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Evaluation of Antifungal Potentials and Antioxidant Capacities of Some Foliose Lichen Species

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Abstract: This work is aimed to assess of antioxidant and antifungal potential of the foliose lichen species: *Flavoparmelia caperata*, *Xanthoparmelia stenophylla* and *Xanthoparmelia conspersa*. The antifungal activity of lichens were studied against some pathogenic fungi by disc diffusion method. The acetonitrile extracts of these lichens were obtained with Soxhlet extraction. While *F. caperata* exhibited maximum antifungal activity (32 mm) against the *C. albicans*, the minimum antifungal activity (10 mm) was obtained from *X. stenophylla* lichen against *S. cerevisiae*. In CUPRAC assay, absorbance values was in order of BHT>*X. conspersa*>*F. caperata*>*X. stenophylla*. As a result of the study, it was concluded that these lichen species may be a potential source for the development of new antifungal and antioxidant compounds.

Bazı Yapraksı Liken Türlerinin Antifungal Potansiyellerinin ve Antioksidan Kapasitelerinin Değerlendirilmesi

Anahtar Kelimeler
Antioksidan aktivite,
Liken,
Antifungal

Özet: Bu çalışma yapraksı liken türleri olan *Flavoparmelia caperata*, *Xanthoparmelia stenophylla* ve *Xanthoparmelia conspersa'*nın antioksidan ve antifungal potansiyellerinin belirlenmesini amaçlamaktadır. Bu likenlerin bazı antifungal aktivitesi bazı patojenik mantarlara karşı antifungal aktivitesi disk difüzyon metodu ile araştırılmıştır. Likenlerin asetonitril ekstraktları Soksalet ekstraksiyonu ile elde edilmiştir. En yüksek antifungal aktivite (32 mm) *F. caperata* tarafından *C. albicans*'a karşı gözlenirken, en düşük antifungal aktivite (10 mm) *X. stenophylla* likeninden *S. cerevisiae*'ye karşı elde edilmiştir. CUPRAC deneyinde absorbans değerleri BHT>*X. conspersa*>*F. caperata*>*X. stenophylla* şeklinde sıralanmaktadır. Çalışma sonucunda, bu liken türlerinin yeni antifungal ve antioksidan bileşik geliştirmede potansiyel bir kaynak olabilecekleri sonucuna varılmıştır.

1. Introduction

Antioxidant term is prominent in human lifetime. Various biological roles such as anti-mutagenicity, anti-aging and anti-carcinogenicity are thought to be based on this concept. The growth of medicinal plants has been connected with phytochemicals which is in their structure. These phytochemicals provide health maintenance and protection against many diseases and cancers [1]. Many studies were revealed on the preventive actions of natural antioxidants against diseases, like AIDS, Parkinson, Alzheimer, diabetes, eye diseases, and cardiovascular diseases [2]. As understanding of the function of free radicals in human diseases has enhanced, antioxidants have gained remarkable concern because of their role in inhibiting harmful effects of free radicals and their help in preventing the body against injury by reactive oxygen species [1].

Infectious which was originated from pathogenic fungi is a crucial hazard on public health. Of late years, increase in immunocompromised patients like organ transplant recipients, HIV and cancer patients cause the rise in frequency of fungal infections. Some commensal fungi like *Candida* species generate infections when their host is immunocomprised. These problem leads to resistance and toxicity to antifungal drugs during prolonged therapy [3].

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Nature provide a large source of medicinal agents for many years and many efficient modern drugs has been isolated from plants. Plants are still the first source of pharmaceutical agents utilized in traditional medicine [4]. Plants possess various secondary compounds, which most of them have antifungal action [3].

Lichens are symbiotic associations that consist of a mycobiont and photosynthetic partners (a photobiont) often from green algae, and/or cyanobacteria. This complex community is a potential source of brand and efficient medicinal agents [5]. Lichens have been utilized in traditional medicines by different cultures in the world commonly for curing wounds, skin illnesses, respiratory, digestive problems and gynecological concerns [6].

Lichens and their metabolites possess plenty of biological activities such as antibiotic, antitumor, ecological roles, antiviral, antiherbivore and enzyme inhibitory [7].

The aim of this study was to bring out to antifungal potential and cupric reducing antioxidant capacity (CUPRAC) of three foliose lichen species namely *Flavoparmelia caperata* (L.) Hale, *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale and *Xanthoparmelia stenophylla* (Ach.) Ahti & D. Hawksw. These tested lichen specimens have sevaral traditional uses (Table 1).

Table 1. Traditional usage of the tested lichens

Lichen	Traditional Usage
F. caperata	*F. caperata* is used to treat the intestinal worms. Dried powder of thallus of it also used on skin burns [10]. Moreover, this lichen has used as decoction to heat in China [11].
X. conspersa	*X. conspersa* is utilized to cure wounds and cuts, therapy of inflammatory gingiva and sore throats [6].
X. stenophylla	*X. stenophylla* is utilized to treat snake bites and venereal diseases like syphilis [12].

2. Material and Method

2.1. Lichen specimens

Lichen specimens of *F. caperata* (Herbarium no: 6398), *X. conspersa* (Herbarium no: 6399) and *X. stenophylla* (Herbarium no: 6400) was collected from Giresun province in 2012. They were kept at the Department of Biology, Faculty of Science, University of Giresun, Turkey. Lichen identification was done with some flora books [8-9].

2.2. Fungi strains

Four fungi strains were used to determine antifungal action of the extracts. *Saccharomyces cerevisiae* was obtained from Giresun Province Control Laboratory; *Candida albicans* and *Candida tropicalis* were obtained from Department of Biology, Fırat University and *Candida parapsilosis* was obtained from Faculty of Education, Giresun University.

2.3. Lichen preparation

Dried and finely grounded lichen samples (30 g) were extracted with acetonitrile (300 ml) in a Soxhlet extractor. The extracts were filtered then concentrated in a rotary evaporator. The crude extracts were kept at -18 °C till further antioxidant and antifungal analyses. The extracts were dissolved in dimethyl sulphoxide (DMSO) for further analysis [13].

2.4. Antifungal activity

Lichen extracts were dissolved in 10% DMSO [14] at 30 mg/ml concentration. Dissolved extracts was sterilized by using 0.45 µm pore sized membrane filter. Standard antifungal flucanozole was utilized to compare inhibition zones of the lichen extracts [15]. The turbidity of fungal suspensions were adjusted with 0.5 Mc Farland standard (10^7 CFU/ml fungi concentration), then, the fungal suspension spread on petri dishes [16]. Discs were put (5 mm diameter) onto the agar and discs were filled with 25 µl *F. caperata* extract, 25 µl *X. stenophylla* extract, 25 µl *X. conspersa* extract, 25 µl flucanozole (30 mg/ml concentration) and 25 µl DMSO, separetely. Plates were incubated at 30 °C for 72 h. Diameter of inhibition zones were measured in millimeters [15].

2.5. Antioxidant activity

2.5.1. Cupric reducing antioxidant capacity (CUPRAC)

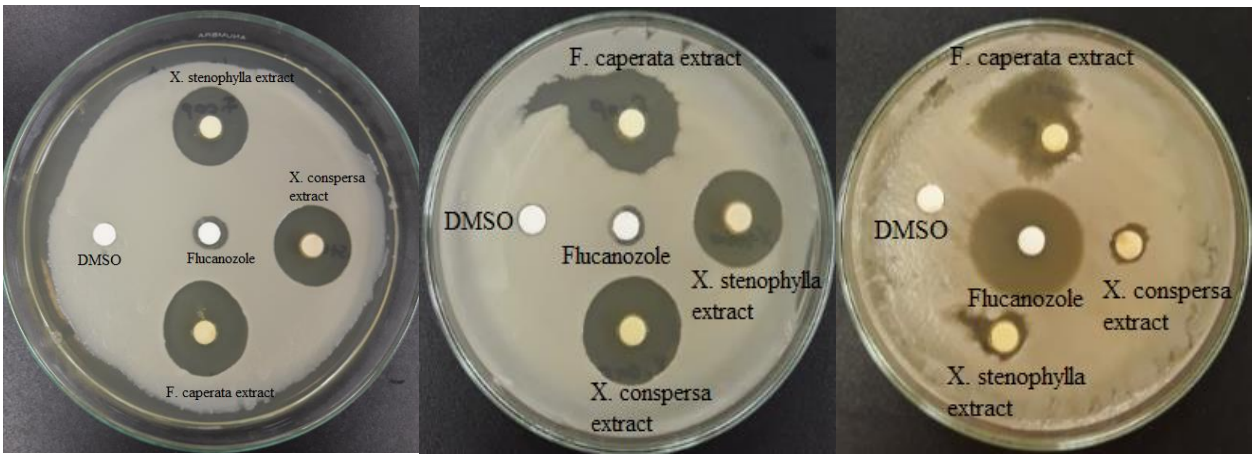
0.5 ml extract (prepared in 50-200 µg/ml), 1.0 ml $CuCl_2$ solution ($1x10^{-2}$), 1.0 ml neocuproine (Nc) solution ($7.5x10^{-3}$ M) and 1.0 ml ammonium acetate buffer (1.0 M, pH: 7.0) were mixed in a test tube. Tube was vortexed and stored in a dark place for 30 min. Absorbance was measured at 450 nm. Butylated hidroxytoluene (BHT) was utilized as standard antioxidant substance [17]. Absorbance values of the extracts were compared with absorbance values of BHT.

3. Results and Discussion

Lichens synthesize many metabolites which knows as lichen substances or lichen metabolites. These substances are dibenzofurans, amino acid derivatives, aromatic compounds, depsides and depsidones. They possess significant biological action such as antiviral, enzyme inhibitory, antioxidant and antibiotic [18].

Table 2. Inhibition zones of the acetonitrile extracts of the lichens (mm)

Fungi	*X. stenophylla*	*F. caperata*	*X. conspersa*	DMSO	Flucanozole
C. albicans	22	25	23	-	10
C. tropicalis	21	25	23	-	12
S. cerevisiae	11	17	10	-	25
C. parapsilosis	-	-	-	-	12



(a) **(b)** **(c)**

Figure 1. Inhibition effects of the tested lichen extracts against a) *C. tropicalis* b) *C. albicans* c) *S. cerevisiae*

Antifungal activity of *F. caperata*, *X. conspersa* and *X. stenophylla* was investigated towards to different yeasts namely *S. cerevisiae*, *C. albicans* and *C. tropicalis* and *C. parapsilosis*. The inhibition zones as demonstrated in Table 2 reveals that the lichen extracts were effective (except for *S. cerevisiae*) in significantly reducing the growth of fungi as compared with flucanozole. DMSO which was used as negative control had no activity against test fungi.

Antifungal activity of the extracts are presented in Table 2. The acetonitrile extracts of the tested lichens showed maximum and minimum activity against *C. albicans* (32 mm) and *S. cerevisiae* (10 mm), respectively. Antifungal action of lichen extracts are increased in the following order: *F. caperata*>*X. conspersa*>*X. stenophylla*.

There are limited studies about antifungal activity of *F. caperata* lichen. For example, Mitrovic et al. [19] reported that methanol extract of *F. caperata* had effective against *C. albicans*. In agreement with this study we also observed that acetonitrile extract of *F. caperata* is active against *C. albicans*. On the other hand, in a study which was conducted by Millot et al [5] it was concluded that acetone extract of *F. caperata* were inactive against *C. albicans*

To the best of our knowledge, there is not any survey about antifungal potential of the extracts of *X. stenophylla* against *C. albicans*.

The degree of the antimicrobial activity changes according to the used extract type, extract concentration, tried microorganisms and localities where plant samples collected [20].

Researches also done about antifungal actions of various lichen species. For example, Halama and Haluwin [21] studied acetone extracts of *Evernia prunastri*, *Hypogymnia physodes* and *Cladonia portentosa* against eight plant pathogenic fungi.

Acetone, methanol and chloroform extracts of *Bulbothrix setschwanensis*, *Everniastrum nepalense*, *Heterodermia diademata* and *Parmelia thamsonii* were screened againt plat pathogenic fungi [22].

Piovani et al. [23] investigated antifungal activity of lichen aromatic metabolites against *C. albicans* and S. cerevisiae.

Methanol, acetone and chloroform extracts of *Usnea florida* was analyzed against *C. albicans* and *C. parapsilosis* [24].

The CUPRAC test of antioxidant activity is attributed to the absorbance reading of Cu(I)-Nc chelate created as a result of the redox reaction of chain-breaking antioxidants by CUPRAC reagent, Cu(II)-Nc [25]. The ferric ion is altered with cupric ion, tripyridyltriazine is replaced by neocupreine and formed color can be observed with a spectrophotometer at 450 nm [26].

The CUPRAC values of the extracts were demonstrated in Table 3. The extract of *X. conspersa* showed the highest reducing power Cu^{2+} to Cu^{+}. The extract of *X. stenophylla* exhibited the lowest activity. Decreasing CUPRAC values is in order of *X. conspersa*>*F. caperata*>*X. stenophylla.*

This is the first report about CUPRAC test of *F. caperata*, *X. stenophylla* and *X. conspersa* lichens.

Table 3. CUPRAC values of the tested lichen extracts (nm)

Lichen	Concentration (µg/ml)	Absorbance values
X. stenophylla	50	0.150±0.012
	100	0.259±0..012
	150	0.377±0.009
	200	0.453±0.003
F. caperata	50	0.170±0.002
	100	0.282±0.019
	150	0.318±0.009
	200	0.473±0.012
X. conspersa	50	0.203±0.010
	100	0.293±0.015
	150	0.385±0.0738
	200	0.520±0.034
BHT	50	0.501±0.007
	100	0.595±0.032
	150	0.718±0.060
	200	0.866±0.013

Studies carried out about CUPRAC activity of different lichen species. Ganesan et al. [27] assessed CUPRAC activity of petroleum ether, benzene, 2-propanol, ethyl acetate and methanol extracts of Ramalina inflata lichen.

Sundararaj et al. [28] searched CUPRAC activity of methanol, petroleum ether and water extracts of *Ramalina nervulosa*.

CUPRAC activity was also studied by Zlatanovic et al. [29] in the acetone extract of *Umbilicaria crustulosa*.

4. Conclusion

Recently, the search for biologically active compounds from plants to improve pharmaceutical, cosmetics and agriculture industry have gained more attraction. The lichen metabolites were studied for their phytochemical features. Lipid peroxidation caused by oxygen leads to deterioration of foods and diseases such as cancer and ageing. Therefore there was an urgent demand for medicines of natural origin for treating many illnesses. As the result of this study we have concluded that tested lichen extracts have antioxidant and antifungal activity. Hereby, *F. caperata*, *X. conspersa* and *X. stenophylla* might be a potential source for improving brand antifungal and antioxidant compounds.

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