar)	*Al-Farsi, A.* 566	Leaves	Hexane	58.08±1.62	32.4±3.08
				Chloroform	69.80±1.55	75.8±1.58
				Ethyl actetate	n.a	n.a.
				Butanol	93.44±1.78	n.a.
				Water		n.a.
Maytenus dhofarensis Sebsebe (Celastraceae)	28-09-2014 (Dhofar)	*Al-Farsi, A.* 566	Stem bark	Hexane	25.1 1±1.14	n.a.
				Chloroform		n.a.
				Ethyl actetate	35.41±1.03	n.a.
				Water		n.a.
Moringa peregrina (Forssk.) Fiori (Moringaceae)	12-05-2015 (Jebel Akhdar)	*Al-Farsi, A.* 595	Leaves	Hexane	57.50±2.54	n.a.
				Chloroform	n.a.	n.a.
				Ethyl actetate	n.a.	n.a.
				Water	n.a.	n.a.
Moringa peregrina (Forssk.) Fiori (Moringaceae)	12-05-2015 (Jebel Akhdar)	*Al-Farsi, A.* 595	Fruits	Hexane	13.13±1.28	n.a.
				Chloroform	n.a.	n.a.
				Ethyl actetate	n.a.	n.a.
				Water	n.a.	n.a.
Polygala senensis Klotzsch var. *senensis* (Polygalaceae)	29-09-2014 (Dhofar)	*Al-Farsi, A.* 569	Whole plant	Hexane	n.a.	n.a.
				Chloroform	n.a.	n.a.
				Ethyl actetate	n.a.	n.a.
				Butanol	n.a.	n.a.
				Water	n.a.	n.a.
Punica granatum L. (Lythraceae)	12-05-2015 (Jebel Akhdar)	*Al-Farsi, A.* 597	Leaves	Hexane	n.a.	n.a.
				Chloroform	n.a.	n.a.
				Ethyl actetate	n.a.	n.a.
				Water	n.a.	n.a.

Species/(Family)	Date Collected/ (site)	Voucher no.	Part used	Extract	IC_{50} (µg/ml)	
					MCAS	MDA MB 231
Rhazya stricta Decne. (Apocynaceae)	30-11-2015 (Wadi Bani Khalid)	*Al-Farsi, A.* 609	Aerial part	Hexane	n.a.	n.a.
				Chloroform	n.a.	n.a.
				Ethyl actetate	n.a.	n.a.
				Water	n.a.	n.a.
Solanum incanum L. (Solanaceae)	28-09-2014 (Dhofar)	*Al-Farsi, A.* 567	Leaves	Hexane	n.a.	n.a.
				Chloroform	10.90±1.02	39.70±2.89
				Ethyl actetate	n.a.	n.a.
				Butanol	35.14±1.69	19.44±1.67
				Water	n.a.	86.05±2.94
Teucrium mascatense Boiss. (Lamiaceae)	12-05-2015 (Jebel Akhdar)	*Al-Farsi, A.* 598	Whole plant	Hexane	15.24±1.25	61.08±2.76
				Chloroform	n.a.	33.80±3.34
				Ethyl actetate	n.a.	n.a.
				Water	n.a.	n.a.
Zataria multiflora Boiss. (Lamiaceae)	30-11-2015 (Wadi Bani Khalid)	*Al-Farsi, A.* 607	Leaves	Hexane	n.a.	n.a.
				Chloroform	n.a.	n.a.
				Ethyl actetate	n.a.	n.a.
				Water	n.a.	n.a.

n.a. = not active (IC_{50} > 100 µg/ml)

2.3.1 Cell culture

Both ovarian (MCAS) and breast (MDA MB231) cancer cell lines were from ATCC (American type culture collection). Both cell lines were cultured in DMEM media. Upon 80-85% confluency the cells were trypsinized and the subsequent pellet was used for the experiments. A 96 well plate was seeded with approximately 15,000 cells/well and allowed to attach and grow for 24 hours. Cell counting was performed using haemocytometer. Upon completion of 24 hours, the cells were treated with each natural product with appropriate dilutions.

2.3.2 Cell viability assay

The cells in the 96 well plates were treated with plant extracts at 11 different concentrations including 50, and 100 – 1000 ug/ml. Each concentration was done in triplicate. Negative controls (cells treated with DMSO only) for each concentration were included. A column of untreated cells treated with fresh media was used as control (blank) and DMSO mixed with media was used as a vehicle control. After adding the treatment to the cells the plate was incubated for 24 hours. Then, the wells were observed under microscope to spot cell death if any (well were imaged using the IN Cell Analyzer 6000 (INCA6000) at 40X magnification (0.60 NA). – GE Healthcare). After this, Alamar blue assay was performed.

2.3.3 Alamar blue assay

After 24-hours, media was refreshed and alamar blue was added to each well. The plate was incubated at 37°C for 1-4 hours. Absorbance was then read at 570 nm with reference wavelength of 600 nm.

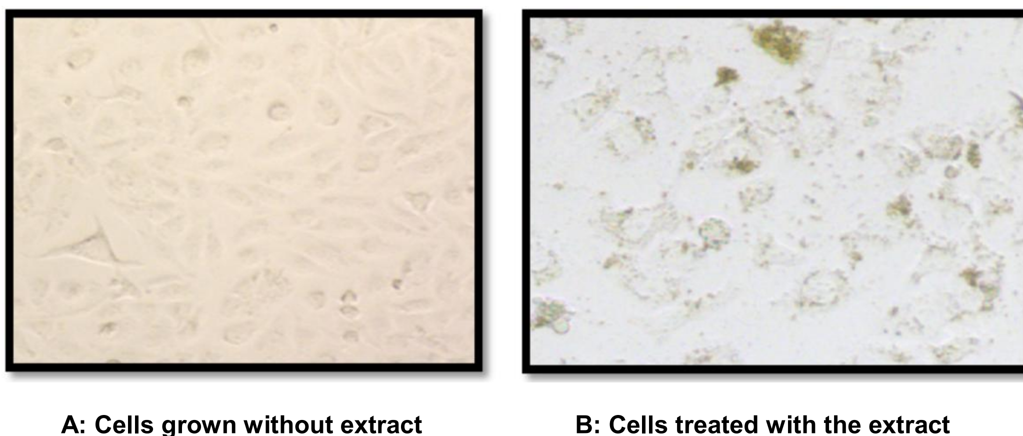
2.4 Data Analysis

The absorbance values of 96 well plates were recorded. The cell viability % was calculated using the average of absorbance values of each treatment. Cell viability% = (sample absorbance/ control absorbance)*100. IC_{50} value of each extract was generated by regression analysis of the % cell viability using Microsoft Excel.

3. RESULTS AND DISCUSSION

Microscopic observation of the plate showed significant inhibition in growth of the cancer cells in wells treated with effective extracts compared to treated controls (Fig. 1).

The evaluated IC_{50} values of tested extracts against the two cell lines MCAS and MDA MB231 are listed in Table 1. As can be seen



A: Cells grown without extract **B: Cells treated with the extract**

Fig. 1. Effect of hexane extract of *T. mascatense* on MCAS cells after 24 hours

from the Table, hexane extracts from leaves of *C. procera, J. servaschanica* and *T. mascatense* induced significant cell death against MCAS (IC_{50} = 26.19, 8.50 and 15.24 µg/ml, respectively). The growth of the ovarian cancer cell was also inhibited by chloroform extracts from leaves of *C. procera* as well as the leaves of *S. incanum*. (IC_{50} = 21.17 and 10.90 µg/ml, respectively). Other extracts that displayed significant activity against MCAS cells were ethyl acetate from stem bark of *M. dhofarensis* and butanol extract from leaves of *S. incanum* (IC_{50} = 35.41 and 35.14 µg/ml, respectively). Products that showed significant cell death on MDA MB231 were hexane exract from leaves of *J. servaschanica* and *M. dhofarensis* (IC_{50} = 11.4 and 32.4 µg/ml, respectively). The growth of these cells were also inhibited by chloroform extract from leaves of *S. incanum*, *T. mascatense* and *J. servaschanica* (IC_{50} = 39.7, 33.8 and 34.05 µg/ml, respectively). Butanol extract of *S. incanum* also induced significant death against MDA MB231 cells (IC_{50} = 19.44 µg/ml).

Comparison of efficacy of tested extracts against the growth of the two cell lines is shown in Fig. 2. Out of the 13 active extracts against MCAS, hexane extract from *Juniperus servaschanica* was found to be the most active followed by chloroform extract from leaves of *Solanum incanum* (Fig. 2). Likewise, hexane extract from *Juniperus servaschanica* was again the most active against MDA MB321 followed by butanol extract of *Solanum incanum.*

Furthermore, results from laboratory experiments to determine toxicity of the active products against normal cells (fibroblast) in comparison to cancer cells MCAS are presented in Fig. 3. As can be seen from the figure fibroblast were almost unaffected by IC_{50} dosage of the active extracts. On the other hand there was significant cell death on MCAS cell lines treated with same concentration as shown in Fig. 4. However, this phenomenon was an exception for organic extracts obtained from the leaves of *M. dhofarensis* and *S. incanum* that showed high toxicity on the normal cell line (Fig. 3).

3.1 Discussion

Organic extracts from five plants *Maytenus dhofarensis*, *Solanum incanum*, *Juniper servaschanica, Teucrium mascatenses* and *Calotropis procera* collected from Oman induced significant cell death on MCAS and MDA MB231 cells. These materials displayed IC_{50} values below the range of 100 ug/ml (Fig. 2). Extracts that showed IC_{50} values above 100 were considered inactive (Table 1).

In vitro anticancer activity observed from the five species collected from Oman is similar to what is reported for related species collected elsewhere. For instance, Wu and his co-workers reported that *S. incanum* extract induced cutaneous squamous cell carcinoma apoptosis via regulation of tumor necrosis factor receptor signaling pathway [11]. However our preliminary observation showed that *S. incanum* extracts possess significant toxicity against normal cells (Fig. 3). Anticancer property of *M. dhofarensis* has so far not been evaluated however; other *Maytenus* species are known to exhibit potent *in vitro* and *in vivo* anticancer activity against various cancer cell lines. For example, organic extracts from *M. royleanus* collected from Pakistan has been found to possess significant

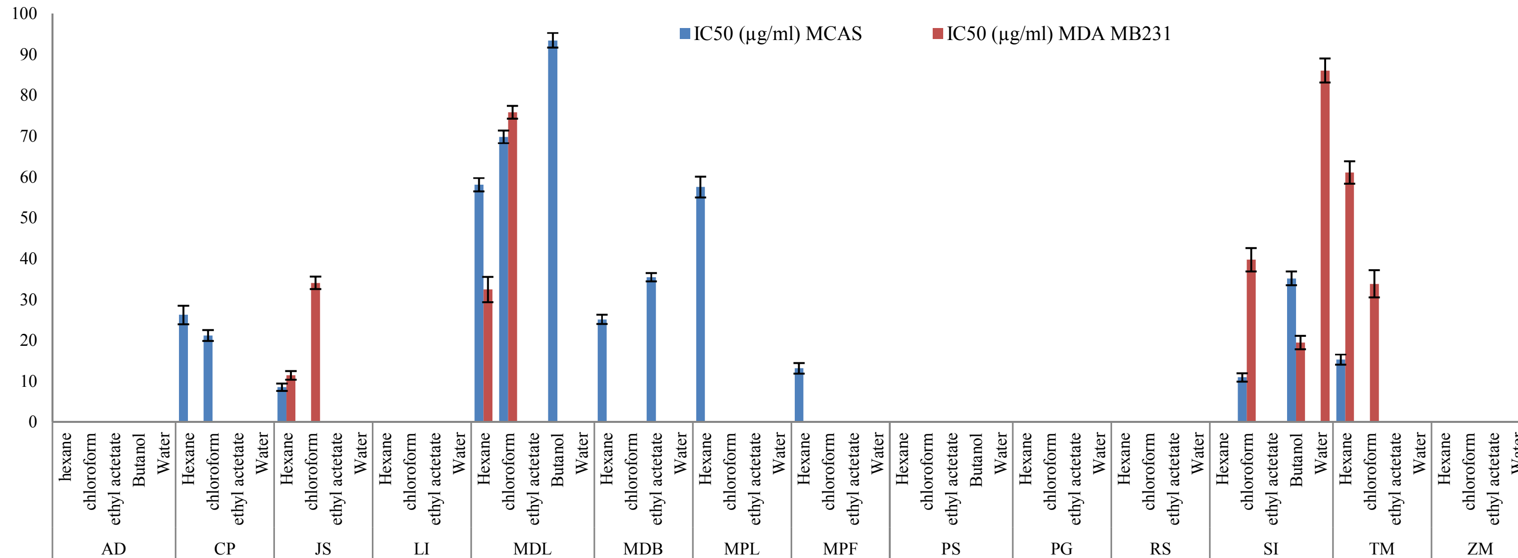


Fig. 2. Comparison of efficacy of tested extracts against MCAS and MDA MB231 cells

AD = Aloe dhufarensis, CP = Calotropis procera, JS = Juniperus servaschanica, LI = Lawsonia inermis, MDL = Maytenus dhofarensis leaves, MDB = Maytenus dhofarensis stem bark, MPL = Moringa peregrine leaves, MPF =Moringa peregrina fruits, PS = Polygala senensis, PG = Punica granatum, RS = Rhazya stricta, SI = Solanum incanum, TM = Teucrium mascatense, ZM = Zataria multiflora

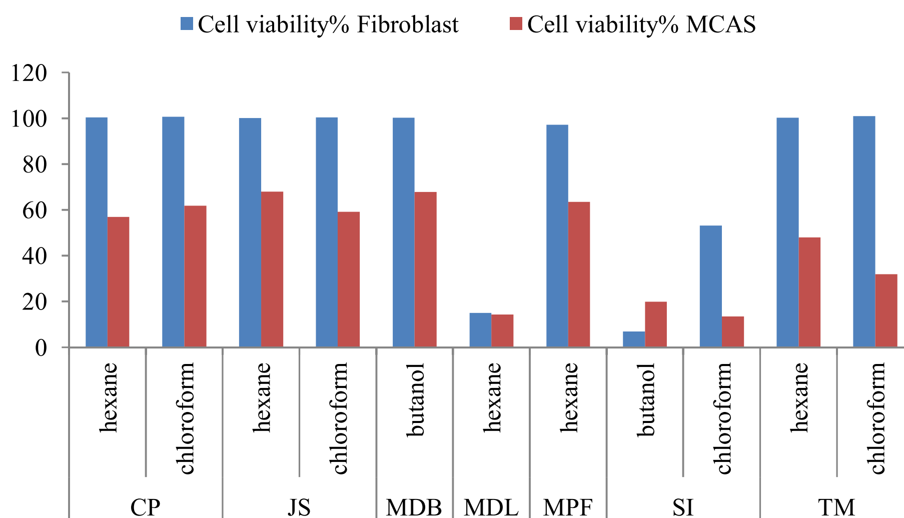
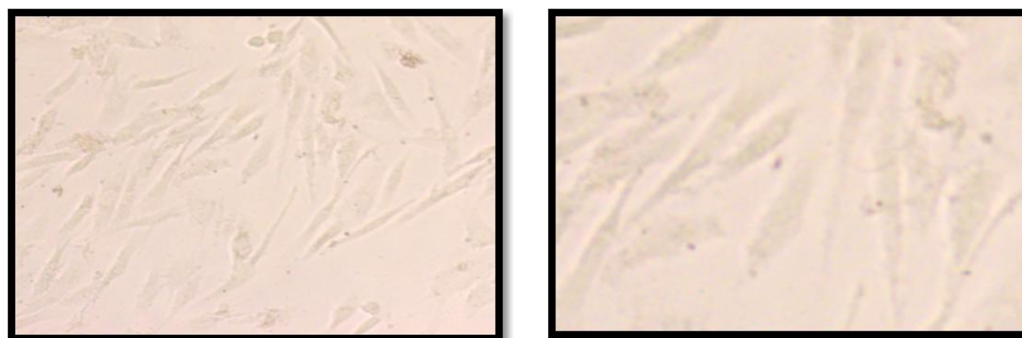


Fig. 3. Cell viability of fibroblast and MCAS cells at IC_{50} dosage of extracts

CP = Calotropis procera, JS = Juniperus servaschanica, MDL = Maytenus dhofarensis leaves, MDB = Maytenus dhofarensis stem bark, MPF =Moringa peregrina fruits, SI = Solanum incanum, TM = Teucrium mascatense



C: Fibroblast grown without extract **D: Fibroblast treated with the extract**

Fig. 4. Effect of hexane extract of *T. mascatense* on fibroblast after 24 hours

activity against prostet cancer in MTT assay [12]. *Tecurium* species are also reported to inhibit the growth of cancer cell lines. MTT assay of seven *Tecurium* species excluding *T. mascatenses* showed good cytotoxic activity on human melanoma Hela cell lines. However, they showed low activity against breast cancer cell, MDA-MB361 IC_{50} > 200 µg/ml) [13]. Likewise, hexane, dichloromethane, ethyl acetate, acetone and methanol extracts from *C. procera* collected from Brazil were found to inhibit the growth of human leukemia cell, HL-60 LEM, human colon cancer cells HCT-8 and murine melanoma cells B-16/F10 in MTT assay (IC_{50} < 30 µg/ml) [14]. So far there is no report on anticancer property of the juniper species growing in Oman. However, other juniper species such as *J. communis* are reported to show anticancer activities including inhibition of the growth of breast cancer cells MDA-MB231 [15].

4. CONCLUSION

Evaluation of *in vitro* anticancer property of 12 medicinal plant from Oman has shown that flora growing in this intemperate desert region are potential source for finding new chemical entities for development of new and more potent cancer chemotherapeutics. It will be interesting to extend this investigation to identify the cytotoxic compounds as well as to understand the mechanism that induces inhibition in growth of the cancer cell lines.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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