Research article

Studies of the in vitro anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island Soqotra

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Abstract

**Background:** Recent years have witnessed that there is a revival of interest in drug discovery from medicinal plants for the maintenance of health in all parts of the world. The aim of this work was to investigate 26 plants belonging to 17 families collected from a unique place in Yemen (Soqotra Island) for their in vitro anticancer, antimicrobial and antioxidant activities.

**Methods:** The 26 plants were extracted with methanol and hot water to yield 52 extracts. Evaluation for in vitro anticancer activity was done against three human cancer cell lines (A-427, 5637 and MCF-7) by using an established microtiter plate assay based on cellular staining with crystal violet. Antimicrobial activity was tested against three Gram-positive bacteria, two Gram-negative bacteria, one yeast species and three multiresistant Staphylococcus strains by using an agar diffusion method and the determination of MIC against three Gram-positive bacteria with the broth micro-dilution assay. Antioxidant activity was investigated by measuring the scavenging activity of the DPPH radical. Moreover, a phytochemical screening of the methanolic extracts was done.

**Results:** Notable cancer cell growth inhibition was observed for extracts from *Ballochia atro-virgata*, *Eureiandra balfourii* and *Hypoestes pubescens*, with IC50 values ranging between 0.8 and 8.2 μg/ml. The methanol extracts of *Acanthospermum hispidum*, *Boswellia dicrocerides*, *Boswellia socotrana*, *Commiphora ornifolia* and *Euphorbia socotrana* also showed noticeable antiproliferative potency with IC50 values < 50 μg/ml. The greatest antimicrobial activity was exhibited by extracts from *Acacia pennivenia*, *Boswellia dicrocerides*, *Boswellia socotrana*, *Commiphora ornifolia*, *Euclea divinorum*, *Euphorbia socotrana*, *Leucas samhaensis*, *Leucas virgata*, *Rhus thyrssiflora*, and *Teucrium sokotranum* with inhibition zones > 15 mm and MIC values ≤ 250 μg/ml. In addition, the methanolic extracts of *Acacia pennivenia*, *Boswellia dicrocerides*, *Boswellia socotrana* and *Commiphora ornifolia* showed good antioxidant potential at low concentrations (more than 80% at 50 μg/ml).

**Conclusion:** Our results show once again that medicinal plants can be promising sources of natural products with potential anticancer, antimicrobial and antioxidative activity. The results will guide the selection of some plant species for further pharmacological and phytochemical investigations.
Background
The worldwide use of natural products including medicinal plants has become more and more important in primary health care especially in developing countries. Many pharmacognostical and pharmacological investigations are carried out to identify new drugs or to find new lead structures for the development of novel therapeutic agents for the treatment of human diseases such as cancer and infectious diseases [1]. In developing countries and particularly in Yemen, a large segment of the population still rely on folk medicine to treat serious diseases including infections, cancers and different types of inflammations.

The Soqotra Archipelago in Yemen has long been a land of mystery. Over the centuries travelers returned from the Indian Ocean isles with bizarre tales of trees yielding dragon’s blood and cucumbers, forests of frankincense and towering pinnacles shrouded in mist [2]. Soqotra is considered the “jewel” of biodiversity in the Arabian Sea. The long geological isolation of the Soqotra archipelago and its fierce heat and many droughts have combined to create a unique and spectacular endemic flora. Surveys have revealed that more than a third of the 800 or so plant species of Soqotra are found nowhere else [2]. Botanists rank the flora of Soqotra among the ten most endangered island flora in the world.

Currently, there is insufficient scientific research on the plants from Soqotra. In previous studies we described investigations of some endemic and non-endemic plants from the island Soqotra for their antimicrobial [3], antiviral [4], enzyme-inhibitory [5] and anticancer activity [6]. Since a literature search indicated the absence of further information regarding biological and phytochemical investigations of plants from Soqotra, this study was carried out as a part of our continued exploration of Yemeni medicinal plants for interesting biological activities. Thus, the main aim of the present project was to carry out a phytochemical and biological investigation on selected plants from the island of Soqotra, especially on those that are endemic and those that find use in the traditional medicine. In this study, 26 plants belonging to 17 families were collected for evaluation of their cytotoxic, antimicrobial and antioxidant activities and the main chemical contents.

Methods

Plant materials
The plants (Table 1) were collected from different locations of the island of Soqotra in the Winter of 2006 and identified at the Pharmacognosy Department, Faculty of Pharmacy, Sana’a University. Part of the identification of the investigated plants was done by Dr. Anthony G. Miller from the Royal Botanic Garden at Edinburgh, UK. Voucher specimens were deposited at the Pharmacognosy Department, Faculty of Pharmacy, Sana’a University.

Extraction of plant materials
The air-dried and powdered plant materials (10 g of each) were extracted with 400 ml methanol (CH₃OH) by Soxhlet extraction for 8 hours. The residue was dried over night and then extracted with 250 ml water (H₂O) by using a shaking water-bath at 70°C for 2 hours. The obtained methanolic and water extracts were filtered and evaporated by using a rotary evaporator and freeze dryer. The dried extracts were stored at -20°C until used.

Determination of antimicrobial activity

Test organisms
The following microorganisms were used as test organisms in the screening: 3 Gram-positive strains namely, Staphylococcus aureus (ATCC 6538), Bacillus subtilis (ATCC 6059), Micrococcus flauus (SBUG 16), 2 Gram-negative strains namely, Escherichia coli (ATCC 11229), Pseudomonas aeruginosa (ATCC 27853) and one fungal strain Candida maltosa (SBUG 700). In addition, three multiresistant Staphylococcus strains namely, Staphylococcus epidermidis 847, Staphylococcus haemolyticus 535, and Staphylococcus aureus North German Epidemic Strain (supply from the Institute of Hygiene of Mecklenburg-Vorpommern, Greifswald, Germany) were also employed as test organisms. Stock cultures were maintained at 4°C on slopes of nutrient agar. The cultures were diluted to achieve optical densities corresponding to 2.0 × 10⁶ colony forming units (CFU/ml) for bacterial and 2.0 × 10⁵ spore/ml for fungal strains.

Antimicrobial assay
The disc-diffusion assay [7] was used to determine the antimicrobial potential of the investigated extracts. Nutrient agar (OXOID LTD, Basingstoke, Hampshire, England) was prepared by dissolving of 27 g/l in water. The sterile nutrient agar was inoculated with microbial cells (200 μl of microbial cell suspension in 20 ml agar medium) and poured into sterile petri dishes. Sterile filter paper discs of 6 mm diameter (Schleicher and Schuell, ref. No. 10321260, lot. DG0274-1) were impregnated with 20 μl of the extract solution (equivalent to 4 mg of the dried extract). The paper discs were dried and placed on the surface of the inoculated agar plates. Plates were kept for 2 hours in refrigerator to enable prediffusion of the extracts into the agar. Then the plates were incubated overnight (18 hours) at 37°C. In contrast, M. flauus was incubated at room temperature for 48 h and C. maltosa was incubated at 28°C for 48 h. Ampicillin, gentamicin and amphotericin B were used as positive control. Negative controls were performed with paper discs loaded with 20 μl of organic solvents (methanol and 5% ethanol) and dried. At the end of the incubation period the antimicibiral
activity was evaluated by measuring the inhibition zones (diameter of inhibition zone plus diameter of the disc). An inhibition zone of 15 mm or more was considered as high antibacterial activity.

**Broth micro-dilution assay for minimum inhibitory concentrations (MIC)**

The broth micro-dilution method described by [8] with modifications was used to determine the MIC of extracts against the three standard Gram-positive strains. With sterile round-bottom 96-well plates, duplicate two-fold serial dilutions of extract (100 μl/well) were prepared to the appropriate broth containing 5% (v/v) DMSO to produce a concentration range of 2000 to 15.6 μg of extract/ml. Two-fold dilutions of ampicillin were used as a positive control. A bacterial cell suspension (prepared in the appropriate broth) of 100 μl, corresponding to 1 × 10^6 CFU/ml, was added in all wells except those in columns 10, 11 and 12, which served as saline, extract and media sterility controls, respectively. Controls for bacterial growth without plant extract were also included on each plate. The final concentration of bacteria in the assay was 5 × 10^5 CFU/ml. The final concentration of extracts ranged between 1000 to 7.8 μg/ml. Plates were then incubated at 37°C for 18 h overnight. After incubation, the MIC of each extract was determined as the lowest concentration at which no growth was observed in the duplicate wells. Twenty microliters of a p-iodonitro-tetrazolium violet solution (0.04%, w/v) (Sigma, USA) was then added to each well. The plates were incubated for a further 30 min, and estimated visually for any change in color from yellow to pink indicating reduction of the dye due to bacte-

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**Table 1: List of plants screened in the study.**

| Plant                      | Voucher specimen no. | Family    | Part tested | Traditional uses a |
|----------------------------|----------------------|-----------|-------------|-------------------|
| *Acacia pennervia* Schweinf. | Mo-Sq28              | Mimosaceae | L           | As a paste around the breast for women with mastitis |
| *Acanthospermum hispidum* DC. | Mo-Sq10              | Astraceae  | L           | Unknown           |
| *Acridocarpus socotranus* Oliv. | Mo-Sq16              | Malpighiaceae | L, S       | Headaches, paralysis and muscle or tendon pain |
| *Aloe perryi* Baker          | Mo-Sq9               | Aloeaceae  | R           | For eye and stomach problems, constipation and malaria |
| *Ballochia atro-virgato* Balf.f. | Mo-Sq15              | Acanthaceae | S, L       | Unknown           |
| *Blepharis spiculifolia* Balf.f. | Mo-Sq13              | Acanthaceae | L, S       | Unknown           |
| *Boswellia dioecorides* Thulin & Gifri | Mo-Sq26          | Burseraceae | B           | Common cold, bronchitis, asthma and rheumatism |
| *Boswellia socotranus* Balf.f. | Mo-Sq24              | Burseraceae | B           | As *Boswellia dioecorides* |
| *Capparis cartilagino Deene.* | Mo-Sq8               | Capparaceae | L           | To treat itching, shortness of breath, head cold and for tumors |
| *Commiphora ornifolia* (Balf.f.) Gillett | Mo-Sq23             | Burseraceae | B           | Antiseptic, diarrhea, dysentery and emmenagoge |
| *Corchorus erodioides* Balf.f. | Mo-Sq27              | Tiliaceae  | L, F        | Diuretic and urinary tract infections |
| *Croton sacotranus* Balf.f.  | Mo-Sq4               | Euphorbiaceae | L, T       | For wounds |
| *Euclea divinorum* Hiern    | Mo-Sq1               | Ebenaceae  | R           | For oral care, tooth ache, fungal diseases, sores, wounds and abscesses |
| *Euphorbia socotranus* Balf.f. | Mo-Sq5               | Euphorbiaceae | L         | For skin diseases and wounds |
| *Eureia ionidion* Cogn.      | Mo-Sq3               | Cucurbitaceae | L         | Unknown |
| *Ficus cordata* Thunb.       | Mo-Sq4               | Moraceae   | L           | Antiseptic and for ulcers and wounds |
| *Glossonema revell* Franch.  | Mo-Sq29              | Asclepiadaceae | L, F    | Increase milk production in breastfeeding women |
| *Hibiscus naq-tangere* A.G.Mill. | Mo-Sq30              | Malvaceae  | L, R        | For snake bite and fever in children |
| *Hypoeus pestes* Balscens* Balf.f. | Mo-Sq12             | Acanthaceae | L           | Fungal skin diseases and scabies |
| *Lannea transulta* (Balf.f.) Radcl.-Sm. | Mo-Sq17         | Anacardiaceae | L          | Haemostatic for wounds, and sores and abrasions |
| *Leucas samhaensis* Cortes-Burns & A.G.Mill. | Mo-Sq23             | Labiatae   | L           | For cough and cold |
| *Leucas virginica* Balf.f.   | Mo-Sq21              | Labiatae   | L           | For persons with heartburn and indigestion and stomach problems |
| *Lycurium sokotranum* Wagner & Vierh. | Mo-Sq20            | Solanaceae | L, S        | For stomach ailments and encourage the wound healing |
| *Marua angolensis* DC.       | Mo-Sq7               | Capparaceae | L           | To treat fever, aches and general malaise |
| *Rhus thysiflora* Balf.f.    | Mo-Sq18              | Anacardiaceae | T, L      | To treat anorexia, general tonic and for painful joints |
| *Teucrium sokotranum* Vierh. | Mo-Sq22              | Labiatae   | F, L        | As flavoring agent and for indigestion |

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

a Most of the information of traditional use has been taken from [2] and native people.
rial growth. The highest dilution (lowest concentration) that remained yellow corresponded to the MIC. Experiments were performed in duplicate.

**Determination of anticancer activity-Cytotoxicity assay on human cancer cell lines**

For the estimation of the in vitro cytotoxic potency of the investigated extracts, an established microtiter plate assay [9] was used with three human cancer cell lines: one lung cancer (A-427), one urinary bladder cancer (5637) and one breast cancer (MCF-7) line. Cell lines were obtained from the DMSZ, Braunschweig, Germany, and culture in RPMI 1640 medium with 10% FCS. Cytotoxicity determinations are based on cellular staining with crystal violet and were performed as previously described in detail. Briefly, a volume of 100 μl of a cell suspension was seeded into 96-well microtiter plates at a density of 1,000 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. Etoposide was used as a positive control. 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 λ = 570 nm with a plate reader. The percent growth values were calculated by the following equation:

\[
\text{Growth} \% = \frac{\text{OD}_T - \text{OD}_{c,0}}{\text{OD}_{c,0}} \times 100
\]

Where OD_T is the mean absorbance of the treated cells, OD_c is the mean absorbance of the controls, and 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.

**Determination of antioxidant activity (Scavenging Activity of DPPH Radical)**

The DPPH free radical scavenging assay was carried out for the evaluation of the antioxidant activity. This assay measures the free radical scavenging capacity of the investigated extracts. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple colour typical for free DPPH radical decays, and the absorbance change at λ = 517 nm is measured. This test provides information on the ability of a compound to donate a hydrogen atom, on the number of electrons a given molecule can donate, and on the mechanism of antioxidant action. The method was carried out as previously described by [10]. The methanolic and aqueous extracts were redissolved in methanol and 5% ethanol, respectively, and various concentrations (10, 50, 100, 500 and 1,000 μg/ml) of each extract were used. Similar concentrations of ascorbic acid were used as positive control. The assay mixture contained in a total volume of 1 ml, 500 μl of the extract, 125 μl prepared DPPH (1 mM in methanol) and 375 μl solvent (methanol or 5% ethanol). After 30 min incubation at 25°C, the decrease in absorbance was measured at λ = 517 nm. The radical scavenging activity was calculated from the equation:

\[
\% \text{ of radical scavenging activity} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100
\]

**Phytochemical screening of the methanolic extracts**

The screening of chemical constituents was carried out with the methanol extracts by using chemical methods and thin-layer chromatography (TLC) using different mixtures of organic solvents as mobile phases. Several chemical reagents e.g. Dragendorf's reagent for alkaloids, borntraeger reagent for anthraquinones and etc. were used in the detection according to previously published methodology [11].

**Results**

In the course of our screening for the antimicrobial, anticancer, and antioxidant activities, a number of plants from different locations of the island Soqotra used in Yemeni traditional medicine were evaluated. A total of 52 extracts representing 26 plant species belonging to 17 families were submitted to biological screening. The botanical names, plant part used and the traditional uses of the plants in the collected areas are presented in Table 1.

**Antimicrobial activity**

Table 2 shows the results of the antimicrobial activity of the investigated extracts in agar diffusion method. An inhibition zone > 15 mm was considered as a high antimicrobial activity. It was observed that the antimicrobial activity of the studied plant extracts was exhibited mainly against the Gram-positive bacteria. Consequently, the MIC-values were determined only against Gram-positive bacteria. The MIC values are reported in Table 3. In general, among the investigated extracts the methanolic extracts exhibited the greatest antimicrobial effect.

Only rarely do plants shown antimicrobial activity against Gram-negative bacteria and Candida maltosa except Acacia pennivenia, Boswellia species and Euclea divinorum. It was apparent that the multiresistant Staphylococcus strains demonstrate more sensitivity to the investigated extracts than the other antibiotic susceptible Gram-positive bacteria (Table 2). Moreover, it was found that with the exception of Boswellia species, Euclea divinorum and Hypoestes pubescens no extract showed antifungal activity against Candida maltosa (Table 2). The most pronounced activity
Table 2: Antimicrobial activity of the investigated plants in agar diffusion test.

| Plant species          | Extracts | Extract yield in % | Microbial strains tested | Multiresistant strains tested |
|------------------------|----------|---------------------|--------------------------|-----------------------------|
|                        |          |                     | S. a. | B. c. | M. f. | E. c. | P. e. | C. m. | S. e. 847 | S. h. 535 | S. a. NGR |
| Acacia pennivenia      | Methanolic | 12.18              | 22    | 12    | 22    | 16    | 14    | -     | 18     | 14     | 20       |
| Basella dioecoides     | Methanolic | 40.45              | 18    | 16    | 20    | 8     | 10    | 10    | 28     | 18     | 38       |
| Basella socotrana      | Methanolic | 37.77              | 31    | 21    | 27    | 14    | 20    | 22    | 28     | 20     | 28       |
| Capparis cartilaginea  | Methanolic | 8.33               | 10    | -     | -     | -     | -     | -     | -      | -      | -        |
| Croton socotrana       | Methanolic | 14.91              | 10    | 8     | 15    | -     | -     | -     | 18     | 10     | 24       |
| Euphorbia divinorum    | Methanolic | 21.85              | 24    | 12    | 18    | 11    | 15    | 10    | 24     | 16     | 26       |
| Ficus balfourii        | Methanolic | 11.60              | 10    | -     | -     | -     | -     | -     | -      | -      | -        |
| Glossonema revoilii    | Methanolic | 16.34              | 20    | -     | -     | -     | -     | -     | -      | -      | -        |
| Hibiscus nol-tangere   | Methanolic | 10.61              | 10    | -     | -     | -     | -     | -     | -      | -      | -        |
| Hypoestes pubescens    | Methanolic | 21.16              | 8     | -     | -     | 15    | -     | -     | -      | -      | -        |
| Laneea transulta       | Methanolic | 8.04               | 13    | -     | 14    | -     | -     | 16    | -      | -      | 16       |
| Leucas samhaensis      | Methanolic | 11.47              | -     | 20    | -     | -     | -     | -     | 14     | 12     | 18       |
| Leucas virgata         | Methanolic | 14.11              | 18    | 11    | 17    | -     | -     | -     | 16     | 12     | 22       |
| Lycium sokotranum      | Methanolic | 10.93              | 20    | -     | 15    | -     | -     | -     | 12     | 12     | 20       |
| Maerua angolensis      | Methanolic | 22.01              | -     | -     | -     | -     | -     | -     | -      | -      | -        |
| Rhus thrysiflora       | Methanolic | 5.88               | 20    | 12    | 18    | -     | -     | 18    | 14     | 24     | -        |
| Teucrium sokotranum    | Methanolic | 12.42              | 17    | 14    | 22    | -     | -     | 14    | 10     | 16     | -        |
| Ampicillin 10 µg/disc   |           | 28                | 24    | 30    | N.T.  | N.T.  | N.T.  | -     | -      | -      | -        |
| Gentamicin 10 µg/disc   |           | N.T.             | N.T.  | N.T.  | N.T.  | 15    | 17    | N.T.  | N.T.   | N.T.   | N.T.     |
| Amphotericin 10 µg/disc |           | N.T.            | N.T.  | N.T.  | N.T.  | N.T.  | N.T.  | 10    | N.T.   | N.T.   | N.T.     |
with inhibition zones greater than 15 mm was found with the methanolic extracts of 10 plants (Table 2). The most effective plant was *Boswellia socotrana*, of which the methanolic and aqueous extracts demonstrated the greatest antimicrobial effect against all tested microorganisms (Table 2). The lowest MIC values were obtained against *Staphylococcus aureus* and *Micrococcus flavus* by the methanolic extracts of *Boswellia* species (125 μg/ml) (Table 3).

**In vitro anticancer activity**
Table 4 presents the IC₅₀ values for the in vitro cytotoxic activity of the investigated 26 methanolic extracts. From our experience, the most aqueous extracts don’t show any notable in vitro anticancer effect at the highest concentration tested (50 μg/ml). Generally, the aqueous extracts may exhibit only a weak cytotoxic effect starting with a concentration of 250 μg/ml. Thus, these extracts were excluded in our screen. The results in Table 4 demonstrate that out of the 26 investigated plants (only methanolic extracts) three plant extracts had noteworthy cytotoxic effects in all three cell lines tested, namely: *Ballochia atrovirgata* (IC₅₀ between 1.5 and 2.8 μg/ml), *Eureiaandra balbouiri* (IC₅₀ between 0.8 and 3.4 μg/ml) and *Hypoestes pubescens* (IC₅₀ between 4.8 and 8.2 μg/ml) (Table 4). Moreover, the extracts of *Acanthospermum hispidum*, *Boswellia dioscorides*, *Boswellia socotrana*, *Commiphora ornifolia* and *Euphorbia socotrana* exhibited a pronounced cytotoxic effect against all tested cell lines (IC₅₀ between 9.3 and 38.5 μg/ml). The other plant extracts demonstrated no significant cytotoxic effect at the highest tested concentration of 50 μg/ml.

**Radical scavenging activity**
The methanol extracts of seven plants, namely: *Acacia pennivenia*, *Acridocarpus socotranus*, *Boswellia dioscorides*, *Boswellia socotrana*, *Commiphora ornifolia*, *Euphorbia socotrana*, and *Lannea transulta* showed a high effective free radical scavenging in the DPPH assay (Table 3). These extracts exhibited a noticeable antioxidant effect at low concentrations (Table 3). So the methanolic extracts of *Acacia pennivenia*, *Boswellia dioscorides*, *Boswellia socotrana* and *Commiphora ornifolia* exhibited a great antioxidant effect at 50 μg/ml (78, 89, 88% and 85%, respectively), comparing with the effect of ascorbic acid at this concentration (Table 3). The hot aqueous extracts of all investigated plants showed only weak antioxidant effect (Table 3).

**Phytochemical screening**
The results of the phytochemical screening of the investigated methanolic extracts indicated the presence of different types of active constituents like flavonoids, terpenoids, tannins, volatile oils, etc... (Table 4).

**Discussion**
In continuation of our search for substances of plant origin with pharmacological effects, we have screened 26 plants collected from the island Soqotra, Yemen for their antimicrobial, cytotoxic and antioxidant activities and for their chemical content. It is important to mention that this work represents the first report on the antimicrobial, cytotoxic and antioxidant activities of extracts from 20 endemic plants (Table 1). The existing knowledge about the other six investigated plants is in many cases very limited.

A correlation was found between the antibacterial activity observed by agar diffusion assay and MIC determination. It is interesting to note that these plant extracts showed more activity on multi-drug resistant *Staphylococcus* strains than the antibiotic susceptible Gram-positive bacteria.

It was demonstrated previously that alcoholic extract of different species of *Acacia* like *A. arabica*, *A. nilotica* and *A. auriculiformis* have antibacterial activity [12-15], and that the isolated saponines from *A. auriculiformis* were responsible for the noticed effect [15]. Previous work indicated that both bark and heartwood extracts of *A. confusa* clearly have strong antioxidant effects [16]. Phenolic compounds were isolated from the bark of *A. confusa*, which were mainly responsible for the antioxidant effect of this plant [17]. Furthermore, triterpenoid saponins were isolated from *A. victoriae*, which induced decreased tumor cell proliferation and induced apoptosis [18]. In addition, a significant reduction in the values of tumor burden and tumor incidence was observed in mice treated by oral gavage with the *A. nilotica* gum and leaf extracts [19]. The antimicrobial and antioxidant effects we have found with extracts from *A. pennivenia* are in accordance with these data. The phytochemical screening revealed the presence of saponins and phenolic compounds like tannins in the methanolic extract of *A. pennivenia*, which could be responsible for these activities. On the other hand, our results of cytotoxic activity were not in agreement with the anticancer effect noted with other *Acacia* species; i.e., at the highest concentration used in our screening (50 μg/ml), the extracts of *A. pennivenia* exhibit no cytotoxic activity.

The antimicrobial effect of leaves and flowers extracts of *Acanthospermum hispidum* has been previously investigated [20]. Moreover, the isolation of sesquiterpene lactones was reported [21,22]. Our screening results confirmed the antimicrobial effect of the alcoholic extract. The hot aqueous extract exhibited a moderate antibacterial effect in contrast with data presented earlier [20]. In addition, the methanolic extract brought about a pronounced growth inhibitory effect against all tested cancer...
| Plant species | Extracts   | Radical scavenging activity in % | MIC in μg/ml |
|--------------|-----------|----------------------------------|-------------|
|              |           | 10 (μg/ml) 50 (μg/ml) 100 (μg/ml) 500 (μg/ml) 1000 (μg/ml) | S. aureus | B. subtilis | M. flavus |
| Acacia pennienia | Methanolic | 25.4 78.5 88.9 95.0 94.0 | 250 500 250 |
| Acanthospermum hispidum | Methanolic | 2.8 13.8 | 25.9 55.5 94.7 | 250 500 500 |
| Ballochia atrovirgata | Methanolic | 3.2 11.6 | 14.4 26.3 27.4 | >1000 >1000 >1000 |
| Aloe perryi | Methanolic | 6.7 19.8 | 28.7 92.4 94.1 | >1000 >1000 >1000 |
| Ballochia atr-virgata | Methanolic | 2.1 5.3 | 45.7 56.8 94.2 | >1000 >1000 >1000 |
| Blepharis spiculifolia | Methanolic | 14.8 13.3 | 25.1 64.6 91.5 | >1000 >1000 >1000 |
| Baswellia diasconides | Methanolic | 25.8 89.8 | 96.1 96.3 96.8 | 125 500 125 |
| Baswellia socotranus | Methanolic | 26.1 88.2 | 94.6 95.1 125 | 250 500 250 |
| Capparis cartilaginea | Methanolic | 0.2 3.8 | 2.1 51.2 87.6 | >1000 >1000 >1000 |
| Commiphora ornifolia | Methanolic | 20.8 85.9 | 95.0 95.4 95.2 | 250 500 250 |
| Corchorus erodoides | Methanolic | 10.4 14.3 | 27.6 95.1 94.1 | >1000 >1000 >1000 |
| Croton socotranus | Methanolic | 2.0 2.7 | 34.5 43.7 36.7 | >1000 >1000 >1000 |
| Eucleria divinorum | Methanolic | 3.1 10.6 | 36.2 93.1 88.6 | 250 1000 500 |
| Euphorbia socotran | Methanolic | 16.7 67.4 | 95.5 96.3 95.2 | 500 1000 500 |
| Euphorbia socotran | Methanolic | 19.1 8.8 | 28.7 36.2 40.1 | >1000 >1000 >1000 |
| Euphorbia socotran | Methanolic | 15.0 17.8 | 8.3 19.1 57.7 | >1000 >1000 >1000 |
| Ficus cordata | Methanolic | 2.5 12.5 | 31.0 89.2 91.6 | >1000 >1000 >1000 |
| Glossonema revolli | Methanolic | 1.7 14.9 | 26.8 38.6 76.4 | >1000 >1000 >1000 |
| Hibiscus noli-tangere | Methanolic | 0.6 7.3 | 18.4 85.9 89.0 | >1000 >1000 >1000 |
| Hypoestes pubescens | Methanolic | 1.5 4.0 | 16.6 38.7 72.6 | >1000 >1000 >1000 |
| Lannea transulta | Methanolic | 21.9 64.2 | 95.4 92.1 98.6 | 1000 >1000 1000 |
| Leucas samhaensis | Methanolic | 2.3 9.6 | 8.0 61.7 88.8 | >1000 >1000 500 |
| Leucas virgata | Methanolic | 3.8 5.6 | 37.3 49.3 47.1 | 500 1000 500 |
| Lycium sokotoanum | Methanolic | 1.5 1.1 | 2.8 27.6 81.9 | 500 >1000 1000 |
| Maerua angolensis | Methanolic | 5.6 27.8 | 29.8 91.4 94.2 | 500 1000 500 |
| Rhus thysiflora | Methanolic | 13.8 19.3 | 32.0 52.5 82.3 | >1000 >1000 >1000 |
| Rhus thysiflora | Methanolic | 29.1 65.1 | 69.1 68.3 73.9 | >1000 >1000 >1000 |
| Rhus thysiflora | Methanolic | 3.8 29.4 | 55.9 95.6 95.4 | >1000 >1000 >1000 |
| Rhus thysiflora | Methanolic | 1.1 9.3 | 12.8 22.4 30.5 | 500 1000 500 |
| Teucrium sokotranum | Methanolic | 3.4 2.8 | 16.4 41.0 92.1 | 500 1000 500 |
| Teucrium sokotranum | Methanolic | 24.2 28.7 | 38.2 47.6 61.4 | 250 1000 500 |
| Ascorbic acid | Methanolic | 42.8 94.2 | 96.8 96.6 96.9 | >1000 >1000 >1000 |
cell lines we tested it against. These effects are most likely due to the presence of sequiterpene lactones identified in our phytochemical screening and in literature data.

Whereas the investigation of *Acridocarpus vivy* showed cytotoxic effect against some cancer cell lines [23], the methanolic extract of *Acridocarpus socotranus* demonstrated no cytotoxic activity in our screen. However the plant extract exhibited a strong antioxidant and moderate antibacterial effects. This finding may be correlated with the presence of flavonoids and terpenoids.

In contrast to Ali and co-workers [24], who found a promising antimicrobial effect for *Aloe perryi*, our extracts of *Aloe perryi* only showed activity against multiresistant bacteria. Although the phytochemical screening illustrated the presence of anthraquinons, which are mostly responsible for cytotoxic and antioxidant activity [25], our results do not indicate any cytotoxic activity at the highest concentration tested (50 μg/ml) and exhibited a moderate antioxidant effect only at high concentrations (500 and 1000 μg/ml).

It is important to consider that we found no published data on the genus *Ballochia* and *Eureiandra*. So this is the first report on pharmacological and chemical investigation of plants of these genera. The remarkable cytotoxic effect of both plants with IC\(_{50}\) values between 0.8 and 3.4 μg/ml is significant. According to the criteria of the American National Cancer Institute, 30 μg/ml is the upper IC\(_{50}\) limit considered promising for purification of a crude extract [26]. Therefore, the highest concentration tested (50 μg/ml) in our screening was slightly above this limit.

The chemical screening showed the presence of terpenoids in both plants (mainly diterpenoids and triterpenoids), which could be responsible for the observed activity in *Eula*.

In our previous studies, the antimicrobial effects of some *Boswellia* species namely *B. elongata* and *B. ameero* were

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### Table 4: IC\(_{50}\) values (μg/ml) for cell growth inhibition of the investigated methanolic extracts on three human cancer cell lines and phytochemical screening.

| Plant species              | Extracts | Cell lines | Phytochemical screening         |
|---------------------------|----------|------------|---------------------------------|
| *Acacia pennienia*        | Methanolic | > 50       | > 50                            |
| *Acanthospermum hispidum* | Methanolic | 9.37 ± 1.98 | 19.92 ± 8.94                   |
| *Acridocarpus socotranus* | Methanolic | > 50       | > 50                            |
| *Aloe perryi*             | Methanolic | > 50       | > 50                            |
| *Ballochia atror-virgata* | Methanolic | 2.83 ± 2.06 | 2.94 ± 0.28                    |
| *Blepharis spiculifolia*  | Methanolic | > 50       | > 50                            |
| *Basella diosconides*     | Methanolic | 29.0 ± 8.26 | > 50                            |
| *Basella socotransa*      | Methanolic | 18.4 ± 4.66 | > 50                            |
| *Capparis cartaginea*     | Methanolic | > 50       | > 50                            |
| *Connolophora ornifolia*  | Methanolic | 30.2 ± 3.12 | 38.5 ± 1.93                    |
| *Corchorus erodioides*    | Methanolic | > 50       | > 50                            |
| *Croton socotransa*       | Methanolic | > 50       | > 50                            |
| *Euclea divinorum*        | Methanolic | > 50       | > 50                            |
| *Euphorbia socotransa*    | Methanolic | 42.0 ± 7.43 | > 50                            |
| *Euleandra balfourii*     | Methanolic | 0.82 ± 0.15 | 3.47 ± 1.18                    |
| *Ficus cordata*           | Methanolic | > 50       | > 50                            |
| *Glossonema revelli*      | Methanolic | > 50       | > 50                            |
| *Hibiscus nak-tangere*    | Methanolic | > 50       | > 50                            |
| *Hypepeus pustescens*     | Methanolic | 8.25 ± 3.49 | 7.03 ± 2.02                    |
| *Lannea transulta*        | Methanolic | > 50       | > 50                            |
| *Leucas samhaensis*       | Methanolic | > 50       | > 50                            |
| *Leucas virgenta*         | Methanolic | > 50       | > 50                            |
| *Lycium sokotransa*       | Methanolic | > 50       | > 50                            |
| *Maerua angolensis*       | Methanolic | > 50       | > 50                            |
| *Rhus thyrsiflora*        | Methanolic | > 50       | > 50                            |
| *Teucrum sokotransa*      | Methanolic | > 50       | > 50                            |
| *Etoposide (μM)*          | 0.54 ± 0.30 | 0.50 ± 0.19 | 0.13 ± 0.10                   |

Values are averages and standard deviations of three or more independent determinations.
investigated [3]. It was found that both plants exhibited a strong antimicrobial effect only against Gram positive bacteria. Both plants demonstrated no cell growth inhibition on five human cancer cell lines [6]. Furthermore, it was reported that the extract of *B. serrata* showed an effect against some bacterial strains [30]. The antimicrobial activity and the chemical content of the volatile oil of some *Boswellia* species like *B. carteri*, *B. papyrifera*, *B. serrata* and *B. rivae* were determined [31]. It was shown that the oils consisted of several monoterpenes, sesquiterpenes and diterpenes are responsible for the effect observed. The *B. socotrana* and *B. dioscorides* we investigated produced extracts with the most effective activity against all tested microorganisms. The essential oils and the terpenoids determined in our phytochemical screening are mostly responsible for this effect. Besides this noticeable antimicrobial effect, the methanolic extracts manifested a considerable cytotoxic effect against two cell lines (5637 and A-427) with IC₅₀ values between 18 and 29 μg/ml. For this effect, boswellic acids, which may represent a considerable part of the chemical content, could contribute to this observed effect. Furthermore, both plants demonstrated even at low concentration (50 μg/ml) a remarkable radical scavenging effect (88 and 89%). This effect may be attributed to the present flavonoids.

Among the most interesting plants, *Commiphora ornifolia* showed similar antimicrobial activities with *Boswellia* plants. The same extract manifested a high antioxidant effect (85%) at 50 μg/ml. The extracts and isolated compounds from *Commiphora opobalsamum* exhibited similar antimicrobial and antioxidant activities [32]. Thus, the estimated antimicrobial and antioxidant effects of our investigated *C. ornifolia* are in accordance with these data. Whereas no cytotoxic activity in *C. myrrha* was found [33], we observed an antiproliferative effect of the methanolic extract against all three cell lines with IC₅₀ values between 30 and 38 μg/ml. The determined effects of *C. ornifolia* are mostly attributed to the essential oil, flavonoids and triterpenes found in the methanolic extract.

Another interesting plant was *Euphorbia socotrana*, which demonstrated considerable antimicrobial, cytotoxic and antioxidant activities. Our data are in agreement with literature data of other *Euphorbia* species as *E. thymifolia*, *E. hirta* and *E. cheiradenia* [34-36]. Studies have demonstrated that some triterpenoids isolated from *Euphorbia* species are responsible for the antimicrobial effect and that some of these compounds were able to induce moderate apoptosis in at least one cancer cell line [37,38]. The presence of such terpenoids in our *E. socotrana* may explain the biological effects seen in our screens.

Some authors reported on investigation with some *Hypoestes* species, where the isolation of two cell growth inhibitory phenanthroindolizidine alkaloids termed hypoestestatin 1 and hypoestestatin 2 from the East African shrub *H. verticillaris* was described [39]. In addition, several antifungal diterpenoids from *H. serpens* were isolated [40]. The methanolic extract of *H. pubescens* tested in our screens showed a remarkable antifungal effect against *Candida maltosa* and an extraordinary cytotoxic effect against all tested cancer cell lines. Apparently these observed activities are correlated with the presence of the terpenoids and alkaloids found in the phytochemical screening.

The notably antimicrobial effect of *Rhus thyrsiflora* and *Teucrium succedaneum* was consistent with literature data of other *Rhus* and *Teucrium* species [41,42]. On the other hand, other authors noted a strong antioxidant effect of some *Teucrium* species and *R. coriaria*, which is not supported by results obtained in our screening [43,44]. Moreover, a cytotoxic activity for the sap of the lacquer tree *R. succedanea* was described previously [45], whereas the investigated *R. thyrsiflora* showed no growth inhibitory effect on the tested cancer cell lines.

**Conclusion**

In conclusion, the results in the present study are agreed to some extent with the traditional uses of the plants investigated. Our results further support the idea that medicinal plants can be promising sources of potential anticancer and antimicrobial agents and antioxidants. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Studies aimed at the isolation and structure elucidation of anticancer, antibacterial and antioxidant active constituents from some plants e.g. *Acacia pennivenia*, *Ballochia atro-virgata*, *Boswellia dioscorides*, *Boswellia socotrana*, *Commiphora ornifolia*, *Euphorbia socotrana*, *Eureandria balfourii* and *Hypoestes pubescens* are in progress.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

RAM carried out the study design, plant collection, experimental work, data collection and interpretation, literature search and manuscript preparation. RG provided assistance in evaluation of the anticancer activity and data interpretation. UL and PJB supervised the work, evaluated the data and corrected the manuscript for publication. All authors read and approved the final manuscript.

**Acknowledgements**

RAM would like to extend deep thanks to the Alexander von Humboldt Foundation for a Georg-Foster-Scholarship enabling a research stay at University of Greifswald. The Soqotra Archipelago Conservation and Develop-
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**Pre-publication history**
The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1472-6882/9/7/prepub