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Anti-yeast properties of the leaves of *Cotinus coggygria* against clinically relevant fungal pathogens

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Abstract
Objective: The extracts obtained from the leaves of *Cotinus coggygria* Scop. (Anacardiaceae) were investigated for their ability to inhibit clinically relevant fungal pathogens.
Methods: Ethanol (EtOH), ethyl acetate (AcOEt) and dicholoromethane (DCM) were screened against six *Candida* species (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13803, *C. guilliermondii* ATCC 6260, *C. krusei* ATCC 20298, *C. glabrata* ATCC 2001, *C. parapsilosis* ATCC 22019), and two *Cryptococcus* species (*C. neoformans* ATCC 90112 and *C. laurentii* ATCC 34142) by the microbroth dilution method.
Results: The plant displayed activity against all yeast cultures tested. Greater activity was observed for the EtOH, AcOEt and DCM extracts of *C. coggygria* against *C. guilliermondii*, *C. krusei* and *C. laurentii*, with MIC values of 30, 60 and 60 µg/mL, respectively. Further separation of active principle from the potent plant will be useful for the control of *Candida* and *Cyptococcus* species.
Conclusion: Our findings support the use of *C. coggygria* in traditional medicine for the treatment against the yeast infections.

Keywords: *Cotinus coggygria* Scop; Anti-yeast Activity; Medicinal Plants

1. Introduction
Cotinus coggygria Scop. (Anacardiaceae) is frequent and locally common in some parts of Turkey. Turkish local names for this plant are Duman Ağacı, Peruke Çalısı and BoyacıSumağı. The leaves are used as antiseptic, antiinflammatory, antimicrobial, antihemorrhagic, wound-healing and against diarrhoea as traditional medicine in Turkey [1]. During our routine field excursions in Turkey, it was found that these plants are used to treat respiratory tract infections, stomachache, and, externally boils and abscesses. The aim of the present study was to screen *C. coggygria* extracts that have been shown earlier to have antimicrobial activity against *Candida* and *Cryptococcus* species.

2. Materals and Methods
2.1. Plant material
The leaves of the plant were collected from Yalova, Turkey during the months of September-October of 2014. The plant were deposited in Department of Medical Biology of Duzce University in the author's personal collection (voucher number GD81-12).

2.2. Preparation of the extracts
The plant leaves were air-dried. Each dry powdered plant material (100 g) was extracted with 80% ethanol (EtOH) over 10 days at room temperature. Following filtration, crude hydro alcoholic extracts were dried under a vacuum. The extracts were subjected to liquid-liquid partition using dicholoromethane (DCM) and ethyl acetate (AcOEt). After solvent removal, all fractions were subjected to biological assays against the test fungi [2].

2.3. Microorganisms
Candida species (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13803, *C. guilliermondii* ATCC 6260, *C. krusei* ATCC 20298, *C. glabrata* ATCC 2001, *C. parapsilosis* ATCC 22019), and *Cryptococcus* species (*C. neoformans* ATCC 90112 and *C. laurentii* ATCC 34142) were used for an anti-yeast evaluation. All fungal strains maintained on Saboraud Dextrose Agar (SDA, Oxoid, Basingstoke, UK) at 4°C.

2.4. Microdilution method

Synthetic RPMI (Sigma, St. Louis, MO, USA) medium with L-glutamine buffered to pH 7.0 with 0.165 morpholine propanesulfonic acid (MOPS, Sigma) was prepared according to the CLSI M27-A2 document (formerly the NCCLS) [3] and used for Minimal Inhibitory Concentration (MIC) determination. Fungal cultures, freshly grown at 35°C, and inoculums suspensions were prepared by the spectrophotometric method with a final inoculums of 1.5 ± 1.0 x 10^3 cfu/mL used for suspectibility testing. Broth microdilution testing was performed in accordance with the guidelines of the CLSI M27-A2 document (formerly the NCCLS) [3]. Susceptibility was determined by the microbroth dilution method performed in sterile flat-bottom 96-well microplates. Extracts and fractions were dissolved in dimethyl sulfoxide (DMSO, Merck, Darmstadt, Germany) after the addition of RPMI. Serial dilutions were then performed, using RPMI as diluents, maintaining a constant volume of 1000 µL per tube. The extracts were tested at eight concentrations that varied from 1000 to 7.8 µg/mL. From each dilution, 100 µL volumes were distributed in microplates. As a control for growth and sterility, RPMI alone was used without extracts or solvents. Amphotericin B was included at concentrations of 25 to 0.03 µg/mL, as positive antifungal controls. After inoculation of fungal strains, plates were incubated at 35°C for 48 h for *Candida* species and 72 h for *Cryptococcus* species. All tests were performed in triplicate. The endpoints were determined visually by comparison with the drug-free growth control well. MICs were defined as the lowest extract concentration for which the well was optically clear, and were expressed in µg/mL.

3. Results

The MICs values concerning in vitro antifungal activities of the extracts are presented in Table 1. Greater activity was observed for the EtOH, AcOEt and DCM extracts of *C. coggygria* against *C. guilliermondii*, *C. krusei* and *C. laurentii*, with MIC values of 30, 60 and 60 µg/mL, respectively. Besides, the EtOH extract has strong effect against *C. glabrata* (MIC 60 µg/mL). *C. tropicalis* and *C. neoformans* was highly resistant to DCM extracts, having an MIC of >1000 µg/mL. EtOH extracts exhibited greater than those of the other extracts.

4. Discussion

The oil composition from leaves of *C. coggygria* has been declared previously [1]. In that study, forty-two components were characterized, representing 99.6% of the total components detected. The major constituents were identified as limonene (48.5%), (*Z*)-β-ocimene (27.9%) and (*E*)-β-ocimene (9.7%). In the study of Novakovic *et al.* [4], essential oils obtained via hydrodistillation of the young branches and leaves of *C. coggygria* were analyzed via GC-MS, which identified 31 components, including monoterpenic hydrocarbons, the dominant component being limonene. The antibacterial and antifungal activities of the oils were examined and compared with Streptomycin and bifonazole, and both oils showed slightly more activity. In a study of the composition of essential oil obtained from the leaves of *C. coggygria*, Demirci *et al.* [5] also reported that the dominant component was limonene, but found that the oil comprised 42 components. Savikin *et al.* [6] determined the total phenol content in extracts from the flowers and leaves of the plant to be 76.5±4.2 and 515.5±8.3 mg $GAEg^{-1}$, and the tannin content to be 13.7%±0.9% and 18.5%±1.1%, respectively. It is important to bear in the test may be correlated with a high activity of its chemical components.

This is the first study to provide data about the extracts of *C. coggygria* evaluated against clinically relevant fungal pathogens. The plant used in this study has been shown earlier to have antimicrobial activity against some bacteria and the yeast cultures [7]. In that study, the ethanolic extract of the plant showed antimicrobial activity against all tested microorganisms with inhibition zones ranged from 10.4 to 22.8 mm for bacteria, 11.8 to 16.2 mm for the yeast cultures. The extract is more effective than those of antibacterial agent chloramphenicol against *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*. Notably, *Staphylococus aureus* is the most susceptible to the extract of the plant among test bacteria. While the extracts have a moderate activity against *Bacillus cereus*, *Escherichia coli* and *Enterobacter aerogenes* in comparison to the standard antibacterial antibiotic, they have weak antibacterial effect against the other bacteria. In case of antifungal activity, the ethanol extract obtained from the plant has a moderate antiyeast effect than the standard antifungal antibiotic clotrimazole. From this aspect, the findings with the ethanol extracts obtained from this study are parallel to the above study.

Tunc *et al* [8] uses the disc diffusion method to examine the antimicrobial activity of the extracts of *C. coggygria* prepared in ethanol, methanol, distilled water, chloroform, acetone, and petroleum ether against the bacteria *Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella typhimurium*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. *Cotinus coggygria* was found to inhibit the reproduction of microorganisms at various rates. The plant extracts in distilled water and methanol were found to be the most effective against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterococcus faecalis*. In addition, Matic *et al.* [9] also reported that the *in vitro* antimicrobial activity of the methanol extract of *C. coggygria* and gallic acid was examined on six different bacterial species and *Candida albicans*, using the cylinder plate and macrobroth dilution method. The antimicrobial studies revealed that methanol extract of *C. coggygria* is more effective against all tested microorganisms than gallic acid. In both the mentioned studies, it is determined that the methanol extracts were found to be the most effective against the tested microorganisms. According to the findings of the present study, the ethanol extract has stronger and broader spectrum of anti-yeast activity. Methanol and ethanol have been proved as effective solvents to extract phenolic compounds [10]. Considering the methanol and ethanol extracts, methanol extract is more polar than ethanol. Ethanol extract is also a good solvent extract for obtaining polyphenolic compounds and is safe for human consumption [11]. From the results, ethanol extract has shown some affinity to extract bioactive compounds even after the use of methanol and it is also evident from the study results. This may be due to extractability of some bioactive compounds in ethanol.

The findings in this study may indicate that *C. coggygria* can be used as natural preservatives against *Candida* and *Cryptococcus* species. According to findings from the National Nosocomial Infection Surveillance System (NNIS), 61% of reported nosocomial fungal infections were due to *Candida albicans*, followed by other *Candida* spp. and *Cryptococcus* spp. [12].

5. Conclusion

This study provides data about the antimicrobial properties of some tropical plant species for therapeutically useful. These extracts may be applied clinically for fungal infection, especially the EtOH extract from the leaf of *C. coggygria*. This explains the use of *C. coggygria* in folk medicine for the treatment of various diseases, some related to microbial infections.

Table 1: MIC values of the leaf extracts of *Cotinus coggygria*

Microorganisms	Minimum inhibitory concentration (MIC) (µg/mL)			
	Extracts of *Cotinus coggygria*			Standard
	EtOH	AcOEt	DCM	Amphotericin B
Candida albicans	125	500	125	1.0
Candida tropicalis	125	500	>1000	0.25
Candida guilliermondii	30	60	60	1.0
Candida krusei	30	60	60	0.5
Candida glabrata	60	250	500	1.0
Candida parapsilosis	500	500	1000	0.5
Cryptococcus neoformans	250	1000	>1000	1.0
Cryptococcus laurentii	30	60	60	2.0

EtOH: ethanolic, AcOEt: ethyl acetate, DCM: dicholoromethane

5. References

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