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Study of the anticancer potential of Yemeni plants used in folk medicine

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Dedicated to Prof. Dr. Thorsten Beyrich on the occasion of his 75th birthday

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The present work evaluated the anticancer activity of methanol extracts from 24 plants used in Yemeni traditional medicine. To evaluate the *in vitro* cytotoxic potency of the investigated extracts, an established microtiter plate assay based on cellular staining with crystal violet was used with 5 human cancer cell lines: two lung cancer (A-427 and LCLC-103H), two urinary bladder carcinoma (5637 and RT-112) and one breast cancer (MCF-7) line. The methanolic extracts of *Dendrosicyos socotrana*, *Withanina aduensis*, *Withania riebeckii*, *Dracena cinnabari* and *Buxus hildebrandtii* exhibited the highest toxicity on all tumor cell lines with IC_{50} values ranging between 0.29 and 5.54 μg/ml. The extracts of *Jatropha unicostata* and *Punica protopunica* showed a moderate potency on the most tumor cell lines.

1. Introduction

For centuries much of the population in Yemen, as elsewhere in many other developing countries, has relied on a system of traditional medicine. Traditional Yemeni medicinal herbs have been used in the treatment of different diseases including cancer. Thus, there have been claims that some traditional healers in Yemen can successfully treat different types of cancer using herbal drugs.

It is well established that there is a long history of the use of different medicinal plants and natural products as anticancer agents in many parts of the world. A variety of medicinal plants have been useful sources of clinically relevant antitumor compounds (Cragg et al. 1994). In addition, it is reported that over 50% of drugs in clinical trials for anticancer activity were isolated from natural sources or are related to them (Cragg and Newman 2000). Thus, much research accomplished today focuses on the investigation of antitumor activity of medicinal plants and on the development of new drugs to treat cancers as well as other incurable diseases (Kamuhabwa et al. 2000; Goun et al. 2002; Itharat et al. 2004; Costa-Lotufo et al. 2005).

The present study aims to evaluate the *in vitro* cytotoxic potential of 24 plant extracts belonging to different families, collected from different locations around the country, on five human cancer cell lines.

2. Investigations, results and discussion

A total of 24 methanolic extracts representing 24 plant species were submitted to screening. The list of the investigated plants, the parts used and their site of collection are shown in Table 1. In order to estimate the antitumor activity of the plant extracts, an established microtiter plate assay was used with 5 human cancer cell lines: two lung cancer (A-427 and LCLC-103H), two urinary bladder carcinoma (5637 and RT-112) and one breast cancer (MCF-7) line (Bracht et al. 2006). Table 2 presents the cytotoxic activity of the 24 investigated methanolic extracts. The results in Table 2 indicate that out of the 24 investigated extracts, five plant extracts exhibited a highly pronounced cytotoxic effect in all of the tested cell lines namely, *Buxus hildebrandtii* (IC_{50} between 0.32 and 15.1 μg/ml), *Dendrosicyos socotrana* (IC_{50} between 0.40 and 1.47 μg/ml), *Dracena cinnabari* (IC_{50} between 2.59 and 5.54 μg/ml), *Withania adunensis* (IC_{50} between 0.30 and 4.30 μg/ml) and *Withania riebeckii* (IC_{50} between 0.29 and 3.78 μg/ml). Out of the 24 tested extracts, four plant extracts showed a cytotoxic effect on three or four of the tested cell lines. These plants are *Carphalea obovata*, *Jatropha unicostata*, *Pulicaria stephanocarpa* and *Punica protopunica* (IC_{50} between 0.97 and >50 μg/ml). Other plant extracts investigated did not demonstrate any significant cytotoxic activity.

It is important to mention that no reports of cytotoxic activity of the investigated plant species on cancer cell lines have been documented so far. We reported in previous studies on the antimicrobial (Mothana and Lindequist 2005) and the antiviral activity (Mothana et al. 2006) of some plant species investigated in this study. The methanolic extracts of the two *Withania* species were found to be the most cytotoxic against all cancer cell lines. A phytochemical screening of the methanolic extracts of both plants showed the presence of withanolids (steroidal lac-

Table 1: List of plants screened for anticancer activity

Plant	Voucher specimen no.	Family	Part used	Site of collection
Acalypha fruticosa Forssk.	YH-05	Euphorbiaceae	L, S	Haja
Boswellia ameero Balf. f.*	SP-M106	Burseraceae	B	Soqotra
Boswellia elongata Balf. f.*	SP-M102	Burseraceae	B	Soqotra
Buddlejja polystaycha Fresen.	YS-01	Loganiaceae	L, F	Sada'a
Buxus hildebrandtii Baillon	SP-M100	Buxaceae	L	Soqotra
Carphalea obovata (Balf.f.) Verdc.	SP-D213	Rubiaceae	L	Soqotra
Cleome schweinfurthii Gilg.	YI-10	Capparaceae	L, T	Ibb
Cleome socotrana Balf. f.*	SP-N030	Capparaceae	L, T	Soqotra
Commiphora parvifolia Balf. f.*	SP-M108	Burseraceae	B	Soqotra
Cystostemon socotranus Balf. f.*	SP-M118	Boraginaceae	L, S	Soqotra
Dendrosicyos socotrana Balf. f.*	SP-A015	Cucurbitaceae	L, S	Soqotra
Dorstenia gigas Schweinf. ex Balf. f.*	SP-M122	Moraceae	S, L	Soqotra
Dracena cinnabari Balf. f.*	SP-D225	Agavaceae	L, F, R	Soqotra
Euryops arabicus Steud.	SP-D203	Compositae	L, F	Soqotra
Exacum affine Balf. f. ex Regel*	SP-M112	Gentianaceae	L, F	Soqotra
Jatropha unicostata Balf. f.*	SP-N035	Euphorbiaceae	B	Soqotra
Kalanchoe farinacea Balf. f.*	SP-D201	Crassulaceae	L, F	Soqotra
Lindenbergia indica (L.) Kuntze	YH-06	Scrophulariaceae	L, S, F	Haja
Pollichia campestris Ait.	YS-12	Caryophyllaceae	L, S	Sana'a
Pulicaria stephanocarpa Balf. f.*	SP-C006	Compositae	L, F	Soqotra
Punica protopunica Balf. f.*	SP-D223	Punicaceae	T, L	Soqotra
Tragia pungens (Forssk.) Muell.-Arg	YT-20	Euphorbiaceae	L, S	Taiz
Withania adunensis Vierh.*	SP-M110	Solanaceae	L, T	Soqotra
Withania riebeckii Schweinf.*	SP-M116	Solanaceae	L, T	Soqotra

* endemic plant, B: Bark, F: Flower, L: Leaves, R: Resin, S: Stems, T: Fruits

Table 2: IC_{50} values (μg/ml) for cell growth inhibition of the investigated plant extracts on five human cancer cell lines

Medicinal plant	Cell lines				
	5637	RT-4	A-427	LCLC-103H	MCF-7
Acalypha fruticosa	>50[a]	>50	>50	>50	>50
Boswellia ameero	>50	>50	>50	>50	>50
Boswellia elongata	>50	>50	>50	>50	>50
Buddlejja polystaycha	>50	>50	>50	>50	>50
Buxus hildebrandtii	10.9 ± 1.93	6.46 ± 1.57	0.32 ± 0.14	10.6 ± 2.4	15.1 ± 3.2
Carphalea obovata	48.9[b]	>50	9.38[b]	> 50	>50
Cleome schweinfurthii	>50	>50	>50	>50	>50
Cleome socotrana	>50	>50	>50	>50	>50
Commiphora parvifolia	41.7[b]	>50	>50	>50	>50
Cystostemon socotranus	>50	>50	>50	>50	>50
Dendrosicyos socotrana	0.40 ± 0.11	1.66 ± 0.32	0.75 ± 0.33	1.33 ± 0.42	1.47 ± 0.29
Dorstenia gigas	>50	>50	>50	>50	>50
Dracena cinnabari	2.59 ± 0.12	3.99 ± 0.27	5.54 ± 1.00	4.77 ± 0.48	3.46 ± 0.21
Euryops arabicus	>50	>50	>50	>50	>50
Exacum affine	>50	>50	>50	>50	>50
Jatropha unicostata	>50	21.6 ± 5.81	0.97 ± 0.66	14.2 ± 8.52	>50
Kalanchoe farinacea	>50	>50	>50	>50	>50
Lindenbergia indica	>50	>50	>50	>50	>50
Pollichia campestris	>50	>50	>50	>50	>50
Pulicaria stephanocarpa	>50	36.4 ± 1.01	36.5 ± 21.8	>50	>50
Punica protopunica	21.3 ± 3.7	37.6 ± 5.4	16.5 ± 1.2	18.8 ± 2.0	>50
Tragia pungens	>50	>50	>50	>50	>50
Withania adunensis	1.07 ± 0.28	4.30 ± 0.95	0.30 ± 0.12	1.07 ± 0.22	0.58 ± 0.13
Withania riebeckii	0.80 ± 0.24	3.78 ± 0.31	0.39 ± 0.10	0.83 ± 0.25	0.29 ± 0.13

Unless otherwise indicated, values are the averages and standard deviations of at least 3 independent determinations.
[a] Cell growth was inhibited by less than 50% at 50 μg/ml in the primary screening; [b] average of 2 independent experiments

tons) (Mothana et al. 2006), which might be responsible for the registered cytotoxic effect. It was previously shown that these substances, which were also isolated from *Withania somnifera*, have antitumor activity (Sharada et al. 1996; Budhiraja et al. 2000). The methanolic extract of *Dendrosicyos socotrana* was also highly cytotoxic against all cancer cell lines tested. This activity can be ascribed to the triterpenoids of the cucurbitan type, which were isolated from this plant in previous work (Hussein et al. 2004). The cytotoxic activity of *Buxus hildebrandtii* could be attributed to the presence of steroidal alkaloids, which were identified in a previous study (Mothana et al. 2006). *Punica protopunica* also exhibited a pronounced cytotoxic effect against four cancer cell lines. It is reported that the extract of the fruits of *Punica granatum* also has antitumor effects, which is attributed to the presence of phenolic compounds including tannins (Mehta and Lansky 2004; Seeram et al. 2004). Thus, the cytotoxic effect of

Punica protopunica could be as well ascribed to the tannins present in this plant.

According to the criteria of the American National Cancer Institute, the upper IC_{50} limit to consider a crude extract promising for further purification is 30 μg/ml (Suffness and Pezzuto 1990). Therefore, the methanolic extracts of *Buxus hildebrandtii*, *Dendrosicyos socotrana*, *Dracena cinnabari*, *Withania adunensis* and *Withania riebeckii* can be considered as highly promising sources of anticancer compounds. In future work, these plants will be evaluated further by isolation and chemical characterization of the antitumor active constituents.

3. Experimental

3.1. Plant material

The plants were collected from different localities of the Republic of Yemen in the beginning of winter 2002 as well as in the summer 2003, and identified at the Pharmacognosy department, Faculty of Pharmacy, Sana'a University. Voucher specimens were deposited at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University.

3.2. Extraction of plant material

The air-dried and powdered plant materials (10 g of each) were extracted successively by shaking with chloroform at room temperature and then with 90% methanol in a water-bath at 50 °C for three to five times. The obtained extracts were filtered and evaporated in a vacuum evaporator or freeze dryer to give the dried extract. Only the methanolic extracts were used in the experiments described.

3.3. Determination of anticancer activity: Cytotoxicity assay on human cancer cell lines

To evaluate the *in vitro* cytotoxic potency of the investigated extracts, an established microtiter plate assay was used with 5 human cancer cell lines: two lung cancer (A-427 and LCLC-103H), two urinary bladder cancer (5637 and RT-112) and one beast cancer (MCF-7) line. Cytotoxicity determinations are based on cellular staining with crystal violet and were performed as previously described in detail (Bracht et al. 2006). Briefly, a volume of 100 μl of a cell suspension was seeded into 96-well microliter plates at a density of 1000 cells per well, except for the LCLC-103H, which were plated out at 250 cell/well. Twenty-four hours later, cells were treated with the plant extracts at five dilutions and exposed continuously to the extracts for the next 96 h. At the end of the exposure time, the medium was removed and the cells were fixed with a glutaraldehyde solution. The cells were then stained with crystal violet and the optical density (OD) was measured at $\lambda = 570$ nm with a plate reader. The percent growth values were calculated by Eq. (1):

$$\text{Growth } (\%) = \text{OD}_\text{T} - \text{OD}_{\text{c},0}/\text{OD}_\text{c} - \text{OD}_{\text{c},0} \times 100 \qquad (1)$$

where OD_T is the mean absorbance of the treated cells, OD_c is the mean absorbance of the controls, $OD_{c,0}$ is the mean absorbance at the time the extract was added. The IC_{50} values were estimated by a linear least-squares regression of the growth values versus the logarithm of the extract concentration; only concentrations that yielded growth values between 10% and 90% were used in the calculation.

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