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Chemical Composition of Apiaceae Family Essential Oils from Different Plants and in-vitro Antimicrobial Activities on Probiotic Microorganisms

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ABSTRACT

Coriander, fennel, caraway and anise species which have major characteristic specialities of Apiaceae family, have antimicrobial activities on pathogene microorganisms. Probiotic microorganisms have fundamental effects on human body and extermination of probiotics causes many diseases. In this study, it was aimed to determine investigate probiotic resistance against natural antimicrobial agents (as essential oils) compare to pathogenes in previous studies. Analysis of essential oils (Eos) from were analyzed by GC-FID and GC/MS, analysis of Eos antimicrobial and antifungal activity from were analyzed by Microdilution test (as described in CLSI). Linalool (%74.927), (E)-anethole (%68.239), carvone (%61.087) and (E)-anethole (%95.577) were found as major compounds of EOs respectively. All essential oils have antimicrobial activities on probiotic microorganisms.

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Introduction

The Apiaceae Family is one of the largest plant families in the World (as known as Umbelliferae Family in the past). This family includes approximately 450 genera and 3700 species worldwide[1]. The members of this family are well known as fundamental vegetables, culinary fruites and medicinal plants such as Coriandrum sativum L.(coriander), Foeniculum vulgare Mill. (fennel), Carum carvi L. (caraway) and Pimpinella anisum L. (anise) etc. Plants of this family usually possess a characteristic pungent or aromatic smell which is owing to the presence of essential oil or oleoresin [2]. Members of Apiaceae possess various different compounds with many important biological activities. Some of the main properties are ability to induce apoptosis, antibacterial, hepatoprotective, vaso-relaxant, cyclooxygenase inhibitory and antitumor [3]. For the family Apiaceae, Turkey is the one of a major center of diversification. In Turkey, the Apiaceae family is represented by 101 genera and 451 species. Turkey with unique climatic conditions has a large variety of plants, especially some unique endemic plants. From the endemism points of view, Apiaceae is an important family in the flora of Turkey with 11224 taxa [4].

Table 1: Pharmacological Action Table of Widely-Used Apiaceae in Previous Studies

Apiaceae Family Species	Pharmacological action
Coriandrum sativum L.	Digestive Aphrodisiac Anti-ulserative and Anti-rheumatismal Treatment of halitosis Carminative, diuretic, tonic, stomachic, antibilious and refrigerant [5]
Foeniculum vulgare Mill.	Treatment of abdominal pains, antiemetic, aperitif, arthritis, cancer and colic Treatment of conjunctivitis, constipation, depurative, diarrhea, dieresis, emmenagogue, fever, flatulence, gastralgia, gastritis, insomnia, irritable colon, kidney ailments, laxative, leucorrhoea, liver pain, mouth ulcer, and stomachache in children [6]
Carum carvi L.	Hypoglyceamic, Hypolipidemic, Anti-oxidant, Adaptogenic, Nootropic, Antimicrobial, Antifertility, Anticolitic, Molluscicital, Insecticidal, Antiinflammatory, anti-cholinesterase (AChE), Anticarcinogenic, Hepatoprotective, Antiulcerogenic [7]
Pimpinella anisum L.	Carminative, disinfectant, galactagogue Antimicrobial, antifungal, antiviral, antioxidant, muscle relaxant, analgesic, anticonvulsant Hypoglycemic, hypolipidemic [8]

Probiotic Because "Antibiotic Age" was created by Lactobacillus sp. probiotics. As known in the medicinal science probiotics are using in Alzheimer's disease(Lactobacillus acidophilus), Parkinson's Disease(Lactobacillus plantarum, Lactobacillus casei), inflammatory bowel diseases(Example: Chron's Disease and Clostridium difficile diseases) and immunmodulatory system. This microorganisms also more resistant than the other patogen microorganisms and fungal microorganisms [9]. In this experiment series you can see which microorganism is more resistant and use them wisely on your health. Don't worry, be healty with "The Power of Nature."microorganisms are valuable resources fort he all humanity from 17th century. Before Alexander Ian Fleming found antibiotic which is "penicilin" from Penicilium notatum French Sciencist Luis Pasteur had isolated lactic acid from Lactobacillus sp. microorganisms. Not only isolation of lactic acid but also identification of first microorganisms is so important in this period.

Table 2: Antimicrobial Activity of Investigated Apiaceae Essential oils on Pathogene Microorganisms in Previous Studies

Essential oil	Inhibited Pathogene Microorganisms
Coriandrum sativum L.	Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Salmonella typhimurium (clinical isolate), Klebsiella pneumonie (clinical isolate), Proteus mirabilis (clinical isolate) and Candida albicans (clinical isolate) [10]
Foeniculum vulgare Mill.	Acinetobacter calcoacetica NCIB 8250, Aeromonas hydrophila NCTC 8049, Alcaligenes faecalis NCIB 815, Bacillus subtilis NCIB 3610, Baneckea natriegens ATCC 14048, Brevibacterium linens NCIB 8456, Brocotrix thermosphacta (clinical isolate), Citrobacter freundii NCIB 11490, Clostridium perfringens NCIB 10696, Enterobacter aerogenes ICTC 10006, Erwinia carotovora NCPPB 312, Escherichia coli NCIB 8879, Flavobacterium suaevolens NCIB 8992, Klebsiella pneumoniae NCIB 418, Leuconostoc cremoris NCDO 543, Micrococcus luteus NCIB 8165, Moraxella catarrhalis NCIB 10762, Proteus vulgaris NCIB 4175, Pseudomonas aeruginosa NCIB 950, Salmonella pullorum NCTC 10704, Serratia marcescens NCIB 1377, Staphylococcus aureus NCIB 6571, Streptococcus faecalis NCTC 775 and Yersinia enterocolitica NCTC 10460 [11]
Carum carvi L.	Staphylococcus epidermidis CIP 106510, S.aureus ATCC 25923, Moraxella luteus NCIMB 8166, Enterococcus feacalis ATCC 29212, Bacillus cereus ATCC 11778, B.cereus ATCC 14579, E.coli ATCC 35218, Listeria monocytogenes ATCC19115, P.aeruginosa ATCC 27853, S.typhimurium LT2 DT104, V. parahaemolyticus ATCC17802, Vibrio alginolyticus ATCC 33787, V.proteolyticus ATCC15338, V.furnisii ATCC 35016, V.mimicus ATCC 33653, V.natrigens ATCC 14048, V.carhiaccae ATCC 35084, V.fluvialis ATCC 33809, Candida albicans ATCC90028, C.glabrata ATCC 90030, C.parapsilosis ATCC22019 and C. krusei ATCC 6258 [12]
Pimpinella anisum L.	Candida albicans ATCC 90 028, Cryptococcus neoformans ATCC 90 113, Aspergillus fumigatus ATCC 90 906, Staphylococcus aureus ATCC 29 213, methicillin-resistant S. aureus ATCC 43 300 (MRS), Pseudomonas aeruginosa ATCC 27 853, Mycobacterium intracellulare ATCC 23 068, M. smegmatis ATCC 607, M. fortuitum ATCC 19 709, M. phlei 11 758, and M. aurum ATCC 23 366 [13]

Materials and Methods

Plant Material

Pharmacopeae essential oils were used as standarts of Apiaceae plants. EOs were selected from Anadolu University, Faculty of Pharmacy, Pharmacognosy Research Laboratory essential oil collection. Microorganisms were bought from Christian Hansen©.

GC-MS Analysis

The GC-MS analysis was provided with an Agilent 5975 GC-MS system. Innowax FSC column (60 m, 0.25 mm film thickness) was prepared with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60 C° for 10 min and set to 220 C° at a rate of 4 C°/min, and kept constant at 220 C° for 10 min. Then, programmed to 240 C° at a rate of 1 C°/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250 C°. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450.

GC Analysis

The GC analysis was set up using an agilent GC system. FID detector temperature was 300 C° to obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of Components

Characterization of the essential oil components was carried out by comparison of their retention times with those of authentic samples or by comparison of their Linear Retention Indices (LRI) to a series of n-alkanes. Computer matching against commercial Wiley GC/MS library (MacLafferty and Stauffer, 1989), MassFinder 3 Library (Koenig et al., 2004) and in house "Baser Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (Joulain and Koenig, 1998: ESO, 2000) was used for the identification.

Antimicrobial and Antifungal Activities with Microdilution Methods

This technique helps to determine MIC (minimal inhibitory concentration) and MLK (minimal lethal concentration) values of antimicrobial drugs. For this purpose, 2 or 10-fold dilutions of antimicrobial drug in Mueller-Hinton Broth are made and dilutions of dense concentrations of drugs are obtained. Ex. drug 256, 128, 64, 32, 16, 8, starting at 256 32g in 1 ml. 4, 2, 1, 0.5, 0.25, 0.12 /g / mL are gradually diluted in three layers. The isolated test is seeded in 100 µL of the 24-48 hours liquid culture culture of the microorganism and incubated at 37 ° C for 24-48 hours. The reproduction in the tubes is evaluated by the eye. Thus, the final dilution without reproduction is accepted as MIC value. However, in order to be precise, it is appropriate to perform the test in three parallel. The average of the most recent results is the MIC or MLK obtained. Esseintial oil fractions of Apiaceae Family were dissoluted in

%10(v,v) DMSO(Merck©, CAS: 67-68-5) and emulsufied in distilled water. Resazurin sodium (Sigma-Aldrich©, CAS No:62758-13-8) is used as indicator for determination of MIC values. Chloramphenicol (Sigma-Aldrich©, CAS: 57-75-7) was used as positive control as indicated in the Clinical Laboratory Standards Institute guide In this study we calculated MIC values as other MIC studies in the literature [14,15].

Results and Discussion

As shown in Table 3, in total 24 constituents were identified. The main components were linalool (%74.927), (E)-anethole (%68.239), carvone (%61.087) and (E)-anethole (%95.577) were found as major compounds of EOs respectively. α -pinene, fanchone, limonene and β-caryophyllene were the second major component in EOs 5,014%, 12,783%, 36,815% and 2,609% resp. The third major component were limonene, t-caveol and methyl cavicol in EOs 2,146%, 6,546%, 0,394% and 0,828% resp. The contents of these EOs show us (E)-anethole is most widely chemical compound in this study.

Table 3: Chemical Components of Apiaceae Essential Oils

Compound Name (EOs)	Coriandrum sativum L.	Foeniculum vulgare Mill.	Carum carvi L.	Pimpinella anisum L.
α-pinene	5.014	3.562	-	-
β-pinene	0.427	-	-	-
myrcene	0.508	-	0.164	-
limonene	2.146	6.546	36.815	-
t-dihydrocarbon	-	-	0.264	-
carvone	-	-	61.087	-
p-cymene	2.112	1.351	-	-
terpinolene	0.423	-	-	-
t-carveol	-	-	0.394	-
linalool	74.927	0.239	-	-
fanchone	-	12.783		
p-allylanisole	-	2.964	-	-
geranyl acetate	3.507	-	-	-
β-caryophyllene	-	-	-	2.609
(E)-anethole	-	68.239	-	95.577
geraniol	1.518	-	-	-
methyl cavicol	-	-	-	0.828
anisaldehyde	-	2.039	-	-
camphene	0.776	-	-	-
cis-1,2-limonene-epolisite	0.100	-	-	-
camphor	4.552	-	-	-
γ-terpineol	3.558	-	-	-
α-terpineol	0.317	-	-	-
farnesol	0.115	-	-	-
Total %	99.683	97.723	98.724	99.014

Antimicrobial Activity of Apiaceae Essential Oils

Table 4: MIC Table of Apiaceae Family EOs

Microorganism / Essential oil (mg/L)	La-5	La-14	L.reu.	L.rh.	L.fer	B.coa.	B.N.	B.cl.	S.sal.	S.ther.	S.b.	S.c.	BB-12
Coriandrum sativum	>128	>128	>128	>128	>128	>128	0.375	2	>128	4	>128	2	48
Foeniculum vulgare	0.25>	0.25>	0.25>	0.25>	0.25>	0.25>	4	0.25	>128	0.25>	>128	1	64
Carum carvi	>128	>128	>128	128	96	>128	>128	0.25>	0.25>	>128	0.25>	>128	>128
Pimpinella anisum	>128	0.25>	>128	16	>128	2	64	0.25>	>128	>128	>128	>128	96
Ketoconazole	8	8	8	0,74	24	16	12	0.25	6	0.25>	-	-	0.25
Chloramphenicol	-	-	-	-	-	-	-	-	-	-	4	4	-

La-5: Lactobacillus acidophilus La-5, La-14: Lactobacillus acidophilus La-14, L.fer.: Lactobacillus fermentum CECT- 5716 L.reu.: Lactobacillus reuteri DSM 17938, L.rh.: Lactobacillus rhamnosus GG, B.coa.: Bacillus coagulans SNZ 1969, B.cl.: Bacillus subtilis var. clausii ATCC9799, B.N.: Bacillus subtilis var. natto BN, S.sal.: Streptococcus salivarius K12, S.ther.: Streptococcus thermophilus

TH-4, S.b.: Saccharomyces cerevisae var. boulardii ATCC – MYA976, S.c.: Saccharomyces cerevisae ATCC – MYA9763, BB-12: Bifidobacterium bifidum BB-12)

For these results, Foeniculum vulgare essential oil ismost effective essential oil against probiotic microorganisms. If it is used on gastrointestinal microflora directly, it can inhibit many microorganisms and cause many gastroinstestinal problems. All of the EOs in this study were shown antimicrobial activity against Bacillus clausii. The antimicrobial study of Apiaceae Family EOs indicated that the natural isolated probiotic microorganisms more resistant than soil isolated microorganisms. When compared all datas about this study probiotic microorganisms generally resistant against Apiaceae EOs. As described in the pathogene microorganisms table section, many microorganisms inhibited with Apiaceae family EOs but probiotic microorganisms are generally resistant on related EOs. This is important to protecting human body against bacterial and fungal infections with symbiotic microorganisms and their fundamental seconder metabolites. This study shows us abilities of probiotic microorganisms to protect human body when natural antimicrobial compounds are taken. In the other hand, probiotic microorganisms can use with antimicrobial agents in the same drug formulations to solve resistant pathogene superinfectious agents in the future.

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