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STUDY OF *IN VITRO* ANTIMICROBIAL AND ANTIPROLIFERATIVE ACTIVITIES OF SELECTED SAHARAN PLANTS

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The aim of the present study was the evaluation of the antimicrobial and antiproliferative activities of selected Saharan species, which are applied in the traditional medicine but not studied thoroughly from chemical and pharmacological point of view. The studied plants, namely *Anthyllis henoniana, Centropodia forskalii, Cornulaca monacantha, Ephedra alata* var. *alenda, Euphorbia guyoniana*, *Helianthemum confertum, Henophyton deserti, Moltkiopsis ciliata* and *Spartidium saharae* were collected from remote areas of North Africa, especially from the Tunisian region of Sahara. After drying and applying the appropriate extraction methods, the plant extracts were tested in antimicrobial screening assay, performed on 19 Gram-positive and -negative strains of microbes. The inhibition zones produced by plant extracts were determined by disc-diffusion method. Remarkable antibacterial activities were exhibited by extracts of *Ephedra alata* var. *alenda* and *Helianthemum confertum* against *B. subtilis, M. catarrhalis* and methicillin-resistant and non-resistant *S. aureus*. Minimum inhibitory concentrations of these two species were also determined. Antiproliferative effects of the extracts were evaluated against 4 human adherent cell lines (HeLa, A431, A2780 and MCF7). Notable cell growth inhibition was found for extract of *Helianthemum confertum* and *Euphorbia guyoniana*. Our results provided data for selection of some plant species for further detailed pharmacological and phytochemical examinations.

Keywords: Saharan plants − *Ephedra alata* var. *alenda* − *Helianthemum confertum* − antibacterial activity − antiproliferative activity

INTRODUCTION

Nowadays the use of natural products including medicinal plants has come to the front in the human therapy, especially in developing countries. In these regions, including the Saharan territory, a large part of the population still apply traditional medicine to treat even serious diseases. A huge number of chemical and pharmacological studies of plant species are performed to find new lead compounds for developing new therapeutic agents of human diseases such as cancer or infections. Despite

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the wide spectra of antimicrobial drugs, the infectious diseases including multidrug resistant pathogens and new infections, are still a worldwide problem, moreover the cancerous diseases are the one of leading causes of death globally. These facts encourage the search for new antimicrobial and anticancer agents with much higher activity and better side-effect spectrum.

Because of the drastic climate conditions, very few number of plant species, mainly bushes, shrubs or sub-shrubs are found in Sahara. The morphological and physiological characteristics of these plants adapted to the arid environment and the limited availability of water [8]. Moreover, the ability of plants to synthetize primary and secondary metabolites can help the survival in these extreme environment and protects against pathogenic attacks. As a consequence of harsh conditions these compounds possibly possess very unique structures with some remarkable biological activities [15].

Despite some studies on the Saharan plants, performed especially in Tunisian arid area, our knowledge about Saharan species are still scant. Most of investigations, performed in recent years on the Saharan flora and vegetation, are purely descriptive, and only a few experimental studies have been performed [1, 18, 24]. Since the literature search showed the absence of information about biological and phytochemical properties of plants from Sahara, the aim of our study was to carry out a pharmacological investigation on selected Saharan plants, which are endemic and/or used in the local traditional medicine. We have limited information about traditional application of these selected species, since they are found mainly in underpopulated area of Saharan desert. *E. guyoniana* is used in local medicines for treatment of skin diseases and *C. monocantha* is applied as laxative, hepatoprotective and antiparasitic medicine by the Moroccan traditional healers [4, 17]. *M. ciliata* is used in veterinary medicine for improving of lactation of camels and *C. forskalii* are known as food for ruminants [7, 26].

In this project nine species were collected for examination of their antimicrobial and cytostatic activities. Excluding three species, namely *Ephedra alata* var. *alenda* (Stapf.) Trabut, *Henophyton deserti* (Coss.&Durieu) and *Euphorbia guyoniana* (Boiss. and Reut.), the other species included in our study were poorly investigated previously as regards their biological effects and chemical constituents responsible for the activities. The present paper reports on the evaluation of antimicrobial and cancer cell growth inhibitory activities of Saharan plants, in order to reveal their therapeutic profile.

MATERIALS AND METHODS

Plant material

The aerial parts of plants and the aerial and underground parts of *H. confertum* were collected in March 2013 from Tunisian rural areas. The plant materials were identified by Prof. M. Chaieb at University of Sfax. The voucher specimens (numbers in Table 1) have been deposited at the Department of Pharmacognosy, University of Szeged, Hungary.

Extraction of plant material

The dried and powdered plant materials (20 g) were extracted with ethanol–water (1:1; 200 mL) mixture by percolation at room temperature for 24 h. The extracts were filtered and evaporated at 40 °C under reduced pressure to remove ethanol, and then lyophilized. The obtained dried powders were stored at –20 °C until used.

Test organisms

The following microorganism were employed as test strains in the screening assay: 8 Gram-positive strains, namely *Bacillus subtilis* (ATCC 6633), *Enterococcus faecium* (QC 2008), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus agalactiae* (ATCC 13813), *Streptococcus pneumoniae* (ATCC 49619), *Streptococcus pyogenes* (ATCC 19613) and 10 Gram-negative strains, namely *Acinetobacter lwolffii* (ATCC 44677), *Citrobacter freundii* (ATCC 34015), *Enterobacter cloaceae* (ATCC 45268), *Escherichia coli* (ATCC 25922), *Haemophilus influenzae* (10211), *Klebsiella pneumoniae* (ATCC 700603), *Moraxella catarrhalis* (ATCC 25238), *Proteus mirabilis* (HNCMB 60076), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enteritidis* (HNCMB 10092), *Shigella sonnei* (ATCC 25201). In addition one multiresistant strain, namely methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 43300) was also used as test organism. The test organisms were cultured at Columbia agar + 5% sheep blood (COS) plates (bioMérieux) at 37 °C. Chocolate agar + PolyVitex™ (PVX) (bioMérieux)) was used for growing of *Haemophilus influenzae*. The culture of bacteria were maintained in their appropriate plates at 4 °C throughout the experiment and used as stock cultures.

Antibacterial screening assay

The antibacterial activity of plant extracts was evaluated with disc-diffusion method [5]. The bacterial isolates for screening assay were prepared by picking single colony from 24-h old plates and it was suspended in sterile, isotonic saline solution (5 mL) to reach 0.5 McFarland standard of optical turbidity, resulting a suspension containing approximately $1–2 \times 10^8$ CFU/mL. The bacterial suspension was spread on sterile appropriate plates by sterile cotton swab. Sterile filter paper discs (6 mm of diameter) were loaded with the extracts (20 μL of dried extracts redissolved in ethanol water (40/60 v/v) at 50 mg/mL) and after drying they were placed on the plates. Amoxicillin combination with clavulanic acid and vancomycin were employed as positive controls. Negative controls were performed with paper discs impregnated with 20 uL of solvent. The plates were then incubated at 37 °C for 24 hours under aerobic conditions. The diameters of inhibition zone produced by the plant extracts were measured and recorded (diameter of the inhibition zone plus diameter of the disc).

Determination of minimum inhibitory concentration

The minimum inhibitory concentrations of plant extracts of *Ephedra alata* var. *alenda* and *Helianthemum confertum* were determined with microdilution method [19]. The single colony of cultured bacteria was suspended in Mueller-Hinton (MH) broth and the suspension was adjusted to 0.5 McFarland standard turbidity. In a sterile 96-well plate, 20 μL of samples from the stock solutions (50 and 100 mg/mL of extracts of *Ephedra alata* var. *alenda* and *Helianthemum confertum*, respectively, dissolved in DMSO) were diluted with MH. Twofold serial dilutions were prepared to produce the final concentration range of 5000 to 4.9 μg/mL and 10,000 to 9.7 μg/mL of extracts of *E. alata* var. *alenda* and *H. confertum,* respectively. The bacterial suspension (100 μL) was then added to the wells. The final volume of each well was 200 μL. Control wells were prepared with culture medium, bacterial suspension only and DMSO in amount corresponding to the highest concentration present. After mixing, the plates were incubated at 37 °C for 24 h in aerobic environment. The MIC value was determined by observing the turbidity of media. The MIC was the lowest concentration of extracts showing no detectable bacterial growth. The experiment was performed twice in triplicate.

Evaluation of antiproliferative activity

Antiproliferative effects of the prepared plant extracts were measured *in vitro* on four human cell lines: HeLa (cervix adenocarcinoma), A431 (skin epidermoid carcinoma), A2780 (ovarian carcinoma) and MCF7 (breast adenocarcinoma) by using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [21]. Briefly, cancer cells were seeded onto a 96-well microplate (5000/well) and attached to the bottom of the wells overnight. On the second day, new medium containing the tested extract was added. After incubation for 72 h, the living cells were assayed by the addition of 20 μL of 5 mg/mL MTT solution. Four h later the medium was removed, and the precipitated crystals were dissolved in 100 μL of dimethyl sulfoxide (DMSO) during a 60-min period of shaking. Finally, the absorbance was measured at 545 nm, using a microplate reader; wells with untreated cells were utilized as controls. All *in vitro* experiments were carried out on two microplates with at least five parallel wells. Cisplatin was used as reference compound. Stock solutions of the tested extracts (10 mg/mL) were prepared with DMSO. The cell lines were treated with extracts at concentrations of 10 or 30 μg/mL. The higher concentration of DMSO (0.3%) did not exert substantial action on the viability of the utilized cell lines [20].

RESULTS

A total of 10 aqueous-alcoholic extracts representing nine plant species were subjected to bioassay. The botanical names with voucher specimen numbers and families are presented in Table 1.

The results obtained in the screening of antimicrobial activity of selected Saharan plants are shown in Table 2. Among 9 plant species included in this experiment, extracts of 6 species exhibited antibacterial effects. It was found that, the activity was shown mainly against Gram-positive strains. Among the tested Gram-negative bacteria only *Moraxella catarrhalis* was susceptible to the investigated plant extracts. In the current study, none of the extracts inhibited *C. freundii, E. faecium, S. epidermidis, S. agalactiae, S. pneumonia, S. pyogenes, A. lwolffii, E. cloaceae, E. coli,*

Table 1
List of plants screened in this study

Plant	Voucher specimen no.	Family	Part tested
Anthyllis henoniana (Coss.)	827	Fabaceae	aerial
Centropodia forskalii (Vahl.)	829	Poaceae	aerial
Cornulaca monacantha (Delile)	835	Amaranthaceae	aerial
Ephedra alata var. *alenda* (Stapf.) Trabut	831	Ephedraceae	aerial
Euphorbia guyoniana (Boiss.&Reut.)	832	Euphorbiaceae	aerial
Helianthemum confertum (Dunal)	828	Cistaceae	aerial and underground
Henophyton deserti (Coss.&Durieu)	830	Brassicaceae	aerial
Moltkiopsis ciliata (Forssk.)	833	Boraginaceae	aerial
Spartidium saharae (Coss.&Durieu)	834	Fabaceae	aerial

Table 2
Antibacterial activity of the investigated plants in disc-diffusion method

Plant	Inhibition zone (mm)			
	Microbial strains			
	B.s.	*M.c.*	*S.a.*	MRSA
A. henoniana	9.5	6.5	9.5	–
H. confertum (aerial part)	16	15	11.5	–
H. confertum (rhizome, root)	11.5	8.5	11.5	–
E. alata var. *alenda*	9.5	7.5	9.5	14.5
E. guyoniana	–	10.5	7	–
M. ciliata	–	7	–	–
Amoxicilin + clavulanic acid	n.d.	32	n.d.	n.d.
Vancomycin	22	n.d.	16	16

Table 3
MIC values of investigated plant extracts

Plant	MIC value (mg/mL)		
	Microbial strains		
	B.s.	*M.c.*	MRSA
H. confertum (aerial part)	0.26	0.13	–
E. alata var. *alenda*	–	–	>5

Data represent the mean value of MIC obtained from two independent experiments. Abbreviations: *B.s.*: *Bacillus subtilis*, *M.c.*: *Moraxella catarrhalis*, MRSA: methicillin-resistant *Staphylococcus aureus*.

H. influenzae, K. pneumoniae, P. mirabilis, P. aeruginosa, S. enteritidis and *S. sonnei*. However, antibacterial activities with low to remarkable potential were detected against *B. subtilis, M. catarrhalis* and *S. aureus*. Moreover, the extracts of *E. alata* var. *alenda* demonstrated notable inhibition against methicillin-resistant *S. aureus*. The antibacterial activities of the extracts of *A. henoniana, H. confertum, E. alata* var. *alenda, E. guyoniana* and *M. ciliata* were observed against the aforementioned bacterial strains. The most pronounced activity with inhibition zone greater than 15 mm was found in case of the extract of *H. confertum,* which was active against *B. subtilis, M. catarrhalis* and *S. aureus* as well.

The MIC values are demonstrated in Table 3. The minimum inhibitory concentrations were determined against *B. subtilis, M. catarrhalis* and methicillin-resistant *S. aureus* for extracts of *H. confertum* and *E. alata* var. *alenda*. MIC values indicate that the extract of aerial part of *H. confertum* possesses remarkable antibacterial potential.

All plant extracts were tested for antiproliferative properties against four human cancer cell lines (HeLa, A431, A2780 and MCF7) at two final concentrations (10 and 30 μg/mL). None of the tested extracts elicited higher than 10% inhibition of cancer cell growth at the lower concentration. At 30 μg/mL extract from *E. guyoniana* exerted a minor antiproliferative action against MCF7 cells. Extract from aerial parts of *H. confertum* also caused a moderate inhibition of ovarian cancer cell growth, while the extract obtained from the root of the plant exerted much more substantial action selectively against the same cell line (Table 4).

Table 4
Antiproliferative properties of the effective plant extracts

Plant	Conc. (μg/mL)	Growth inhibition (%) ± SEM	
		MCF7	A2780
E. guyoniana	30	18.26 + 0.81	–*
H. confertum (aerial part)	30	–	12.21 ± 2.65
H. confertum (underground)	30	–	91.62 ± 2.52

*Extracts eliciting less than 10% inhibition of proliferation were considered ineffective (data are not given).

DISCUSSION

In summary, our screening results confirmed that six plant species exhibited remarkable antibacterial activity against four bacterial strains, and notable cancer cell growth inhibition was observed for two extracts.

Helianthemum confertum produced the most effective activity against *B. subtilis, M. catarrhalis* and *S. aureus*. Besides the notable antimicrobial activity of *H. confertum*, the extract of its underground part displayed a considerable cytotoxic effect against ovarian cancer cell line A2780. It is important to notice that no published data on pharmacological or phytochemical investigation of *H. confertum* was found. So, this is the first report on antibacterial and antitumor effects of this species. However, a previous work indicated that some *Helianthemum* species possess antimicrobial effects. The 80% methanolic extracts of *H. alypoides, H. manifolium, H. cinereum* and *H. hirtum* showed good inhibitory activities against *S. aureus, E. faecalis, L. monocytogenes, E. coli* and *S. enterica* [28]. Another research group demonstrated a strong antibacterial effect of *H. kahiricum* as well [6]. Any publication about the cytotoxic effects of *Helianthemum* species has not been found in the literature, so this is also first time it has been noted. However, the antiproliferative effects of *Cistus* species, which are taxonomically related to *Helianthemum* species, were recently demonstrated [25].

The extract of *E. alata* var. *alenda* exhibited moderate antibacterial activity against three bacterial strains and strong inhibitory effect against methicillin-resistant *S. aureus*. In the case of this species, screening results of its antimicrobial capacity were partly in agreement with the findings reported earlier. Ghanem et al. proved the antibacterial effect of acetonitrile extract of *E. alata* against *S. aureus, P. aeruginosa, B. subtilis* and *E. coli* [11]. In our study, the extract of *Ephedra* species was inactive against *P. aeruginosa* and *E. coli*. The probable explanation of this difference is that a subspecies of *Ephedra alata* was included in our investigation, and the different solvent, used for extraction process can also be the reason of the different activities. The presence of alkaloids, including ephedrine and pseudoephedrine, lignans, flavonoids and other phenolic compounds were referred in the extracts of *E. alata,* but the compounds responsible for the antibacterial activity has not been yet identified [22, 23]. The antimicrobial effects of other *Ephedra* species were also described earlier. The water and ethanol extracts of *Ephedra intermedia* inhibited the growth of *S. aureus* with MIC of 0.5 mg/mL [16]. Transtorine, considered as a pharmacologically active compound of *E. transitoria,* was also identified. This quinoline alkaloid exhibited growth inhibitory activity against the common bacteria, *E. cloaceae, E. coli, P. aeruginosa* and *S. aureus* [3].

Euphorbia genus is a well-investigated taxon of the plant kingdom. A less known species, *E. guyoniana* from this genus was tested in our experiment. Moderate antibacterial and noteworthy cytotoxic activity of its extract were observed. Some publications about chemical compositions of *E. guyoniana* were found in the literature. The characteristic compounds of *Euphorbia* genus, the diterpenoids were isolated from *E. guyoniana* as well. *Ent*-abietane and jatrophane polyester diterpenes were

identified in the root and aerial parts of *E. guyoniana* [2, 13, 14]. The pharmacological activity of this species was also reported. Antibacterial capacity and cytotoxic activity against HEK293 cell line of *E. guyoniana* were demonstrated, these results are in agreement with our findings [4, 10].

The existing knowledge about *Anthyllis* species is very limited. Antioxidant activity and phenolic compounds production of two related species, *A. aurea* and *A. vulneraria* were noted in the literature [12, 29]. Any publication on *A. henoniana*, tested in our screening was not found, so this is the first report about moderate antibacterial activity of this species.

Besides the pyrrolizidine alkaloid content of *Moltkiopsis ciliata*, any other chemical and biological information about this species is not available in the literature [26, 27]. In our screening, aqueous ethanolic extract of *M. ciliata* demonstrated a mild antibacterial activity against one Gram-negative strain, namely *M. catarrhalis*.

Any antibacterial and cytotoxic activities were not observed in case of the extracts of other species *(Centropodia forskalii, Cornulaca monacantha, Henophyton deserti* and *Spartidium saharae)*. References about the biological activity of these plants could not be found in the literature, except *H. deserti*. The seed extracts of *H. deserti* showed good inhibitory capacity against *S. aureus, P aeruginosa, E. coli, S. enterica* and *B. subtilis* [9]. The seed extract probably contains special antimicrobial compounds missing in the aerial part, which was inactive in our screening assay.

As concerns the antiproliferative activity, promising cell growth inhibitory results was exerted by the extract of underground part of *H. confertum,* which indicate that this plant is worthy for detailed phytochemical, pharmacological studies, in order to identify compounds responsible for the antiproliferative effect.

In conclusion, the antimicrobial and anticancer activities of some Saharan plant species have been clearly demonstrated against Gram-positive, Gram-negative bacteria and human cancer cell lines. Our screening study proved that Saharan plant species are promising sources of potential antibacterial and antitumor agents. Our findings serve as starting points for selection of plant species for further investigation. The detailed examination of the pharmacologically active plant species, e.g. *H. confertum* and *E. alata* var. *alenda* aimed at isolation and structure elucidation of its bioactive compounds are in progress.

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