

A PURIFIED EXTRACT FROM BROWN TRUFFLES OF THE SPECIES *TERFEZIA CLAVERYI* CHATIN AND ITS ANTIMICROBIAL ACTIVITY

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Abstract

A purified aqueous extract of *Terfezia claveryi* Chatin brown truffles was obtained by water extraction, concentration and purification by ultrafiltration. The concentrated extract was tested for antimicrobial activity against three microorganisms: a Gram-negative bacterium (*Escherichia coli* ATCC 8739) and two Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538P and *Staphylococcus epidermidis* ATCC 12228), using the agar diffusion method and it presented a very good activity in all three cases, with growth inhibition zones of 20.5 mm, 21 mm and 26.5 mm respectively. The brown truffle extract showed an antimicrobial activity comparable with that of 300 - 400 µg/mL and 25 µg/mL gentamicin sulphate chosen as reference, respectively.

Rezumat

Extractul purificat din trufe brune *Terfezia claveryi* Chatin a fost obținut prin extracție apoasă, concentrare și purificare prin ultrafiltrare. Extractul concentrat a fost testat pentru activitatea antimicrobiană față de trei microorganisme: o bacterie Gram-negativă (*Escherichia coli* ATCC 8739) și două bacterii Gram-pozitive (*Staphylococcus aureus* ATCC 6538P și *Staphylococcus epidermidis* ATCC 12228), folosind metoda de difuzie în agar și a prezentat o activitate foarte bună în toate cele trei cazuri, cu zone de inhibiție a creșterii de 20.5 mm, 21 mm și, respectiv, 26.5 mm. Extractul din trufe brune a prezentat o activitate antimicrobiană comparabilă cu cea a sulfatului de gentamicină ales ca referință, în concentrații de 300 - 400 µg/mL și, respectiv, 25 µg/mL.

Keywords: *Terfezia claveryi* Chatin, brown truffles extract, antimicrobial activity

Introduction

Desert truffles are a rich source of protein, amino acids, fatty acids, minerals and carbohydrates [1, 5, 6, 7]. Like other fungi, desert truffles comprise a vast and yet largely unexploited source of new pharmaceutical products.

Searching for new therapeutic alternatives, in modern medicine, truffles are considered a large source of therapeutic compounds with anti-inflammatory, immunosuppressor, antimutagenic, anticarcinogenic [14], antioxidant properties [20], and antimicrobial properties [15, 16]. Many eye infections such as bacterial conjunctivitis, blepharitis and dacrocystitis are caused by *Staphylococcus aureus* [19]. Chronic bacterial conjunctivitis is most commonly caused by *Staphylococcus aureus* but may also be due to Gram-negative rods such as *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Serratia marcescens*, and *Moraxella lacunata*

[27]. Blepharitis, which is the inflammation of the eyelid and chronic blepharoconjunctivitis are also caused by *Staphylococci* [8, 21]. For the treatment of the above mentioned affections, antibiotics or antivirals are used, both local and systemic [10]. Many of these preparations can cause adverse or allergic reactions, having unpleasant or even serious effects. Moreover, the healing can be accompanied by proliferative processes, with upsetting scars, affecting sight. Many antibacterial drugs are used to treat eye infections, such as chloramphenicol, fusidic acid, fluoroquinolones, oxacillin and aminoglycosides, e.g. neomycin, tobramycin, gentamicin [18]. Most of these antibiotics have serious side effects: burning, stinging, irritation, itching, redness, blurred vision, eyelid itching, eyelid swelling, sensitivity to light [2, 4, 12, 17, 21, 22, 24]. Prolonged use of topical antibiotics may give rise to overgrowth of non-susceptible organisms including fungi [24].

Bacterial and fungal corneal ulcers have developed during treatment with gentamicin ophthalmic preparations. The most frequently reported adverse reactions are ocular burning and irritation upon drug instillation, non-specific conjunctivitis, conjunctival epithelial defects and conjunctival hyperaemia [3, 9, 11].

With the increasing resistance of many micro-organisms to the currently used antibiotics and the high cost of production of synthetic compounds, this study is an attempt to find an alternative antibacterial preparation from natural desert truffles which can be used for the treatment of eye infections.

Thus, the preparation of a natural origin product by a procedure involving pre-established steps and parameters, as well as antimicrobial activities of a purified aqueous extract of the *Terfezia* type fungi are presented in this study.

Materials and Methods

Preparation of truffle extract

Fresh or frozen truffles were extracted in demineralized water at 4°C by a ratio of 2-3:1 (v/w) for 24 h. The extract was separated by filtration on a filter aid layer (celite) or by centrifugation and it is submitted to purification by ultrafiltration on a Biomax-10 cassette (0.5 m²) membrane with a cut-off limit of 10 kDa (Millipore). The obtained permeate solution was then concentrated under vacuum to 1/6-1/9 (v/v), at max. 40°C.

Microbiological studies

Inoculum preparation

The three test-microorganisms were grown on casein soy broth agar medium (CaSoA). Before any experiment, the strains were activated by transferring some bacterial cells, with a sterile loop, on fresh CaSoA medium and incubated for 18-24 hours at 30 - 35°C.

Antimicrobial assay

Antimicrobial activity

Antimicrobial activities of the resulted solutions (filtrate and retentate) after ultrafiltration of the raw *Terfezia clavaryi* Chatin extract, as well as the final extract (permeate solution after concentration), were tested against three strains of bacteria: a Gram-negative bacterium (*Escherichia coli* ATCC 8739) and two Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538P and *Staphylococcus epidermidis* ATCC 12228).

The tests were performed in sterile Petri dishes, each containing 15 - 20 mL of appropriate culture medium (CaSoA), previously inoculated with 10⁴-10⁵ colony forming units (CFU)/mL from one bacterial strain. On each dish, 4 stainless steel cylinders of 8 mm diameter were placed on the solidified surface of the medium. Afterwards, in each

cylinder, 0.2 mL of sample were added, either truffle extract as it was (9.3 % dry matter) and aqueous dilution (extract/water 1:5 v/v) or different concentrations of the antibiotic gentamicin sulphate (EIPICO).

The Petri dishes were incubated 24 hours at 33 ± 2°C. After the incubation period, the growth inhibition zones were measured and the results were expressed as the arithmetic mean of three measurements for each sample.

Antimicrobial activity was valued on the basis of the diameter of the growth inhibition zone as follows: < 10 mm – no antimicrobial activity; 10 - 15 mm – weak antimicrobial activity; 16 - 20 mm – moderate antimicrobial activity; 20 mm > – high antimicrobial activity [25].

In the present study, we assayed the activity of the aqueous extract as it is and of a five times dilution extract, of it in order to compare it with the results obtained after testing different concentration of gentamicin sulphate, varying from 25 µg/mL to 400 µg/mL.

Results and Discussion

We determined that the extract was the most effective against *S. epidermidis* and had a lesser effect against *Escherichia coli* or *Staphylococcus aureus*. In all cases there was a significant decrease in antibacterial activity after diluting the extract, as it can be observed in Table I.

Previous studies [15, 16] showed that both aqueous and methanolic extracts from *Terfezia clavaryi* Chatin have a significant antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The authors assumed that the antibacterial agent may be a peptide, having in view that they noticed as active a protein fraction obtained by ammonium sulphate precipitation and purified by gel filtration and ion exchange chromatography.

The observation that only the permeate obtained by ultrafiltration showed antimicrobial activity suggests a molecular weight of the active extract component(s) less than 10 kDa. The retentate, containing macromolecules with MW (molecular weight) higher than 10 kDa was inactive.

The present study confirmed that the brown truffle extract has a high effect of growth inhibition against *Staphylococcus aureus* and also against *E. coli* and *Staphylococcus epidermidis*, the latter being a common cause of eye infection. However, as it can be observed from the data in Table I, the extract has to be used as it is, dilution causing a substantial loss of antibacterial effect, with the growth inhibition zones decreasing from 20.5 - 26.5 mm to only 10 - 12.5 mm.

On the other hand, *Terfezia clavaryi* Chatin may not be the only one in its family that has anti-microbial

activity, the aqueous extract from *Terfezia boudieri* was shown to have a minimal inhibitory concentration of 6 mg/mL against *Escherichia coli*

ATCC 19115 and 8 mg/mL against *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228 [13].

Table I

Antimicrobial activity of the purified aqueous truffle extract compared with different concentrations of gentamicin sulphate

No.	Sample	Bacterial strain	Inhibition zone (mm)
1	Truffle extract	<i>Staphylococcus aureus</i> ATCC 6538P	20.5
		<i>Escherichia coli</i> ATCC 8739	21
		<i>Staphylococcus epidermidis</i> ATCC 12228	26.5
2	Truffle extract (1:5 dilution)	<i>Staphylococcus aureus</i> ATCC 6538P	10.5
		<i>Escherichia coli</i> ATCC 8739	< 10
		<i>Staphylococcus epidermidis</i> ATCC 12228	12.5
3	Gentamicin sulphate 400 µg/mL	<i>Escherichia coli</i> ATCC 8739	22
4	Gentamicin sulphate 300 µg/mL	<i>Escherichia coli</i> ATCC 8739	20
5	Gentamicin sulphate 25 µg/mL	<i>Staphylococcus aureus</i> ATCC 6538P	21
6	Gentamicin sulphate 25 µg/mL	<i>Staphylococcus epidermidis</i> ATCC 12228	25

As it can be seen in Table I, the purified aqueous extract from brown truffles has an activity comparable with a concentration of antibiotic equal to 25 µg/mL against the two Gram-positive bacteria. Against *E. coli*, however, it has an activity almost as gentamicin sulphate in 300 - 400 µg/mL concentration, due to the outer membrane of Gram-negative bacteria, which is composed of lipids, proteins and lipopolysaccharides, less fluid and permeable, thus being more difficult for exterior substances to enter the cells [23].

Conclusions

A purified aqueous extract of *Terfezia clavervyi* Chatin brown truffles was obtained by ultra-filtration of a crude one on 10 kDa membranes. The product showed antimicrobial activity against *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 8739 and *Staphylococcus epidermidis* ATCC 12228.

The aqueous extract of the truffle *Terfezia clavervyi* Chatin contains a potent antibacterial agent with a molecular weight (MW) less than 10 kDa which may be used in the treatment of eye infections caused by *Staphylococcus aureus*, *Escherichia coli* or *Staphylococcus epidermidis*, thereby helping to reduce the use of chemically synthesized antibiotics and the development of drug resistance bacteria.

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