

ANTIFUNGAL POTENTIAL OF *PIMPINELLA ANISUM*, *CARUM CARVI* AND *CORIANDRUM SATIVUM* EXTRACTS. A COMPARATIVE STUDY WITH FOCUS ON THE PHENOLIC COMPOSITION

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Manuscript received: December 2019

Abstract

The present study aimed to characterize the phenolic extracts obtained from fruits of *Pimpinella anisum* L., *Carum carvi* L. and *Coriandrum sativum* L. (*Apiaceae*), and to assess their antifungal activity against main etiological agents involved in onychomycosis and other dermatomycoses (*Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum canis*, *M. gypseum* and *Candida albicans*). Total phenolic and flavonoid contents varied from 3.80 ± 0.49 to 24.16 ± 0.82 mg gallic acid equivalents (GAE)/100 g dw, and 12.51 ± 0.14 to 37.75 ± 0.21 mg catechin equivalents (CE)/100 g dw, respectively. HPLC-DAD-ESI-Q-TOF-MS/MS analysis revealed the presence of chlorogenic acid, dicaffeoylquinic acid, luteolin and apigenin pentoside in all analysed samples. The extracts showed antifungal activity against all tested strains, with minimal inhibitory concentration (MIC) values in the range of 1.25-5 mg/mL. These results provide evidence that phenolic extracts obtained from fruits of *P. anisum*, *C. carvi* and *C. sativum* might be sources of antifungal agents with putative use in onychomycosis.

Rezumat

Obiectivul studiului de față a fost caracterizarea extractelor polifenolice obținute din fructe de *Pimpinella anisum* L., *Carum carvi* L. și *Coriandrum sativum* L. (*Apiaceae*) și determinarea activității lor antifungice asupra agenților etiologici majori implicați în onicomicoze și alte dermatomicoze (*Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum canis*, *M. gypseum* și *Candida albicans*). Conținutul în polifenoli totali și flavonoide a variat între $3,80 \pm 0,49$ și $24,16 \pm 0,82$ mg echivalenți acid galic (GAE)/100 g produs vegetal, și $12,51 \pm 0,14$ și $37,75 \pm 0,21$ mg echivalenți catehină (CE)/100 g produs vegetal, respectiv. Analiza HPLC-DAD-ESI-Q-TOF-MS/MS a identificat prezența acidului clorogenic, acidului dicafeoilchinic, luteolinei și apigenol-pentozidei în toate probele investigate. Extractele au prezentat activitate antifungică asupra tuturor tulpinilor testate, cu valori ale concentrației minime inhibitorii cuprinse în intervalul 1,25 - 5 mg/mL. În concluzie, extractele polifenolice obținute din fructe de *P. anisum*, *C. carvi* and *C. sativum* reprezintă surse de agenți antifungici cu potențială utilizare în tratamentul onicomicozelor.

Keywords: *Pimpinella anisum* L., *Carum carvi* L., *Coriandrum sativum* L., *Apiaceae*, phenolics, chemical composition, antifungal activity

Introduction

Pimpinella anisum L. (anise), *Carum carvi* L. (caraway) and *Coriandrum sativum* L. (coriander) are plant species belonging to the *Apiaceae* family, widely used for their medicinal and culinary properties. Even though their health benefits have been mainly associated with the essential oil [15, 18], these species contain significant amounts of phenolic compounds. The later have been recognized to possess many biological effects including anti-inflammatory, anti-

microbial, hypolipidemic, antioxidant, anti-mutagenic and anticarcinogenic activity [1, 6].

In order to identify natural compounds with putative use in onychomycosis, we screened the antifungal properties of phenolic extracts obtained from anise, caraway and coriander fruits.

Onychomycoses are fungal infections of the finger or toenails, mainly caused by fungi of *Trichophyton*, *Microsporum* and *Epidermophyton* genera, and some *Candida* species. Onychomycoses are among the most common nail disorders in adults (15 - 40% of all

nail diseases) and are often intractable, with relapses due to poor adherence to long-term treatment regimens and development of antifungal resistance mechanisms [11]. Current available therapies consist of both oral and topical treatment, but their efficacy is hampered by side effects and poor nail plate penetration [8]. Therefore, the development of novel antifungal drugs targeting structures that are unique to fungal pathogens and ensure nail permeation is a challenge. In the search for new antifungal lead structures, interest in natural product-based screening has gained much attention [21]. Among the plant-derived products, phenolics are one of the most diverse classes of specialized metabolites that play an important role in plant growth and reproduction, and, more, are involved in the plant defensive response to various insults, including microbial attacks [14].

Therefore, the present study was designed to evaluate the *in vitro* antifungal properties of phenolic extracts obtained from anise, caraway and coriander fruits against several pathogenic dermatophytes (*Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum canis*, and *Microsporum gypseum*) and yeasts (*Candida albicans*) involved in the aetiology of onychomycosis and other dermatomycoses. To the best of our knowledge, the effects of phenolic extracts on dermatophytes have not been investigated. Herein, an analysis of individual phenolic compounds was performed, correlating these phytochemicals with the antifungal activity of the *Apiaceae* extracts.

Materials and Methods

Chemicals

Aluminium chloride, (+)-catechin, dimethyl sulfoxide (DMSO), Folin-Ciocalteu's phenol reagent, gallic acid, methanol, sodium hydroxide, sodium nitrite, sodium carbonate were purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile and formic acid for LC-MS were purchased from J.T. Baker Inc. (Deventer, The Netherlands). Sabouraud dextrose agar (SDA) was purchased from Biolab (Hungary).

Plant material and extraction

Raw plant material (fruits of anise, caraway and coriander) was purchased from a local pharmacy. Voucher specimens (s20180901, s20180902 and s20180903) were deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Iași, Romania. The fruits (5 g) were powdered and extracted three times with methanol (50 mL) for 30 min by sonication at 60°C. The obtained solutions were evaporated to dryness under reduced pressure at 40°C. The extraction yields were 11.96% for anise, 10.22% for caraway, and 5.09% for coriander.

Total phenolic and flavonoid contents

The extracts were re-dissolved in DMSO (10 mg/mL) and the total phenolic content (TPC) was assayed by Folin-Ciocalteu method [19], whereas total flavonoid

content (TFC) was estimated according to Trifan *et al.* [20]. TPC was expressed as mg GAE/100 g dry weight (dw) and TFC were calculated as mg CE/100 g dw.

HPLC-DAD-ESI-Q-TOF-MS/MS analysis

Qualitative high-performance liquid chromatography with diode array detector coupled to electrospray ionization quadrupole time-of-flight tandem mass spectrometry (HPLC-DAD-ESI-Q-TOF-MS/MS) experiments were conducted on an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, California, USA) equipped with an auto-sampler (G1329A), a degasser (G1379B), a binary pump (G1312C), a column oven (G1316B), a DAD detector (G1315B) and a Q-TOF-MS (G6530B). The separations were carried out on a Phenomenex Gemini C18 (100 × 2 mm, 3 μm) column, following the method described in Luca *et al.* [9]. All compounds were identified according to their mass, fragmentation pattern and UV-VIS characteristics by comparison with literature data.

Fungal strains

For experimental validation, the methanolic extracts of anise, caraway and coriander were evaluated for antifungal activity against five standard strains, namely *Trichophyton rubrum* ATCC 28188, *Trichophyton mentagrophytes* ATCC 36107, *Microsporum canis* CECT 20190, *Microsporum gypseum* CECT 2098 and *Candida albicans* ATCC 90028. Thus, fungi were grown on SDA and were incubated for 48 hours (yeast strain) or 15 days at 30°C (dermatophyte strains). All of the strains were cultured until the maximal numbers of conidia were formed.

Preparation of standard inoculum and agar dilution assay

The antifungal screening of the methanolic extracts was performed by determining the minimum inhibitory concentrations (MICs) using an agar dilution method. Testing plates were prepared by mixing 20 mL of melted SDA with an appropriate volume of extracts eluted in DMSO. The final concentrations of methanolic extracts were 1.25, 2.5, and 5 mg/mL respectively. Control agar plates with 5% DMSO were also tested. Itraconazole was used as control at concentrations of 0.03, 0.06, 0.125, and 0.25 mg/mL. The plates were kept for 10 minutes at room temperature and were subsequently inoculated with the fungal strains as follows: 10 μL of standardized suspension (10⁵ CFU/mL) was inoculated onto the control plates and the media incorporating the investigated extracts. The inoculated plates were incubated at 30°C for 48 hours or 15 days depending on the type of fungi (until mature colonies were formed on the control plates). The MIC was defined as 100% inhibition of the fungi. Experiments were performed in triplicate.

Statistical analysis

All experiments were performed in triplicate and the results were expressed as mean ± standard deviation (SD).

Results and Discussion

Anise presented the highest content in phenolics (24.16 mg GAE/100 g dw) and flavonoids (37.75 mg CE/100 g dw), in comparison with caraway and

coriander (Table I). Our data are within the range reported in literature regarding TPC and TFC in *Apiaceae* fruits [6, 10, 13].

Table I
TPC and TFC of *Apiaceae* fruits

Extract	TPC (mg GAE/100 g dw)	TFC (mg CE/100 g dw)
<i>Pimpinella anisum</i> L.	24.16 ± 0.82	37.75 ± 0.21
<i>Carum carvi</i> L.	22.26 ± 0.76	24.02 ± 0.08
<i>Coriandrum sativum</i> L.	3.80 ± 0.49	12.51 ± 0.14

Data of the tentative identification, retention time, molecular ion, molecular formula, and main MS/MS fragment ions of phenolic compounds in anise,

caraway and coriander fruits are presented in Table II. Figure 1 shows the base peak chromatograms of phenolic profiles belonging to investigated samples.

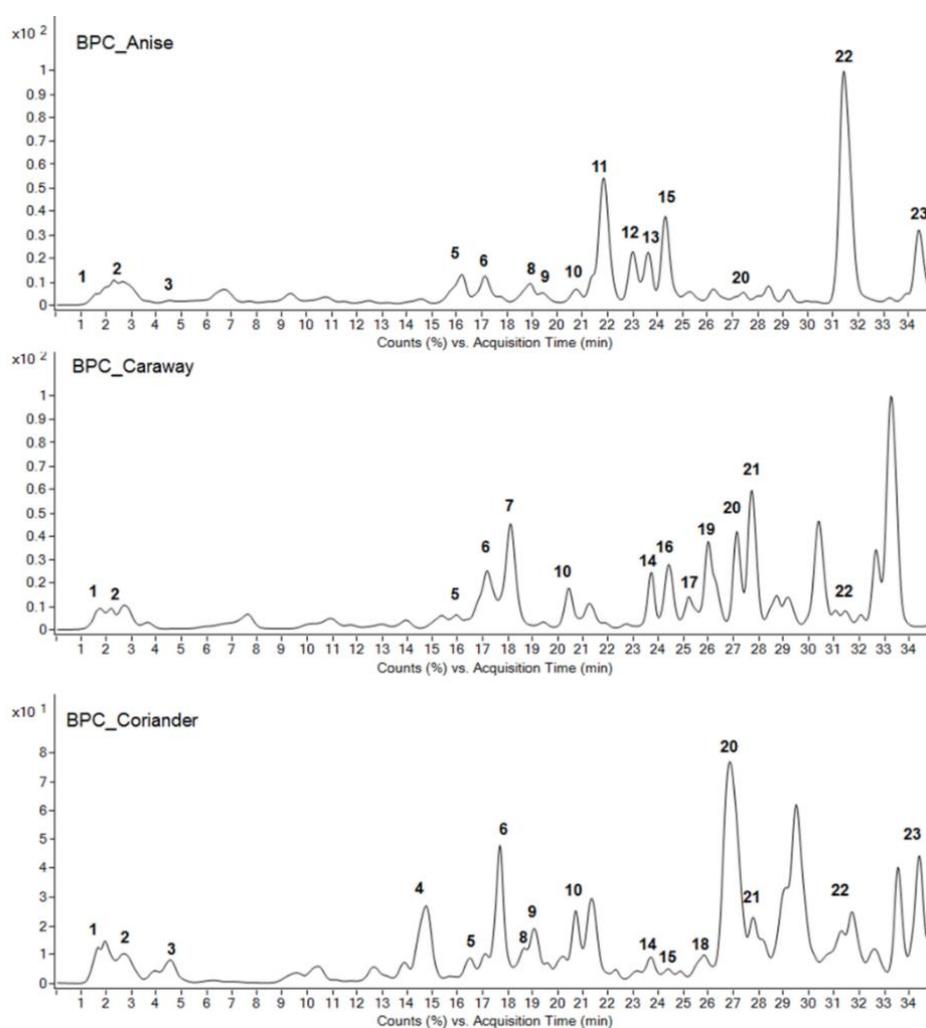


Figure 1.

Chromatographic profiles of *Apiaceae* fruit extracts obtained by HPLC-DAD-ESI-Q-TOF-MS/MS (base peak chromatograms); identity of compounds as in Table II.

The phenolic profile of *Apiaceae* fruits presented hydroxycinnamic acid derivatives (caffeic, quinic, ferulic and hydroxybenzoic acid derivatives), flavone derivatives (apigenin, luteolin derivatives) and flavonol derivatives (quercetin and kaempferol derivatives),

alongside two polyols (sorbitol and malic acid) (Table II). Our study revealed the presence of chlorogenic acid, dicaffeoylquinic acid, luteolin and apigenin pentoside in all analysed samples.

Table II

MS and MS/MS data of constituents of methanolic extracts from <i>Apiaceae</i> fruits											
No.	Proposed identity	Rt (min)	[M-H] ⁻ _{exp.} (m/z)	[M-H] ⁻ _{calcd.} (m/z)	Δ (ppm)	Molecular formula	MS/MS fragments (m/z)	P.a.	C.c	C.s	Refs
1	Sorbitol	1.6	181.0714	181.0718	1.99	C ₆ H ₁₄ O ₆	163.0607	✓	✓	✓	[4]
2	Malic acid	2.3	133.0145	133.0142	-1.89	C ₄ H ₆ O ₅	115.0039	✓	✓	✓	[4]
3	Hydroxybenzoic acid hexoside	4.5	299.0775	299.0762	-0.86	C ₁₃ H ₁₆ O ₈	137.0211	✓	✗	✓	[4]
4	Hydroxybenzoic acid	14.7	137.0246	137.0244	-0.60	C ₇ H ₆ O ₃	–	✗	✗	✓	[4]
5	Apigenin pentoside	16.3	401.0889	401.0878	-2.72	C ₂₀ H ₁₈ O ₉	269.0992	✓	✓	✓	[9]
6	Chlorogenic acid	17.6	353.0882	353.0878	-1.11	C ₁₆ H ₁₈ O ₉	191.0507, 179.0269, 173.0409, 135.0417	✓	✓	✓	[3]
7	Thymoquinol hexoside	18.1	327.1448	327.1449	0.39	C ₁₆ H ₂₄ O ₇	165.0919, 161.0487	✗	✓	✗	–
8	Caffeic acid	18.7	179.0353	179.0350	-1.76	C ₉ H ₈ O ₄	135.0456	✗	✗	✓	[3]
9	Neochlorogenic acid	19.1	353.0890	353.0878	-3.40	C ₁₆ H ₁₈ O ₉	191.0507, 179.0269, 135.0417	✗	✗	✓	[3]
10	Feruloylquinic acid	20.4	367.1045	367.1035	-2.84	C ₁₇ H ₂₀ O ₉	191.0604	✗	✓	✓	[7]
11	Luteolin-C-hexoside	21.8	447.0938	447.0933	-1.15	C ₂₁ H ₂₀ O ₁₁	357.1021, 327.0919, 297.0686, 285.0552	✓	✗	✗	[10]
12	Apigenin-C-hexoside-O-pentoside	23.0	563.1396	563.1406	1.82	C ₂₆ H ₂₈ O ₁₄	443.0975, 413.0986, 311.0590, 293.0491, 269.0459	✓	✗	✗	[3]
13	Apigenin-C-hexoside	23.6	431.0986	431.0984	-0.53	C ₂₁ H ₂₀ O ₁₀	413.0859, 341.0578, 311.0478	✓	✗	✗	[3]
14	Quercetin-O-rutinoside	23.7	609.1459	609.1461	0.34	C ₂₇ H ₃₀ O ₁₆	301.0371, 277.0311, 255.0319, 151.0011	✗	✓	✓	[3]
15	Luteolin-O-hexoside	24.3	447.0913	447.0933	4.43	C ₂₁ H ₂₀ O ₁₁	285.0381, 227.0221, 199.0356, 151.0026	✓	✗	✓	[10]
16	Quercetin-O-hexoside	24.4	463.0869	463.0882	2.80	C ₂₁ H ₂₀ O ₁₂	301.0370, 271.0239, 255.0302, 151.0041	✗	✓	✗	[3]
17	Kaempferol-O-rutinoside	25.2	593.1515	593.1515	-0.52	C ₂₇ H ₃₀ O ₁₅	285.0317, 255.0232, 227.0259	✗	✓	✗	[3]
18	Ferulic acid	25.9	193.0518	193.0506	-0.86	C ₁₀ H ₁₀ O ₄	178.0292, 161.0235	✗	✗	✓	[3]
19	Kaempferol-O-hexoside	26.0	447.0926	447.0933	-1.56	C ₂₁ H ₂₀ O ₁₁	285.0408, 255.0263, 227.0370	✗	✓	✗	[3]
20	Dicaffeoylquinic acid	27.0	515.1215	515.1195	-3.88	C ₂₅ H ₂₄ O ₁₂	353.0896, 191.0545, 179.0360	✓	✓	✓	[3]

No.	Proposed identity	Rt (min)	[M-H] ⁻ _{exp.} (m/z)	[M-H] ⁻ _{calcd.} (m/z)	Δ (ppm)	Molecular formula	MS/MS fragments (m/z)	P.a.	C.c.	C.s.	Refs
21	Quercetin di-O-hexoside	27.7	625.1422	625.1410	-1.88	C ₂₇ H ₃₀ O ₁₇	463.0893, 301.0249, 255.0329, 151.0046	×	✓	✓	-
22	Luteolin	31.4	285.0415	285.0405	-3.63	C ₁₅ H ₁₀ O ₆	239.0509, 223.0601, 201.0462, 175.0551, 133.0435	✓	✓	✓	[12]
23	Apigenin	34.4	269.0465	269.0455	-3.53	C ₁₅ H ₁₀ O ₅	229.0647, 151.0047, 117.0324	✓	×	✓	[12]

P.a. *Pimpinella anisum*, C.c. *Carum carvi*, C.s. *Coriandrum sativum*

It is noteworthy that ferulic acid derivatives were identified only in the coriander extract; the presence of these compounds was previously described in coriander fruits by El-Zaiedi *et al.* and Barros *et al.* [3, 7]. C- and O,C-glycosylated flavones (luteolin and apigenin derivatives) were characteristic for anise extract, being identified according to the fragmentation patterns described by Martins *et al.* and Barros *et al.* [3, 10]. O-glycosylated flavonols were also tentatively identified, namely kaempferol derivatives, which were found only in the caraway extract, and quercetin derivatives that were present both in caraway and coriander extracts. O-glycosylated flavones (luteolin derivatives) were identified only in anise and coriander fruits.

The antifungal activity of anise, caraway and coriander extracts is presented in Table III; the MIC values ranged from 1.25 to 5 mg/mL. Anise and caraway evidenced the highest antifungal activity against all tested strains, although less pronounced than the positive control, namely itraconazole. Overall, the extracts were more effective against *T. rubrum* and *M. gypseum* with MIC values of 1.25 mg/mL and showed slight antifungal effects against *T. mentagrophytes*. *C.*

albicans was more susceptible to anise and caraway, meanwhile coriander and anise proved fungicidal activity against *M. canis* (Table III). The antifungal effects of the investigated spices are due to their content in phenolic compounds such as apigenin, luteolin, quercetin, kaempferol and their derivatives, caffeic and chlorogenic acids, which have been previously reported to possess significant antimicrobial properties. Thus, apigenin and its derivative apigenin-7-O-glucoside proved antifungal activity against *C. albicans* strains (MIC values of 0.05 - 0.15 mg/mL) [16]. Alves *et al.* showed the efficacy of luteolin and quercetin against *Candida* strains (MIC = 0.625 - 1.25 mg/mL) and more, that these flavonoids were able to inhibit the fungal biofilm formation [2]. Caffeic acid (MIC 1 mg/mL) displayed antifungal effects against *C. albicans*, activity that was related to inhibition of isocitrate lyase, key-enzyme essential for the virulence of the yeast [5]. More, a derivative of caffeic acid, chlorogenic acid was able to inhibit the fungal growth of *C. albicans* at micromolar concentrations (MIC 80 µg/mL), due to the disruption of cell membrane structure [17].

Table III

Antifungal activity of phenolic extracts from anise, caraway and coriander fruits

Sample/Positive control	MIC (mg/mL)				
	<i>T. rubrum</i> ATCC 28188	<i>T. mentagrophytes</i> ATCC 36107	<i>M. canis</i> CECT 20190	<i>M. gypseum</i> CECT 2098	<i>C. albicans</i> ATCC 90028
<i>Pimpinella anisum</i> L.	1.25	2.5	1.25	1.25	5
<i>Carum carvi</i> L.	1.25	5	1.25	1.25	1.25
<i>Coriandrum sativum</i> L.	1.25	1.25	5	1.25	1.25
Itraconazole	0.125	0.125	0.06	0.125	0.03

Despite the fact that the three species belong to the same family, there were differences in their chemical composition and antifungal activity, thus emphasizing the heterogeneity in the bioactive constituents of plants from the same botanical family. Indeed, these *Apiaceae* plants have different medicinal uses. It is also important to note that culture and pedo-climatic conditions, harvesting time, but also storage and manipulation procedures, significantly affect the

phytochemical composition, and, consequently, their bioactivity. Among them, phenolic compounds act as contributors to the antifungal potential of plant extracts, this being evidenced in the present study: anise and caraway extracts provided the most prominent antifungal effect mainly conferred by their content in phenolic compounds.

Conclusions

The phenolic extracts obtained from anise, caraway and coriander fruits exhibited antifungal activities, thus underlining their putative use as therapeutic agents in onychomycosis. Nevertheless, further studies are necessary to assess the *in vivo* efficacy of the studied plant extracts, as also to deepen knowledge on their mechanism of action.

Acknowledgement

This research was financially supported by “Grigore T. Popa” University of Medicine and Pharmacy Iași, Romania, grant no. 7246/12.04.2018.

Conflict of interest

The authors declare no conflict of interest.

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