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Antifungal Activity of *Euclea divinorum* Root and Study of its Ethnobotany and Phytopharmacology

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Abstract: The ethnobotanical survey of *Euclea divinorum* Hiern (Ebenaceae) was conducted on Soqotra Island, Yemen. The root bark is used to treat mouth, dental, dermal and blood diseases in the traditional medicine of the island. The study is the first report about the effect of the plant root barks against six human pathogenic fungi. The non-polar dichloromethane extract of *Euclea divinorum* root bark showed stronger antifungal activities compared to polar direct and sequential methanolic extracts. These extracts showed significant broad antifungal activity against *Absidia corymbifera, Aspergillus fumigatus, Candida krusei, Microsporum gypseum, Mucor* sp. and *Trichophyton mentagrophytes* compared to the standard antibiotic drug nystatin. Thin-layer chromatography (TLC) revealed the presence of the naphthoquinones in the extracts. The results showed an extraction process to separate most antifungal naphthoquinones from the root bark by using non-polar solvent dichloromethane, while flavonoids remained in the polar methanolic extracts; therefore, the polar sequential and direct methanolic extracts recorded higher antioxidant activity than the non-polar extracts with less toxicity. The biological and chemical results identified the presence of antifungal and antioxidant constituents in the root bark and confirm its traditional use in Soqotra Island as crude powder to treat dental and dermal diseases and to clean teeth. Moreover, our results are compared with literature review on ethnobotany and phytopharmacology of the *E. divinorum* to present a medicinal monograph about the plant.

Keywords: *Euclea divinorum*; ethnobotany; naphthoquinones; antifungal; antioxidant; Soqotra

1. Introduction

Fungal infections of the mouth and skin are common diseases in tropical developing countries, including Yemen. Medicinal plants are a potential and natural source for treatment of fungal infections. For treatment of these tropical fungal diseases, many traditional plant species have been reported in different traditional medicine systems in the world, such as *Dracaena cinnabari* [1], *Piper guineense* [2]. Toothbrush and/or chewing pieces (*miswak*) are the most frequently traditional modes prepared from the plant parts and used for teeth and mouth problems. These used parts can be classified into: a) plant toothbrush with colouring properties such as such as *Euclea divinorum* [3], *Euclea natalensis* [4] and *Diospyros lyciodes* [5]; b) plant toothbrush without colouring agents such as *Salvadora persica* [6].

Euclea divinorum Hiern (Ebenaceae) is a famous plant in the ethnomedicine of Soqotra Island, the root bark is used mostly as a toothbrush or as powder for cleaning of the teeth by rubbing on the teeth and chewing or rubbing to dye the lips and mouth red. Similar traditional uses of *Euclea divinorum* root were found in the ethnobotany of Namibia and Kenya [5,7,8]. However, in the ethnomedicine systems of some African countries, the roots of different *Euclea* species were reported in use as medicines, such as *Euclea undulata* roots that are used to relieve toothache in Botswana [9], and to clean teeth in Zanzibar [10], while *Euclea pseudebenu*s roots are used to clean teeth on the

southwest coast of Africa [11]. In addition, in other tropical African countries the most traditional uses of *E. divinorum* were reported for internal applications [8]. The root decoction of *E. divinorum* is used in ethnomedicine in Zambia to treat different genital and oral diseases in the case of HIV/AIDS-related diseases [12] and the root bark to treat diarrhoea, cancer and dermal ailments in Zimbabwe [13]. In Kenya, the decoction or infusion of *E. divinorum* root are used to induce or augment labor [14]. Fruits are chewed to treat abdominal upsets, skin, kidney and respiratory disorders [15]. In Ethiopia, urine retention is treated by root decoction of *E. divinorum* [16] while the leaves are used to treat malaria, leprosy, gonorrhea, syphilis and tapeworm [17].

Preliminary phytochemical investigations identified previously secondary bioactive metabolites in different parts of *E. divinorum*. Methanol and aqueous extracts of the *E. divinorum* root have been reported to contain polyphenols, saponins, tannins, flavonoids, steroids, terpenoids, glycosides and alkaloids [5,16]. Polyphenols and glycosides have been identified in the methanol extracts of leaves, stems and fruits [5,17]. Some triterpenoids including lupeol, botulin and lupene [13,18] and naphthoquinones including methyljuglone, mamegakinone, diospyrin and isodiospyrin [13,18,19] have been previously identified and isolated as main constituents of the non-polar chloroform extract of the *E. divinorum* root [13,18,19]. In contrast, the leaves, stems and fruits contain no naphthoquinones [13,19]. However, from the ethyl acetate extract of the plant leaf, two naphthalene derivatives, Eucleanal A and B, were isolated [8]. From the methanol extract of the aerial parts, some flavonoids including myricitrin, quercetin and kaempferol were isolated [20].

The cytotoxic, antibacterial, oxytocic, and diuretic activity of the root of *E. divinorum* have been demonstrated [3,5,14,16] (Table1). Moreover, different biological and pharmacological investigations of the other parts of *E. divinorum* including leaves, fruits and barks of the plant were previously studied and reported, which are presented in Table 1.

This is the first report about the antifungal activity of *E. divinorum* root bark against six human pathogenic fungal strains to validate the ethnomedicinal uses of the plant on Soqotra Island, Yemen.

Table 1. Summary of previous biological and pharmacological studies of *Euclea divinorum* Hiern.

Used part/ Extract solvent	Pharmacological activities/details [references]	Model of test
1. Antibacterial activity against oral pathogens		
Root/Methanol	Activity against *Streptococcus mutans* and *S. sanguinis* by MIC < 2.5 mg/mL [5]	*in vitro*
Stick (Stem)/ Dichloromethane	Weak activity against *Staphyloccocus aureus*, *Escherichia coli, Bacillus subtilis* [21]	*in vitro*
Stem/Aqueous	Inhibition of enzymes activity of *Porphyromonas gingivalis*, *Treponema denticola*; *Bacteroides gingivalis*, *B. intermedius* up to 200 μg/mL [22]	*in vivo*
Bark, Leaves/Ethanol	Activity against *Actinomyces naeslundii, Streptococcus mutans* and *Actinobacillus actinomycetemcomitans* (MIC: 6.2–25.0 mg/mL) [23]	*in vitro*
Fruit/Methanol	Moderate activity against *Neisseria gonorrhoea* (250–2000 μg/mL) [17]	*in vitro*
2. Antifungal activity		
Leaves/Hexane	No activity against *Candida albicans*, and *C. krusei* [24]	*in vitro*
Leaves/Methanol	Weak activity against *C. albicans*; *Cryptococcus neoformans* (4000 μg/mL). No activity against *Aspergillus flavus; A. niger; Tricophyton mentagrophytes and T. violacium* [17]	*in vitro*
Bark, Leaves/Ethanol	No effect on *C. albicans* [23]	*in vitro*
3. Cytotoxicity activity		
Bark, Leaves/Ethanol	Moderate cytotoxicity (inhibitory concentration (IC_{50}): 142.3 μg/mL) on vero cell line [23]	*in vitro*

Root/Petrol ether/ Ethyl acetate 1:1; Methanol	The lipophilic extract displayed marked toxicity (IC_{50} value 11.6 μg/mL) [3]; The polar extract showed less toxicity (IC_{50} 36.0 μg/mL) against ECV-304 cells [3]	*in vitro*
	4. Oxytocic activity	
Root, Bark/Aqueous, Ethanol	Direct stimulation of the uterus contraction [14]	*in vivo*
	5. Diuretic activity	
Root/80% Methanol	Diuretic effect at 200–400 mg/kg [16]	*in vivo*
	6. Antioxidant activity	
Root/Aqueous; Methanol	Antioxidant activity at 2000 μg/mL with 74.5-82.5% DPPH inhibition [25]	*in vitro*
	7. Toxicity effect	
Root/80% Methanol	Safe extract at 2000 mg/kg [16]	*in vivo*
	8. **Antidote effect**	
Leaves/Methanol	Antidote for nephrotoxicity caused by gentamicin at (100 mg/kg) [25]	*in vivo*

MIC, minimal inhibition concentration.

2. Materials and Methods

2.1. Ethnobotanical Survey

The ethnobotanical survey of *E. divinorum* was conducted in one city (Hadibou) and in nine villages on Soqotra Island (Reyged, Qalansieh, Haggeher, Noged, Mori, Qubah, Qadheb, Dihamidh and Shuub) during February–March 1990. Fifty indigenous peoples were interviewed (male 45; female 5) in the Arabic language. 80% of the informants were above 35 years old. Ten informants were in primary schools between 12–16 years. All interviewees were local people and spoke Soqotri and Arabic language. Informants asked to identify the Soqotri local name of the plant tree in the nature and the specimen (root) collected during the survey.

2.2. Plant Material

The plant parts samples of *Euclea divinorum* (Ebenaceae) were collected from Reyged, Soqotra Island, Yemen, in February 1990 for taxonomic identification. The plant species was identified by the Pharmacognosy Department, Aden University, Yemen and at the personal Herbarium of the author.

2.3. Extraction

Roots of *E. divinorum* were collected for the extraction in March 2006. The dried root barks were powdered in a grinder.

1. Direct extract: 2 g powdered root barks were extracted direct with 100 ml methanol (DM)
2. Sequential extracts: a sequential extraction way was demonstrated to produce different subextracts from the dried root bark; 30 g powdered root bark was extracted with different solvents of increasing polarity, 300 mL for each: dichloromethane, ethyl acetate and methanol successively at room temperature for 8 hours.

   The crude and sequential extracts (direct methanolic extract (DM), sequential dichloromethane extract (SD), sequential ethyl acetate extract (SE), sequential methanolic extract (SM) were concentrated under reduced pressure at 40 °C. They were dried by lyophilization-freezing and stored in exsiccator (Figure 1).

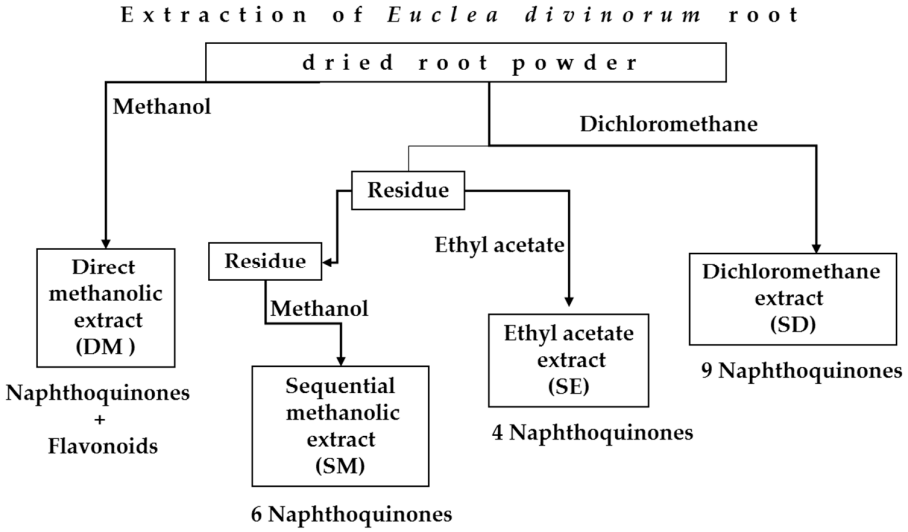

Figure 1. The extraction process of *Euclea divinorum* root bark.

2.4. Antifungal Test—Agar Diffusion Test

The antifungal activities of the direct and sequential extracts of *E. divinorum* (2 mg dried extract per disc) were identified using the agar diffusion assay [6,26]. Inhibition zone diameters include diameter of the disc (6 mm). The tested human pathogenic fungi strains: *Absidia corymbifera* (100798), *Aspergillus fumigatus* (13550/99), *Candida krusei* (ATCC 90878), *Microsporum gypseum*, *Mucor* spp. and *Trichophyton mentagrophytes* (05/2004). Nystatin was used as positive control (100 μg/disc).

2.5. Determination of Antioxidant Activity

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay were used to determine the quantitative of radical scavenging activity of the extracts. Quantitative estimation was performed according to the method described by Brand et al. [27]. Ascorbic acid was used as positive control. The DPPH radical concentration (antioxidant activity) was calculated using this equation:

Scavenging activity (%) = [(Absorbance control – Absorbance sample)/Absorbance control] × 100.

Antioxidant activity was also expressed as inhibitory concentration (IC_{50}), defined as the concentration of phenolic compounds in the extract required to cause a 50% decrease in initial DPPH radical absorbance at 517 nm.

2.6. Determination of Cytotoxicity Activity

The cytotoxicity of the extracts was estimated by the neutral red uptake assay, using FL-cells, a human amniotic epithel cell line [28]. Quantitative determination was carried out as described in our previous study [1].

2.7. Identification of Naphthoquinones on Thin-Layer Chromatography (TLC)

Extracts were analyzed by silica gel thin layer chromatography (TLC) according to Wagner and Bladt [29] to identify the presence of naphthoquinones in the extracts. The following mobile phases of TLC were used: A: toluene-formic acid (9:1); B: ethyl acetate-glacial acetic acid-formic acid-water (100:1:11:26). The following indicators were used to detect the spots on TLC: a) Physical method: visual and ultraviolet (UV) light (254 and 365 nm), b) Chemical reagents used as spray: 10% methanolic potassium hydroxide KOH and vanillin–sulphuric acid reagent [29].

3. Results

3.1. Ethnobotany of Euclea divinorum

Table 2 shows the collected data of ethnobotanical survey for *Euclea divinorum* Hiern (Ebenaceae) and its traditional uses in Soqotra Island compared with the literature [3,30]. In the present study, we interviewed 50 informants (female: 5; male: 45) in different locations of Soqotra Island, Yemen. The root bark is the most frequently used part of the plant. All respondents (100%) knew and/or used *E. divinorum* for medicinal purposes in all different selected 10 localities in the Island. According to our results (Table 2), the plant is recognized to treat dental and dermal ailments. Furthermore, the root bark is used as cosmetic red colouring agent for the lips and mouth cited by (90%) of informants (Table 2). The plant is the second famous and most important medicinal plant from Soqotra Island after *Dracaena cinnabari* that used in Soqotra Island; whereas both are transported as medicines into inland Yemen [1].

Table 2. Data of ethnobotanical survey of *Euclea divinorum* on Soqotra Island.

Used part/ Preparation form/ administration route	Ethnobotanical uses	Ct %	Ref
1. Medicinal uses			
1.1. Dental diseases		100	
Root/dried, raw, cut pieces are used as toothbrush (*misawk*)/topically	dental diseases: mouth and dental fungal infection		[3]
Root/dried powder of root barks/ rubbing on the teeth	tooth cleaning		[3,30],
Root/root bark is powdered with little water and salt and inserted into the tooth cavity	toothache, tooth cavity		[or], [30]
1.2. Dermal diseases		70	
Roots/dried powder of the root bark in little water as paste/topically	fresh wound , abscesses and kin diseases		[3,30]
Root/a piece is chewed and salvia used or Root barks powdered and mixed in little water ass paste/ topically	skin complaints , sores, fungal skin complaints, (ringworm)		[or], [30]
1.3. Blood diseases		20	
Root/a piece of root is chewed and the produced salvia is swallowed/internal	to purify blood		[30]
2. Cosmetic		90	
Root pieces/fresh or dried root pieces used as chewing/ topically	red colouring of lip and mouth		[or], [30]
3. Economic uses		50	
Leaves, fruit/ dried or fresh parts	fodder for livestock and for firewood		[or], [30]
Dried stems	firewood		[or]
Dried stems	house ceiling		[or]

Ct, citations percentage: number of informants that cited traditional use(s) for the plant from the total informants number; [or], oral interview with local people on Soqotra Island; Ref, References; [3]: Al-Fatimi, et al., 2005; [30]: Miller and Morris, 2004.

3.2. Antifungal Activity

The antifungal activity results are reported in Table 3 and Figure 2. This study demonstrated the antifungal activity of *E. divinorum* root bark by measuring the inhibition zone of the fungi growth in

a paper disc diffusion assay. The tested extracts showed a clear inhibition on all tested fungi. The best specific strong antifungal activity was demonstrated by the dichloromethane extract of *E. divinorum* against *Trichophyton mentagrophytes* (30 mm, minimal inhibition concentration (MIC): 200 μg) and *Microsporum gypseum (*25 mm) followed by the direct methanolic extract (20 and 25 mm) (MIC: 500 μg), compared with nystatin.

Table 3. Antifungal activity of the root bark extracts of *Euclea divinorum* in an agar diffusion test.

Fungi strains	IZ Diameter (mm)				
	SD	SE	SM	DM	Nys
Absidia corymbifera	17	10	17	18	25
Aspergillus fumigatus	16	15	16	15	24
Candida krusei	16	15	16	18	24
Microsporum gypseum	25	16	15	20	22
Mucor sp.	10	10	10	15	21
Trichophyton mentagrophytes	30	20	18	25	25

IZ: inhibition zone; SD: sequential dichloromethane extract; SE: sequential ethyl acetate extract; SM: sequential methanolic extract; DM: direct methanolic extract of *E. divinorum*; Nys, Nystatin (100 μg/disc).

3.3. Antioxidant Activity

The antioxidant activity results are shown in Table 4 comparable with ascorbic acid. The direct sequential and direct methanolic extracts showed significant antioxidant activities (IC_{50} = 225.0, 550.0 μg/mL) respectively. Non-polar extracts: dichloromethane and ethyl acetate extract showed moderate antioxidant activity (IC_{50} > 600 μg/mL).

Table 4. Cytotoxic and antioxidant activity of root barks extracts of *Euclea divinorum.*

Extracts solvents	IC_{50} (μg/mL)	
	Antioxidant activity	Cytotoxicity
Dichloromethane	690.5	240.0
Sequential ethyl acetate	680.8	387.7
Sequential methanol	225.0	800.6
Direct methanol	550.0	900.5
Ascorbic acid [a]	15.5	-

[a] Positive control; IC_{50}: a concentration required for 50% reduction in absorbance compared with control values.

3.4. Cytotoxic Activity

The concentration of each extract that is required for 50% growth inhibition of FL-cells, was measured using neutral red uptake assay. The sequential and direct methanolic extract showed less cytotoxicity. (IC_{50} > 800 μg/mL) than dichloromethane and ethyl acetate extracts (IC_{50} < 240.0 μg/mL) (Table 4).

3.5. Identification of Naphthoquinones

The dichloromethane, ethyl acetate and methanolic extracts showed positive results to naphthoquinones as described by Wagner and Bladt [29]: A) four spots by R_f values 0.3 to 0.5 reacted positive with potassium hydroxide KOH and vanillin–sulphuric acid to give red, orange and violet in each extract. In the thin-layer chromatography (TLC) of dichloromethane extract, three spots showed orange red to yellow colors under ultraviolet–visible (UV–vis) light; quenching under 245 nm and red-brown to red fluorescence under 365 nm.

4. Discussion

Euclea divinorum Hiern (Ebenaceae) is the botanical name of a wild tree growing to 4 m [26] on Soqotra Island. The plant is abundantly grown in different localities in Soqotra Island, such as Reyged and Shauub. "*Kala*" is the local name of the *E. divinorum* tree in Soqotra. "*Doreeb*" is the Soqotri name of the used part, "root", that is employed as a traditional crude drug. The plant is also tropically widespread in Africa and the Old World. The family, Ebenaceae has only two genera with 500 species; fourteen species belong to *Euclea* genus [30]. It is also native to South and East Africa [16]. In Yemen, there are two plant species which are most frequently used as toothbrushes "*miswak*" and found in the markets of the inland; the famous common plant *Salvadora persica* besides *E. divinorum*. *Salvadora persica* is called locally "*erhik*" in Soqotra Island and "*rak*" in the inland. We interviewed informants in ten different localities in the Island, where the medicinal use of the plant is known in all the Island parts, even in the Inland Yemen. This widespread traditional knowledge of the plant indicates its high importance as a famous medicinal plant. Tooth cleaning is the most cited ailment by all informants (100%), followed by wound and dermal infectious diseases (70%). The most frequent administration route of the root bark is topically, while fewer citations were documented for oral application to clean the blood (Table 2). In the inland, we documented the traditional use of the root bark as an infusion to treat jaundice besides its traditional uses as powder or toothbrush to clean teeth. We compared our present ethnobotanical survey with some traditional uses of the plant have been previously reported by Miller [30] and in our previous paper [3].

Generally, naphthoquinones were reported as antibacterial agents [5,17,21–23] and to inhibit viral infection [31]. Some naphthoquinone derivatives have been identified and isolated from the plant root [13,18,19]. The plant root contains a large amount of naphthalene, which is responsible for the red color on the lips and mouth by using the root pieces as "*miswak*". Due to its popularity as a traditional medicinal herb in Soqotra Island and inland Yemen, the roots of *E. divinorum* are available in the markets in the inland. It may be used as substitutional adulterant for the red resin of *Dracaena cinnabari* that is used also in the traditional herbal medicine in Yemen for treat oral diseases including tooth ailments [1].

The low polar dichloromethane and ethyl acetate extracts showed considerable antifungal activity (10 to 30 mm) against all tested pathogenic fungi strains. Previous phytochemical investigation of the non-polar chloroform extract of *E. divinorum* root reported the isolation of four coloring naphthoquinones: 7-methyljuglone, diospyrin, isodiospyrin, shinanolone, [13,18] besides three triterpenoids lupeol, lupene, and botulin [13,18,19]. Moreover, nine naphthoquinones have been previously isolated from different Ebenaceae species [32–35].

However, these naphthoquinones were reported to have the ability of extraction by different polarity of solvents; they have been previously isolated from the roots of different species of *Euclea*, *Diospyros*, and *Drosera*. For instance, 8-hydroxyisodiospyrin, diospyrin from polar methanolic extract [36]; shinanolone, from the ethanolic extract [37]; 7-methyljuglone from the methanolic [38] and dichloromethane extracts [39]. These lead to the fact that the aforementioned naphthoquinones could be extracted, identified and isolated from different plant species using a different polarity of solvents: either non-polar (e.g. chloroform, dichloromethane) or polar (e.g. methanol, ethanol, acetone). By the partitioned extraction between polar solvents and non-polar solvents, these naphthoquinones showed a high ability to separate from polar solvent into non-polar solvents, example, partition from the methanol phase to dichloromethane or chloroform phase [36,37,39].

The spots of the *E. divinorum* obtained from the three extracts on TLC, showed positive reaction with the UV and chemical reagents that used to identify naphthoquinones in the extracts according to method used by Wagner and Bladt [29]. The study identified for the first time, the presence of the naphthoquinones in three polar and non-polar extracts of *E. divinorum* root bark collected from Soqotra Island. This result is consistent with the previous studies that reported the identification and isolation of seven pure naphthoquinones from the roots of *E. divinorum* samples collected from different African localities, in Ethiopia [19], Zimbabwe [13] and Mozambique [18]. Moreover, this study result confirms the chemotaxonomy of Ebenaceae.

The naphthoquinones are mostly stored in the roots of different Ebenaceae species and investigated for their antifungal activities. Previous biological examination of the naphthoquinones 7-methyljuglone, diospyrin and isodiospyrin isolated from the root acetone extract of *Diospyros virginiana* showed antifungal activities on some plant pathogenic fungi [40]. Against human pathogenic fungi, only three pure isolated naphthoquinones: 7-methyljuglone, plumbagin and shinanolone were reported to have antifungal activity, which were isolated from the methanolic extract of *Diospyros maritima* bark [38]. 7-Methyljuglone showed high activity against *Aspergillus niger*, *Candida albicans*, and *Saccharomyces cerevisiae* with growth inhibition of 99.9% at concentration 20, 1, 1, 300 μg/mL, respectively [38]. From the root bark of *E. natalensis*, shinanolone and octahydroeuclein were isolated and showed significant inhibition against *Aspergillus niger* and *Cladosporium cladosporioides* by 0.01 mg/mL [41]. Ten naphthoquinone derivatives of dehydroiso-ß-lapachone were isolated from a dichloromethane extract of *Newbouldia laevis* roots, and reported with antifungal activity against *Cladosporium cucumerinum* and *Candida albicans* [42]. Shikonin and deoxyshikonin were isolated from roots of *Lithospermum erythrorhizon* showed strong antifungal activities, against *Candida krusei* (MIC: 4 mg/mL) [43]. The antifungal activities of 30 different naphthoquinones against some *Candida* species such as *C. albicans* were demonstrated [44]. Thirteen synthetic naphthoquinones containing sulphur atoms showed higher antifungal activity than amphotericin B against *Candida tropicalis*, *Aspergillus niger*, *Trychophyton tonsurans* and *Fusarum oxysporum* [45]. In addition, previous biological examinations of the triterpenoids lupeol, Lupene and betulin isolated from the of *E. natalensis* root bark showed antifungal activity against *Aspergillus flavus*, *A. niger* and *Cladosporium cladosporioides* (0.01 mg/mL) [41]. These results suggest that the four naphthoquinones 7-methyljuglone, diospyrin, isodiospyrin and shinalone and the three triterpenes lupeol, lupene, and botulin that were previously isolated from the root chloroform extract of *E. divinorum* [13,18], must be responsible for the specific antifungal activity of the root dichloromethane extract of *E. divinorum* tested in the present study. Therefore, both groups of naphthoquinones and triterpenes showed synergistic effects on the antifungal activity of the dichloromethane extract. While the naphthoquinones were reported as the main constituents in the plant root [13], the plant leaves were reported to be naphthoquinones free [5]. Furthermore, two naphthalene derivatives, Eucleanal A and B have been previously isolated from the leaves' ethyl acetate extract [8]; however, the leaves extracts were reported to give negative or weak antifungal effects on some fungi including *Candida albicans* and *Tricophyton mentagrophytes* (Table 1) [17,23]. This observation leads to suggest that the responsible chemical part for the strong antifungal activity is related to the ketone functional groups that are found in the general chemical structure of naphthoquinones, while lack in the naphthalene derivatives: Eucleanal A and B.

On the other hand, the direct methanolic extract showed higher antifungal activity (15–30 mm) than the sequential methanolic extract (15–18 mm). This led us to suggest that the differences in antifungal activity between the two extracts were essentially due to more naphthoquinones content in direct methanolic extract. The antifungal naphthoquinones of the root powder are separated in the first step of the sequential extraction process in the dichloromethane extract, and therefore they are lacking in the sequential methanolic extract. However, by the direct extraction of the root powder, most naphthoquinones are soluble also in methanol and can be separated in the methanol extract (Figure 2). The direct methanol extract contains naphthoquinones besides other phenolic compounds, and therefore it showed stronger antifungal activity than the sequential methanol and ethyl acetate extracts. In previous chemical investigation, many phenolic compounds have been investigated in the methanol extract of *E. divinorum* root with different functional groups, flavonoids and tannins [5,16]. From the leaves, pure flavonoids have been isolated (myricitin, quercetin, kaempferol and tannins including gallic acid and catechin [20]. These polyphenols as the main constituents of the polar extract might account for the moderate antifungal activity of sequential methanolic fraction and for the additive action on the antifungal activities of direct methanolic extracts presented in this study.

Figure 2. Antifungal activity of the extracts (sequential dichloromethane (SD) sequential ethyl acetate (SE), sequential methanolic (SM), direct methanolic (DM)) against *Trichophyton mentagrophytes* as the inhibition zone in agar diffusion (mm).

The present study shows for the first time the antifungal activity of *E. divinorum* on six selected human pathogenic fungi. The study indicated the suitable method to separate the antifungal extracts with no-polar solvents to obtain higher antifungal activity; these non-polar extracts were identified previously to contain antifungal naphthoquinones as their main constituents. On the other hand, the antibacterial activities of the plant extracts against some bacterial strains have been determined against the oral pathogens to tooth surface *Streptococcus mutans, S. sanguinis* [4,5] and *Bacteroides gingivalis* [22]. According to the previously antibacterial activities reports [4,5,22], the tested fungi strains demonstrated more sensibility to root extracts than to the bacteria. However, some naphthoquinones identified in *Euclea natalensis* root were reported with significant antimycobacterial activity against *Mycobacterium tuberculosis* including diospyrin (MIC values 8.0 μg/mL), 7-methyljuglone (0.5 μg/mL) [39] and shinanolone (0.1 mg/mL) [46].

Methanolic extracts of the root were previously investigated to contain many polyphenols such as tannins and flavonoids [5,16]. Some flavonoids (myricitrin, quercetin, kaempherol) and tannins (catechin and gallic acid) were isolated as major constituents from the plant leaves [20]. These polyphenols are known antioxidant compounds [35], which might be responsible for the significant antioxidant effect of polar methanolic fractions. Furthermore, methanolic extract of *E. divinorum* leaves was reported to have antioxidant activity at 2000 μg/mL; which acts as an antidote against the nephrotoxicity caused by gentamicin [25].

The low toxicity of both polar methanolic extracts against FL-cells confirms the safe traditional medicinal uses of the root bark as an entire crude powder. This indicates that the flavonoids in combination with naphthoquinones in a methanolic extract showed antagonist effect on the cytotoxic activity. Our previous study reported the cytotoxic activity of different of *E. divinorum* extracts on the human ECV-304 cells [3]. The methanol extract exhibited markedly low toxicity (IC50 value 36 μg/mL) against ECV-304 cells, while the lipophilic (Petrol ether/ethyl acetate) (1:1) extract of *E. divinorum* root displayed strong toxicity IC_{50} 11.6 μg/mL [3]. These results suggest the antifungal activity of the direct methanolic extract of the root in a safer dose than the dichloromethane extract, due presence of the four identified naphthoquinones, some of them were reported with high cytotoxic activity such as 7-methyljuglone. The high cytotoxic activity of 7-methyljuglone was previously reported against five cell lines including murine lymphocyticleukemia 0.1 and human breast cancer 2.2 μg/mL [13].

The antifungal and antioxidant activities and the low toxicity of the direct methanolic extract support the traditional Soqotri use of the plant root to treat fungal infection in the mouth and to clean teeth. Furthermore, the results confirm the administration mode of the root as raw powder to rub on teeth, because the crude powder contains all the bioactive constituents of the entire root bark: the antifungal naphthoquinones and the antioxidant phenols and flavonoids.

5. Conclusions

The ethnobotanical documentation of a medicinal plant is an important guide for the biological examinations and phytochemical isolation of bioactive compounds that lead to the discovery of new natural drugs. Therefore, the identification of new plant species regarding ethnomedicinal uses is also an essential process to isolate new antifungal compounds. The extraction type is a practical process to separate the bioactive fractions from a traditional medicinal plant. The root bark of *E. divinorum* is rich with naphthalene derivatives, naphthoquinones, tannins, flavonoids and other polyphenols, which have antimicrobial activities especially against dental microbes. Naphthoquinones are the main constituents of the *E. divinorum* root bark, which could be identified in the Soqotri sample using the TLC method. They can be separated with either the non-polar or the polar solvents. The low polar or non-polar dichloromethane extract was identified with high content of naphthoquinones; it showed significant and broad antifungal activity. The polar ethyl acetate and methanolic extracts contain fewer naphthoquinones but more flavonoids and tannins; therefore, they showed weak antifungal but more antioxidant activity with safe toxicity. The combination of different polyphenols (flavonoids and naphthoquinones) in the plant root can insert strong synergist effect on antifungal activity against the human pathogenic fungi. These findings validate the use of the traditional preparations of the root bark as a crude powder and/or as toothbrush, which contain both antifungal naphthoquinones and antioxidant flavonoids, to treat dental and mouth diseases in the tropical Soqotra Island. We suggest further research to investigate the natural naphthoquinones, especially 7-methyljugloneas semisynthetic derivatives on other broad human pathogenic fungi. These survey and experimental results are an additional part of the ethnobotany and phytopharmacology of *E. divinorum*, that were also reviewed based on literature to form an essential monograph for this important medicinal plant.

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