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SCREENING OF EXTRACTS FROM ENDEMIC SOCOTRAEN MEDICINAL PLANTS FOR ANTIRADICAL AND ANTIFUNGL ACTIVITY

Nasser A. Awadh Ali[1, 2*], Hassan A. Samaha[1], Norbert Arnold[3], Mehdi Alhaj[4], and Ludger Wessjohann[2]

[1]Pharmacognosy Department, Faculty of clinical Pharmacy Albaha University, KSA.

[2]Department of Pharmacognosy and Pharmaceutics, Faculty of Pharmacy, University of Sana'a, Sana'a, Yemen.

[3]Leibniz Institute of Plant Biochemistry, Department of Bio-organic Chemistry, Weinberg 3, 06120 Halle/Saale, Germany.

[4]Medicinal Chemistry Department, Faculty of Pharmacy, Aden University, Yemen.

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***Correspondence for Author**
Dr Nasser A. Awadh Ali
Pharmacognosy Department, Faculty of clinical Pharmacy Albaha University, KSA.

ABSTRACT

A total of fifty different extracts from eighteen endemic Soqotran medicinal plants belonging to twelve plant families were screened for their antioxidant activity, using the DPPH free radical scavenger assay and their antiphytofungal activity against the phytopathogenic fungus *Cladosporium cucumerinum,* using a microbioassay on TLC plates. Of the extracts tested, 8 out of 25 methanol extracts showed more than 50% antiradical activity at a concentration of 200 µg/ml. Extracts of *Kalanchoe farinaceae*, *Caralluma socotrana***,** and *Boswellia socotrana* were the most active ones. Nine out of fifty extracts exhibited antifungal activity. Chloroform extracts of *Pulicaria stephanocarpa* leaves and roots showed at concentration of 400µg inhibition zones of 20 and 18 mm, respectively. The chloroform extract of *Kalanchoe farinaceae* leaves exhibited stronger antifungal activity (18 mm inhibition zone) than the methanolic extract (15 mm inhibition zone). The most active methanolic extract was obtained from *Acridocarpus socotranus* leaves with 18 mm inhibition zone for 400 µg applied.

KEYWORDS: Plant extracts; Antioxidant; Antifungal; *Cladosporium cucumerinum***;** Soqotra.

INTRODUCTION

Soqotra is the largest and most easterly island of a small Yemeni archipelago in the Indian Ocean. The island is home to 307 endemic plants of about 850 plants described [1] and represents a wealthy treasure for finding biologically new active compounds. One such activity of considerable recent interest is the antioxidant behaviour of phytoconstituents, with the aim to replace synthetic ones such as butylated hydroxyanisole or hydroxytoluene, which found to possess toxic and mutagenic effects. Besides, screening for antioxidant activity is important because free radicals are involved in a number of pathological conditions such as inflammatory diseases, atherosclerosis, cerebral ischaemia, and cancer [2,3]. Other useful biological properties expected from plant metabolites, are new antiphytofungal agents, since phytofungal diseases still are an obstacle to the economic production of plants such as pepper, cinnamon, turmeric etc. Application of synthetic phytofungicides is considered as the most inexpensive and common method in plant disease control. However, their adverse effects on human health, the environment, and the resistance development of pathogenic microorganisms against chemical fungicides promoted man to search for finding new natural fungicides [4,5]. Biologically active compounds such as flavonoids, phenols, tannins, alkaloids, quinons, terpenes, saponins and sterols, found in plants appear to be more adaptable, acceptable and safer than synthetic compounds and display a wealthy source of potential pathogens control agents [6,7]. There are a few studies on the effects of Soqotraen medicinal plants on antiradical and antiphytofungal activity [8-12]. Therefore the aim of this study is to evaluate *in vitro* the antiradical and antiphytofungal activities of extracts obtained from some endemic Socotraen plants against *Cladosporium cucumerinum* that attack and cause heavy losses on important crops.

MATERIALS AND METHODS

Plant Material

The plant material was collected by the first author in March 2006 from different locations on Soqotra island (Table 1). The plants were taxonomically identified at the Centre of Soqotra Archipelago Conservation and Development Program (SCDP). Species names are according to International Plant Name Index (IPNI) (http://www.ipni.org). Voucher specimens of the plant material are deposited at the Pharmacognosy Department, Aden University, Yemen.

Preparation of Plant Extracts

Air-dried and powdered plant material (10 g) was extracted under shaking at room temperature successively with $CHCl_3$ (4 x 100 ml), followed by MeOH (4 x 100 ml). The obtained extracts were filtered, and evaporated to dryness *in vacuo* at 40 °C. The resulting crude extracts were stored at 4 °C.

Table I: Investigated Plant Parts

Species	Plant Family	Part tested[a]	Voucher no.	Local name
Acridocarpus socotranus Olive	Malpighiaceae	b, l	SP-Ma-01	kirilloh
Boswellia ameero Balf. fil.	Burseraceae	b, re	SP-Bu-01	Ameero
Boswellia socotranao Balf. fil.	Burseraceae	b, re	SP-Bu-03	taliy'oh
Boswellia elongata Balf. fil.	Burseraceae	re	SP-Bu-02	zaihil
Caralluma socotrana N. Br.	Asclepediaceae	ap	SP-As-0.3	mishherihimn
Cephalocroton socotranus Balf.	Euphorbiaceae	b, l	SP-Eu-05	tan
Commiphora ornifolia (Balf. fil.) J. B. Gillett	Burseraceae	B, re	SMP-Bu-08	ikshih
Croton socotranus Balf. fil.	Euphorbiaceae	b	SP-Eu-09	mitrer
Dendrosicyos socotrana Balf.	Cucurbitaceae	b, l	SP-Cu-03	qamhiyn
Dorstenia gigas Schweinf. ex Balf. fil.	Moraceae	l	SP-Mo-03	bownino
Eureiandra balfourii Cogn. & Balf. fil.	Cucurbitaceae	t	SP-Cu-05	diah'shwah
Kalanchoe farinacea Balf. fil.	Crassulaceae	l	SP-Cr-08	bigilihan
Ledebouria grandifolia (Balf. fil.) A.G.Mill. & D. Alexander	Hyacinthaceae	bu	SP-Hy-06	difitaher
Limonium sokotranum (Vierh.) Radcl.-Sm.	Plumbaginaceae	l	SP-Pl-05	lezibih
Ochradenus socotranus A.G. Mill.	Resedaceae	f	SP-Re-04	gershiy'oh
Oldenlandia pulvinata Vierh.	Rubiaceae	ap	SP-Ru1-01	digezgaz
Pulicaria diversifolia Balf. fil.	Asteraceae	ap	SP-Co-12	di'alimlihum
Pulicaria stephanocarpa Balf. Fil.	Asteraceae	l, r	SP–Co-13	dirbeb

[a] ap, aerial parts: b: bark; bu: bulb; f: fruits; l: leaves; re,:resins; r, roots; t: tubers.

Determination of Antioxidant Activity: Estimation of a radical scavenging effect was carried out by using a DPPH free radical scavenger assay in 96 well micro titre plates (MTP) according to the modified method of [13]. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of methanol, and the solution was kept in the dark at 4 °C until its use. Stock solutions of the extracts were prepared at 2 mg/ml and diluted to a concentration series. 5 µl of methanolic DPPH solution (final concentration 300 µM) was added to each well. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and stored in the dark. After 30 min the optical density (OD) of the solution was measured at the wavelength of 517 nm using a Tecan GeniosPro micro plate reader. A methanolic solution of DPPH served as a control. Percentage inhibition was calculated using the following formula:

% Inhibition = 100 – [(DPPH + sample) x 100/DPPH]. All tests were carried out in duplicates. Ascorbic acid was used as a positive control. An overview of the antioxidative activity of the crude methanolic plant extracts is given in Table 2.

Antiphytofungal Assay: Initial tests of fungicidal activity were carried out by the method described in [8]. This semiquantitative test allows a relative estimation of the activity of extracts or compounds with similar diffusion characteristics. The phytopathogenic fungus *Cladosporium cucumerinum* Ell. et Arth. was used as test organism. Antifungal tests were performed on TLC plates (glass plates, 20 x 20 cm, silica gel 60 HF254, thickness 0.5 mm (Merck). The extract was applied by using microsyringes on the TLC plate at concentrations of (50µg, 100µg, 200µg und 400µg) as individual spots (diameter 1 cm, corresponding to a surface of 78 mm^2). Subsequently, the plates were dried in a warm air stream, in order to evaporate remaining solvents. Each plate was covered with approx. 10 ml spore suspension of *C. cucumerinum* (approx. 2.5 × 106 spores/ml). Afterwards the plates were dried at room temperature for some minutes, placed into a TLC chamber lined with water soaked filter paper and covered. After 48 h incubation at 25 °C in an incubator a dark grey mycelium had developed. Benomyl (Riedel-de-Haen, Germany) was used as positive control. The evaluation of the antifungal effect was based on the area of the white spots corresponding to fungus growth inhibition. Three independent tests were performed and an average of the observations was calculated (n= 3).

RESULTS AND DISCUSSIONS

Fifty chloroform and methanol extracts of various parts of eighteen endemic plants, belonging to twelve plant families collected on Soqotra Island were investigated about their bioactive properties. Table 1 gives the names of the plants investigated, their voucher specimen no., their families, and their local names. The methanol extracts were screened for their antioxidant activity in a DPPH based free radical scavenger assay (Table 2). Eight out of twenty five methanol extracts tested, demonstrated variable antioxidant activity in the DPPH assay extending, from 56 % to 89 % at concentration of 200 µg/ml. The antiradical activity of *A. Socotranus, C. Ornifolia and B. Socotranus* was lower than those reported in literature[12]. This difference may be due to the geographical origin or time of collection, which may play an important role in the formation of plant constituents and in particular of the phenolics quantitatively.

Table II: Antioxidative Activity of the Crude Methanolic Plant Extracts (Radical Scavenging Activity in % with DPPH)

Species (Parts used[a])	% yield[b]	10 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
Acridocarpus socotranus (l)	(15.5)	32	39	47	56
Acridocarpus socotranus (b)	(5.4)	25	34	44	59
Boswellia ameero (b)	(14.3)	11	37	50	63
Boswellia socotrana (b)	(9.95)	36	53	65	69
Caralluma socotrana (ap)	(13.4)	31	67	69	73
Commiphora ornifolia (b)	(6.5)	34	42	50	62
Kalanchoe farinaceae (l)	(12.7)	23	51	67	89
Ochradenus socotranus (f)	(8.41)	25	34	42	59
ascorbic acid		53	98	98	98

[a] ap: aerial parts; b : bark; f: fruits; l: leaves;

[b]Percentage extract yield (w/w) was estimated as dry extract weight/dry starting material weight x 100

No reports were found regarding the antiradical activity of *B. Ameero*, *C. Socotrana*, *K. farinaceae* and *O. Socotranus*. *K. farinaceae* extract, with an inhibition value of 89 % thus has an activity comparable to the reference compound ascorbic acid (Table 2).The active extracts showed positive reactions with $FeCl_3$, which strongly suggest the presence of (ortho) phenolic constituents, which may be responsible for the antiradical activity [14].

Nine (18 %) out of 50 extracts tested showed antifungal activity against the phytopathogenic fungus *Cladosporium cucumerinum*(Table 3). Among those were four chloroform and two methanolic extracts that showed noteworthy effects. The highest antifungal activity was found in chloroform extract of *P. stephanocarpa* leaves (20 mm inhibition zone). This may be attributed partially to the presence of components of the essential oil [15] It was found that terpenes typical for such essential oils such as α-terpineol, α-copaene, and nerolidol showed antifungal activity against C. *cucumerinum*[16]. Phytochemical screening of the active extracts revealed the presence of triterpenes, volatile oils and flavonoids which may be involved in the antifungal activity against *Cladosporium* species as was reported elsewhere [17, 18, 19]. Antimicrobial activity of *K. farinaceae* and *P. stephanocarpa* were reported. Extracts of both plants showed no antifungal activity against *C. maltosa* [12]. The differences in

Table III: Antifungal activity of plant extracts against *C. cucumerinum*: Inhibition area in mm² of crude extracts after application of 100 µg to 400 µg (spot 0.78 mm²). A larger area roughly correlates to higher activity.

Species (Part tested[a])	Extract and % (yield)[b]	400 µg	200 µg	100 µg
Acridocarpus socotranus (l)	MeOH (15.5)	18	12	8
Kalanchoe farinaceae (l)	MeOH (12.7)	15	8	nd
Boswellia socotrana (b)	MeOH (18.3)	11	nd	nd
Acridocarpus socotranus (l)	CH_3Cl (5.3)	12	nd	nd
Ledebouria grandifolia (bu)	CH_3Cl (2.03)	14	11	nd
Kalanchoe farinaceae (l)	CH_3Cl (5.3)	18	14	nd
Ochradenus socotranus (f)	CH_3Cl (2.21)	12	nd	nd
Pulicaria stephanocarpa(l)	CH_3Cl (4.30)	20	15	13
Pulicaria stephanocarpa (r)	CH_3Cl (3.21)	18	14	12

[a] b: bark; bu: bulb; f: fruits; l.leaves; r: roots;

[b]Percentage extract yield (w/w) was estimated as dry extract weight/dry starting material weight x 100.

Antifungal activity on *C. maltosa* vs. C. *cucumerinum* may be explained by the different assay or by different compound properties, e. g. bioavailability of the active constituents in the pathogenic fungal cells. Also, it has been reported that the presence of carboxylic moieties in plant constituents may constitute a precondition for activity against *C. cucumerinum*, whereas methyl esters were inactive to the fungus, but were active against the human pathogenic yeast *Candida albicans*. [20].

To our knowledge, the phytochemical studies of P. stephanocarpa and A. socotranus have not been investigated so far. Therefore, the bioactivity-guided isolation and characterization of their antifungal compounds are under current investigation.

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