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Research Article

Fatty acid composition and antimicrobial activity of *Asphodelus aestivus* seeds

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Abstract:
Objective: In this study, the fatty-acid profiles and antimicrobial activity of *Asphodelus aestivus* seeds were examined. Material and Methods: The fatty acid composition of *Asphodelus aestivus* seeds was investigated by gas chromatography (GS). The antimicrobial activity of the isolated oil of the seeds was assessed against Gram (+) and Gram (-) bacteria and three yeast strains by disc diffusion and broth microdilution tests. Results: Butyric (76.26%) and nervoic acid (3.65%) were identified as major components by GC-FID and GC/MS analysis. Saturated fatty acids (76.84%) were found in higher amounts than unsaturated fatty acids (4.97%). The oil showed moderate antibacterial activity against *Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 29998, *Klebsiella pnemoniae* ATCC 13883, and *Pseudomonas aeruginosa* ATCC 27853, and also showed antifungal activity against *Candida albicans* ATCC 10239 and *C. krusei* ATCC 6258. Conclusion: The present study confirms the seeds of *Asphodelus aestivus* contain high percentage of butyric acid and has potential antibacterial and antifungal application.

Key Words: *Asphodelus aestivus*; fatty acid composition; antimicrobial activity

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Introduction

Asphodellus aestivus Brot. is one of the *Asphodelus* L species in Turkey. The genus *Asphodelus* is locally known as "çiriş otu and yalancı çiriş" and represented by three species in the flora of Turkey (Matthews 1984; Baytop 1999). *Asphodellus aestivus* Brot. have been used as food material, and in traditional medicine due to their diuretic, wound healing, antihemoroidal, menstruation facilitative activities and curative properties on alopecia, eczema and abscess (Baytop 1999; Ugurlu et al 2009). Recent studies reported gastroprotective (Gurbuz et al 2002), antifungal (Peksel et al 2002), antioxidant (Peksel et al 2002; Aslanturk & Celik 2013), cytotoxic and apoptopic (Aslanturk & Celik 2013) activities of various extracts of *A. aestivus*. Relatedly, many researches showed that *Asphodelus* species contain a variety of valuable chemical compounds such as anthranoids, flavonoids, steroids, triterpens and arylcoumarins, anthraquinones and glycosides (Adinolfi et al 1989; Adinolfi et al 1991; Rizk et al 1972; Van Wyk at al 1995; Calis et al 2005; El-Seedi 2007). Besides valuable constituents and beneficial effects of *A. aestivus*, some veterinarian researchers declared a severe neurologic syndrome which had been occured in sheep after grazing *A. aestivus* seeds in Turkey. The disease was characterized by some neurological symptoms, such as tremors, paresis, ataxia, oral and nasal discharge and severe respiratory distress (Birincioglu et al 2005; Birincioglu et al 2012). Since ancient times, extracted oils from various plants have been consumed as food or food ingredients, massage oils, additive substance in skin care products and some other cosmetics. Natural fats and dietary oils widely contain fatty acids that include nutritious and biologically active components and metabolites (Kabara et al 1977; Kabara 1979).

The aim of this study was to elucidate fatty acid composition and to evaluate antimicrobial activity of the oil obtained from the seeds of *A. aestivus*.

Materials and methods

Plant material: *Asphodelus aestivus* Brot. (*Liliaceae*) was collected from Aydın by S. Serap Birincioğlu, Turkey in August 2013. The plant was identified by Bijen Kıvçak and a voucher specimen (No.1520) was deposited in Herbarium of the Ege University, Faculty of Pharmacy, Department of Pharmacognosy.

Oil extraction and fatty acid methyl esters (FAMEs) preparation
The oil extraction of dried and powdered seeds (40 g) was carried out at 60ºC for 6 h by Soxhlet extractor using petroleum ether as a solvent. Then, the solvent was evaporated by a rotary evaporator. The extracted oil was esterified to determine the fatty acid composition. The fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5N

methanolic NaOH and transesterified with 14% BF3 (v/v) in methanol (IUPAC, 1979).

Fatty acid analysis by GC

Fatty acid methyl esters (FAMEs) were analyzed on a HP (Hewlett Packard) Agilent 6890 N model gas chromatograph (GC), equipped with a flame ionization detector (FID) and fitted to a Supelco SP-2380 Fased Silica capillary column (60 m, 0.25 mm i.d. and 0.2 µm). Injector and detector temperatures were set at 250ºC and 260ºC, respectively. The oven was programmed at an initial temperature of 140ºC and an initial time of 5 min. Thereafter the temperature was increased up to 240°C at a rate of 3°C/min^{-1}. The total run time was 41.33 min. Helium was used as the carrier gas (1 ml min^{-1}). Identification of fatty acids was carried out by comparing sample FAME peak relative retention times. The results were expressed as FID response area in the relative percentages. Each reported result was given as the average value of three GC analyses. The results are offered as means±S.D.

Antimicrobial activity

Antimicrobial activities of the sample were tested by Kirby Bauer disc diffusion and broth microdilution tests according to the recommendations of Clinical and Laboratory Standards Institute (CLSI M44-A, 2004; CLSI M27-A3, 2008; CLSI M02-A10, 2009; CLSI M07-A8 2009) against nine bacteria strains (*Bacillus cereus* ATCC 7064, S*taphylococcus aureus* ATCC 6538-p, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 29998, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* CCM 5445, and *Salmonella enterica* ATCC 13311) and three yeast strains (*Candida albicans* ATCC 10239, *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019).

Disc diffusion assay

Freshly cultured test organisms were adjusted to a 0.5 Mc Farland standard turbidity, and then the bacteria and yeast strains were inoculated on Mueller Hinton (MH) agar and Sabouraud Dextrose (SD) agar plates, respectively. The solution of the oil (4mg/ml) was prepared in 20% dimethyl sulphoxide (DMSO), and it was embedded onto 6 mm sterile paper discs (BD BBL Susceptibility Blank Paper Discs) to obtain 120 µg oil/disc concentration. The impregnated discs were placed onto inoculated agar plates and incubated at 35°C for 24-48 h. At the end of the incubation time, the diameter of the zone sides of the paper discs were recorded in millimeters. All tests were performed under sterile conditions in triplicate. Ampicillin (Oxoid, 10 µg/disc), Ciprofloxacin (5 µg/disc) and Fluconazole discs (Oxoid, 25 µg/ml) were used as positive controls.

Microdilution method

The suspensions of the test organisms were adjusted to a 0.5 McFarland standart; afterwards they were diluted 100 fold (v/v) in MH broth and SD broth, respectively. Dilution series of the oil were prepared from 2048 to16 µg/ml by using MH broth and SD broth medium in 96-well microtiter plates. Final concentrations in the medium were 1024 to 8 µg/ml, when the suspensions of the microorganisms were added to the plates. Microtiter plates were incubated at 35°C for 24-48 h. All the tests were performed under sterile conditions in triplicate. The "minimum inhibitory concentration" (MIC) was defined as the lowest concentration of an antimicrobial test that inhibited the visible growth of a microorganism after incubation.

Ampicillin (Sigma Aldrich Chemical Co. St Louis, USA) and Ciprofloxacin (Sigma Aldrich Chemical Co. St Louis, USA) were used as standard antibacterial agents, whereas Fluconazole (Sigma Aldrich Chemical Co. St Louis, USA) was used as a standard antifungal agent. Their dilutions were prepared from 128 to 0.015 µg/ml concentrations in microtiter plates.

Data analysis

Three analytical replicates were carried out on each sample. The results of antimicrobial activity were analyzed and measured using one-way ANOVA. Measurements were averaged, and results are given as mean ± standard deviation, calculated by analysis of variance using the Microsoft excel.

Table 1. Fatty acid composition of *A. aestivus* (%)

Fatty acids	Content (%)
C 4:0	76.26±0.00[a]
C 6:0	0.33±0.02
C 8:0	0.09±0.01
C 10:0	0.01±0.01
C 16:0	0.05±0.02
C 18:0	0.06±0.03
C 21:0	0.03±0.01
C 24:0	0.01±0.01
∑SFA[b]	76.84
C 14:1	0.06±0.02
C 15:1	0.05±0.01
C 18:1n9t	0.63±0.02
C 20:1	0.04±0.03
C 24:1c	3.65±0.02
∑MUFA[b]	Apr-43
C 18:2	0.17±0.02
C 18:2n6t	0.03±0.05
C 18:2n6c	0.07±0.02
C 20:2n6	0.03±0.01
C 20:3n3	0.03±0.02
C 22:6n3	0.21±0.01
∑n-3	0.24
∑n-6	0.13
n-3/n-6	1.85
∑PUFA[b]	0.54
Unknown	18.19

[a]Average of three lots analysed.

[b] Values reported are means ±SD.

SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids.

Results

The results of fatty acid composition of *Asphodelus aestivus* seeds from Turkey were shown in Table 1.

Unsaturated fatty acids (UFAs) amounted to 4.97% of the total fatty acids (TFAs), while the saturated fatty acids (SFAs) were 76.84%. Polyunsaturated fatty acids (PUFAs) of *Asphodelus aestivus* amounted 0.54% of the TFAs, while the monounsaturated fatty acids (MUFAs) was 4.43% and SFAs was about 76.26%. A significant amount of SFAs was found in the total lipid extract of *A. aestivus* seeds, whereby butyric acid (C 4:0, 76.26%) was the major component. The fat tested as significant source of SFAs. The other major component present in SFAs was nervonic acid (C 24:1c, 3.65%).

Table 2. Antimicrobial activity test results of the fatty acid extract of *A. aestivus*

Microorganisms	Zone diameter (mm±SD)		MIC (µg/mL±SD)	
Gram (+) Bacteria	FA (120 µg/D)	Amp (10 µg/D)	FA	Amp
***Bacillus cereus* ATCC 7064**	-	17.7±0.6	-	9.3±6.1
***Staphylococcus aureus* ATCC 6538-p**	8.3±0.6	38.0±1.0	512.0±0.0	1.3±0.6
***S. epidermidis* ATCC 12228**	-	26.0±1.0	-	1.7±0.6
Streptococcus faecalis ATCC 29212	7.7±0.6	22.7±0.6	512.0±0.0	2.0±0
Gram (-) Bacteria	FA (120 µg/D)	Cip (5 µg/D)	FA	Cip
***Escherichia coli* ATCC 29998**	6.7±0.6	37.3±0.6	-	<0.015±0
***Klebsiella pneumoniae* ATCC 13883**	9.3±0.6	32.7±0.6	512.0±0.0	0.015±0
***Pseudomonas aeruginosa* ATCC 27853**	7.0±0.0	34.3±0.6	-	0.100±0
***Salmonella typhimurium* CCM 5445**	-	36±0.0	-	0.015±0
S. enterica ATCC 13311	-	39.3±0.6	-	<0.015±0
Yeast strains	FA (120 µg/D)	Flu (25 µg/D)	FA	Flu
***Candida albicans* ATCC 10239**	7.7±0.6	22.0±1.0	512.0±0.0	0.2±0.1
***C. krusei* ATCC 6258**	6.7±0.3	24.3±0.6	-	1.7±0.6
***C. parapsilozis* ATCC 22019**	-	23.0±0.0	-	3.3±1.2

D: disc, SD: Standard deviation, FA: fatty acid, Amp: Ampicillin, Cip: Ciprofloxacin, Flu: Fluconazole, MIC: Minimum inhibitory concentration.

The antimicrobial activities of *A. aestivus* fatty acid extract against microorganisms examined in the present study and its potency was qualitatively and quantitavely assessed by measuring the inhibition zones and determining the MIC values (Table 2).

Discussion

In a previous study on the fatty acids of *Asphodelus tenuifolius,* it was established that lauric (C 12:0), palmitic (C 16:0), α-linoleic (C 18:3n3), dihomo-γ-linoleic (C 20:3n6), and tricosylic (C 23:0) acids were the main components of the SFAs (Freije et al 2013). Fell et al. (1968) also isolated myristic (C 14:0), palmitic (C 16:0), stearic (C 18:0), oleic and linoleic acids (C 20:3n6) from the seeds of *A. microcarpus Viviai* and *A. fistulosus* L. from Egypt. Moreover some of the fatty acid contents of the seeds oil of *A. fistulosus* L. from Pakistan was identified with linoleic (54.9%), oleic (33.1%), palmitic (5.7%) and stearic (3.7%) as major constituents (Khan et al 1961). The chemical composition of the total methyl esters of fatty acids from the extracts of *A. tenuifolius*, *A. microcarpus*, and *A. fistulosus* have very similar profile. Whereas, in this study, butyric acid was found as the major component of the SFA in *A. aestivum*. According to our finding, the *A. aestivum* oil may be a good source of butyric acid. In previous studies, neither butyric nor nervoic acid were found (Freije et al 2013; Fell et al 1968; Khan et al 1961).

The antimicrobial activity of short-chain fatty acids against pathogenic bacteria seems depend on the type of fatty acid, form, pH, exposure time, degree of sensitivity of specific types of pathogens, and quantitiy used (Fukushi et al 2003; Zgoda & Porter 2001). In this study, n-butyric acid (76.26%) was found a major component in the total lipid extract of *A. aestivus* seeds. Butyric acid, a short-chain fatty acid; has been shown to be effective on single strains of *Salmonella typhimirium* from chickens and *Pseudomanas aeruginosa* from a patient (Abdul & Lloyd 1985; Levison 1973). In contrast, in our study the fatty acid extract including n-butyric acid (76.26%) showed no activity against *S. typhimirium* CCM 5445 and exhibited little or no antibacterial activity against *P. aeruginosa* ATCC 27853 by the disc diffusion and microdilution methods probably due to the specific types of pathogen. Short-chain organic acids have a specific antimicrobial activity that is pH dependent (Knarreborg et al 2002). Butyric acid is strongly lipofilic and can diffuse across the membranes of bacteria (especially Gram-negative). Butyric acid is found in colon as well as other parts of the digestive tract. SCFA found in the fecal material can inhibit the growth of *Salmonella* (Baskett & Hentges 1973). Butyric acid has the ability to induce HIV reactivation (Kurita-Ochiai et al 2008). The low molecular weight esters of butyric acid are used as food and perfume additives as they have pleasent aromas and tastes. Butyric acid and its esters forms are also used as animal feed supplements, due to the ability to reduce pathogenic bacterial colonization (Van Immerseel at al 2005).

As reported that in Table 2, when compared with the standard antimicrobials, fatty acid extract of *A. aestivus* exhibited low antimicrobial activity. However, it showed significant effect against *S. aureus* ATCC 6538-p, *S. faecalis* ATCC 29212, *E. coli* ATCC 29998, *K. pneumoniae* ATCC 13883 and *P. aeruginosa* ATCC 27853 and moderate activity against *C. albicans* ATCC 10239 and *C. krusei* ATCC 6258 when compared with each other.

There are some reports regarding the antimicrobial activity of different extracts *A. aestivus* from Manisa and Kahramanmaraş, Turkey (Oskay et al 2007; Ilcim & Digrak 1998). In a previous study by Oskay et al (2007), the ethanol and n-butanol extracts of the aerial parts of *A. aestivus* exerted high antimicrobial activity against *S. aureus, Pseudomonas fluorescens, E. coli, Yarrawia lipolytica* and slight activity against *K. pneumonia*, *S. typhimirium, Proteus vulgaris* and the fungi *Saccharomyces cerevisiae*. Ilcim & Digrak (1998) showed that the chloroform extract of *A. aestivus* leaves did not inhibit the growth of the bacteria *Bacillus megaterium, B. subtilis, B. brevis, E. coli, K. pneumonia, Enterobacter aerogenes, P. aeruginosa, S. aureus, Listeria monocytogenes* and the fungi *Candida albicans* and *S.cerevisiae.*

Conclusion

The present results showed moderate antimicrobial activity against Gram (+), Gram (-) bacteria and the yeast strain when compared the commercial antimicrobials. The fat of *A. aestivus* seeds could be considered as significant source of SFAs. In this study, the antimicrobial activity and fatty acid analysis of *A. aestivus* seeds has been carried out for the first time. Thus our study confirms that, *A. aestivus* seeds contain higher percentage of the above mentioned fatty acids such as butyric acid that has potential antibacterial and antifungal principle for clinical application.

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