Araştırma Makalesi – Original Article

GC/MS Analysis of Essential oil distilled from *Arum rupicola* Boiss. var. *rupicola* (Araceae)*

Arum rupicola Boiss. var. *rupicola* (Araceae)'dan distile edilen uçucu yağın GK/KS analizi

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ABSTRACT

Aim: *Arum rupicola* Boiss. var. *rupicola* with a vernacular name of Dağ Sorsalı, are used etnobotanically for hemorroid, eczema, rheumatism, and cancer. Essential oil combination of *A. rupicola* var. *rupicola*, under the lower risk (lc) threatened category because of the excess gathering was investigated.

Methods: The above ground samples of *Arum rupicola* Boiss. var. *rupicola* was collected from Palamut province of Antalya (Kaş) and essential oil was taken by hydrodistillation method with Clevenger apparatus. The essential oil composition was analysed by Gas chromatography (GC) and Gas chromatography/Mass Spectrometry (GC/MS) systems simultaneously.

Results: As a result of, (E)-geranyl acetone (15%) was the main compound while hexahydrofarnesyl acetone (9.2%), nonanal (6.8%), heptacosan (6.5%), farnesyl acetone (5.0%), tricosan (4.9%), ve 2-dodecanone (3.8%) followed.

Conclusion: Farnesyl acetone (5%) and hexahydrofarnesyl acetone (9.2%) sesquiterpenes were new records for *A. rupicola* var. *rupicola* volatile oil content. The only species is *Arum creticum* consisting α-farnesene (95%) as its taxonomical feature. *A. rupicola* var. *rupicola* has farnesyl acetone and hexahydrofarnesyl aceton being known a flagy and odourless species.

Keywords: *A. rupicola* var. *rupicola*, Araceae, Essential oil, (E)-geranyl aseton, GC/MS

ÖZET

Amaç: *Arum rupicola* Boiss. var. *rupicola* yöresel adıyla Dağ Sorsalının, halk ilacı olarak hemorroit, egzema, romatizma, ve özellikle kanser hastalıklarında kullanıldığı bilinmektedir. Bu nedenle aşırı toplanma sonucunda tehdit altındaki türler kategorisine dahil edilen bitkinin toprak üstü kısımlarının uçucu yağ bileşiminin incelenmesi amaçlanmıştır.

Yöntem: *Arum rupicola* Boiss. var. *rupicola*'nın topraküstü kısımları Antalya (Kaş)'nın, Palamut köyünden toplanmış ve uçucu yağı Clevenger apareyi kullanılarak su distilasyonu yöntemi ile elde edilmiştir. Elde edilen uçucu yağın bileşimi eş zamanlı olarak gaz Kromatografisi (GK) ve gaz kromatografisi/ kütle spektrometresi (GK/KS) sistemleri ile tayin edildi.

Bulgular: Ana bileşik (E)-geranil aseton (%15) olurken, hekzahidrofarnesil aseton (%9.2), nonanal (%6.8), heptakosan (%6.5), farnesil aseton (%5.0), trikosan (%4.9), ve 2-dodekanon (%3.8) diğer önemli bileşikler olarak tespit edilmiştir.

Sonuç: *A. rupicola* var. *rupicola*' nın uçucu yağ içeriğinde farnesil aseton (%5) ve hekzahidrofarnesil aseton (%9.2) varlığı yeni bir kayıttır. Taksonomik özellik olarak α-farnesen (%95) içeren tek tür *Arum creticum* dur. Bayraklı ve kokusuz bir tür olarak bilinen *A. rupicola* var. *rupicola*'da da farnesil aseton ve hekzahidrofarnesil aseton bulunmaktadır.

Anahtar Kelimeler: *Arum rupicola* var. *rupicola*, Araceae, Uçucu bileşikler, (E)-geranil aseton, GK/KS

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Introduction

Araceae Juss. is a monocotyledonous family represented by 117 genera and approximately 4000 species worldwide.[1-6] A specific character of all aroids is the spadix inflorescence structure, and lacking bracteoles on it. In most cases, the spadix is attached by the spathe. Araceae are pollinated mostly by insects and, lestly, by bees.[7] Natural habitat of the genus *Arum* L. is the basin of Mediterranean and the Central and North Europe, Middle East, and Central Asia.[4,8-10]

The genus *Arum* is wide spread in almost all over the Türkiye, mainly Mediterranean and Black Sea, and is defined by 14 species, and subspecies and total 17 taxa in previous studies.[8,11] By the way, the latest *Arum* revision within the Illustrated Flora of Türkiye, the genus *Arum* is represented by 12 species in Türkiye.[12,13] These are; *Arum sintenisii* (Engl.) P.C. Boyce, *A. concinnatum* Schott., *A. maculatum* L., *A. italicum* Mill., *A. euxinum* R.R. Mill., *A. hygrophilum* Boiss., *A. creticum* Boiss. & Heldr., *A. rupicola* Boiss., *A. cylindraceum* Gasp., *A. orientale* M. Bieb, *A. dioscoridis* Sm., *A. megobrebi* Lobin, M. Neumann, Bogner & P.C. Boyce'dir.[13] In recent years, a new *Arum* species was described from Osmaniye province in South Anatolia / Türkiye.[14] After adding this species, the number of *Arum* species rose to 13 in Türkiye.[13,14] Of these species, only *A. euxinum* R.R. Mill. and recently identified *A. meremianum* Yıldırım are endemic to Türkiye.

Arum rupicola is a widely distributed taxon in Greece, Belarus, Syria, Iraq, Iran, Cyprus, Lebanon, and Israel; especially the sizes of spathe and spadix structures are quite variable.[4] *A. rupicola* has two varietes as *A. rupicola* Boiss. var. *rupicola* and *A. rupicola* var. *virescens* (Stapf) P.C.Boyce.[4,13] *A. rupicola* Boiss. var. *rupicola* is under the lower risk (lc) threatened category according to the 1994 IUCN categories.

Arum rupicola Boiss. var. *rupicola* belongs to *Arum* subsect. Tenuifila with scentless and flag named inflorescence type and pistillode and staminode bases approximately smooth, from pale green to cream colour; conic-cylindric and sometimes massively spadix appendix and their tubers are discoid.[14] Peduncle longer than the petioles. According to field studies peduncle length is the descriptive feature of pollinator group. Although floral odors are attracting pollinators in *Arum* species: *A. dioscorides*, *A. maculatum*, *A. creticum*, *A. palaestinum*, *A. higrophilum*; some species of *Arum*: *A. rupicola*, *A. korolkowii*, and *A. jacquemontii* have not any odor. Biting midges (Culicoides sp.) which mimic the midge pheromone are the pollinators of them.[6] Bicyclogermacrene occurred 95% of species, but the chamber odor of *A. creticum* occurs in a similar ratio of α-farnesene which has a taxonomic or biological significance.[15,16]

Although there were not any article about the volatiles of *Arum rupicola* var. *rupicola* in the literature survey, 36 compounds like butanoïc acid esters, 1-decene, terpenes, p-cresol, methyl salicylate, indole, and 2-heptanone were identified from different *Arum* species.[16] *A. rupicola* Boiss. var. *rupicola* with a vernacular name of Dağ Sorsalı, are etnobotanically common for treatment of hemorroid, eczema, rheumatism, and cancer.[17]

The air dried leaves of *A. rupicola* var. *rupicola* collected from Van distinct and its isolated fatty acids: Palmitic acid, oleic acid, phytol acetate, stearic acid, α-glyceryl linolenate, behenic alcohol were shown as an activator of the glutathione reductase a flavoenzyme activity.[18] Also, antimicrobial and antioxidant bioactivities of the plant were proved from the phenolic compounds and fatty acid composition.[19]

Anticarcinogenic activities of distilled water extracts of *A. rupicola* var. *rupicola* tubers were reported to have cytotoxic activity.[20]

In this study, hydrodistilled essential oil ingredients of this species spreaded Antalya was investigated by the GC and GC combination of mass spectrophotometry.

Materials and Method

Aerial parts of the *A. rupicola* var. *rupicola* were collected from the Antalya Kaş Palamut basin of the Rahat plateau roadside at an altitude of 1950 m in June 2016 by Prof. Dr. Hasan Yıldırım. The aerial parts were dried on air and powdered. Herbarium voucher number is EGE43219.

Isolation of essential oil

The above-ground samples of the plant were getting to a drug by drying at room temperature. The essential oil was extracted with hydrodistillation technique during 3 hours by Clevenger apparatus. After the extracted oil were filtered over anhydrous sodium sulfate, kept in refrigerator +4˚C.

GC and GC/MS Analysis

Essential oil analysis was performed according to the method used in a previous study.[21]

Table 1. The Composition of the Essential Oil of *Arum rupicola* var. *rupicola*.

RRI	Compound	%	IM
1244	2-Pentylfuran	0.6	Ms
1400	Nonanal	6.8	Ms
1553	Linalool	tr	t_R, Ms
1562	Octanol	0.8	t_R, Ms
1600	Hexadecane	0.3	t_R, Ms
1604	2-Undecanone	0.1	Ms
1638	*β*-Cyclocitral	0.9	Ms
1700	Heptadecane	0.3	t_R, Ms
1715	2-Dodecanone	3.8	Ms
1766	1-Decanol	0.3	Ms
1800	Octadecane	0.1	t_R, Ms
1815	2-Tridecanone	1.1	Ms
1868	(*E*)-Geranyl acetone	15.0	Ms
1900	Nonadecane	0.3	t_R, Ms
1973	1-Dodecanol	1.6	Ms
2100	Heneicosane	1.6	t_R, Ms
2131	Hexahydrofarnesyl acetone	9.2	t_R, Ms
2179	1-Tetradecanol	1.4	Ms
2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	1.1	Ms
2200	Docosane	0.8	t_R, Ms
2300	Tricosane	4.9	t_R, Ms
2384	Farnesyl acetone	5.0	Ms
2400	Tetracosane	0.7	t_R, Ms
2512	Benzophenone	1.1	t_R, Ms
2600	Hexacosane	0.2	t_R, Ms
2607	1-Octadecanol	0.6	Ms
2622	Phytol	0.5	Ms
2670	Tetradecanoic acid	tr	t_R, Ms
2700	Heptacosane	6.5	t_R, Ms
2900	Nonacosane	1.5	t_R, Ms
2931	Hexadecanoic acid	1.5	t_R, Ms
	Oxygenated Monoterpenes	0.9	
	Oxygenated Sesquiterpenes	0.9	
	Fatty acid+esters	1.5	
	Diterpenes	0.5	
	Alkane+Alkanes	22.2	
	Others	43.5	
	Total	69.5	
	%Yield	0.05	

RRI: Relative retention indices calculated against *n*-alkanes,
%: calculated from Flama Ionization Dedector tr: Trace (< 0.1 %)
IM: Identification method based on the retention indices (tR) of authentic compounds on the HP Innowax column; Ms, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

Results

The light yellow essential oil was taken by hydrodistillation of aerial parts of *A. rupicola* var. *rupicola* yielded 0.05%. 32 compounds were isolated, representing about 69.5%, and their relative percentages and linear retention indices (***Table 1***). The oil of *A. rupicola* var. *rupicola* comprised oxygenated monoterpenes, sesquiterpenes, and other hydrocarbons. Also -cosanes are present with carbon numbers of 20-29. Tetradecanoic (myristic) acid in trace amounts. (E)-Geranyl acetone (15%), hexahydrofarnesyl acetone (9.2%), nonanal (6.8%), heptacosane (6.5%), farnesyl acetone (5%), tricosane (4.9%), and 2-dodecanone (3.8%) are the ingredients.

Discussion

The chemical combination of essential oils of leaves of *Arum maculatum* (Kardeh) collected from Iran (Shiraz) was also obtained by hydrodistillation method.[22] While palmitic acid (23.31%), phytol (13.02 %), methyl 9,12,15-octadecatrienoate (10.34 %), and methyl linolenate (8.64 %) were the major compound of *A. maculatum*; nonanal (1.21%), geranyl acetone (0.52%), hexahydrofarnesyl acetone (5.6%), phytol (13.02%) and palmitic acid (23,31%) presence was similar with *A. rupicola* var. *rupicola* volatile oil components with the different ratios of each of them (***Table 1***). Researchers also detected antioxidant and antimicrobial effects of *A. maculatum* oxygenated terpenes as a source of protecting lipids in foods.

The leaves of *A. canophalloides* Kotschy ex Schott (syn. *Arum rupicola* Boiss., *A. virescens* Stapf.) naturally grown in Iran essential oil composition was reported as mainly T-muurolol (25.4%), Ionone (12.6%), nonanal (17.7%), T-cadinol 8.9(%), phytol (7.9%), and methyl palmitate (7.0%).[23] The composition of essential oil components has been shown some similarities with the presence of 2-pentyl furan (1.0%), nonanal (17.7%), geranyl acetone (1.0%), β-cyclocitral (2.9%), and methyl palmitate (7.0%) in *A. canophalloides* leaves hydrodistilled 4 hours with a Clevenger type apparatus, but the ratios of components were different in *A. rupicola* var. *rupicola* (***Table 1***).

The chemical profile of *A. dioscoridis* spreading naturally in Mersin, was determined by GC/MS after Soxhlet extraction with methanol for 4 hours. α-Tocopherol (99%), linolenic acid (99%), linoleic acid ethyl ester (99%), elaol (95%), p-vinylguaiacol (95%), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (94%), neophytadiene (91%), 1-docosanol (91%), palmitic acid (86%), and stearyl alcohol (1-octadecanol) (58%) were the main constituents of volatile oil.[24] 1-Docosanol (91%), palmitic acid (86%), and stearyl alcohol (1-octadecanol) (58%) were seen as similar compounds with different ratios of *A. rupicola* var. *rupicola* volatiles of aerial parts.

Linalool (1.85%), methyl palmitate (0.31%), tricosane (0.34%), heptacosane (0.73%), docosane (2.06%), pentacosane (1.61%), nonacosane (1.61%) were similar volatile constituents of flowers of compounds of *A. cyreniacum* from Libya.[25] But the main ingredient was 1,8-cineole as a major compound (43.32%), the second class is oxygenated sesquiterpenes (18.54%), in which spathulenol is the highest one (12.12%) followed by the esters group where it constitute about 11.86% with the principle compound viz. linalyl acetate.

It would be interesting that the presence of farnesyl acetone (5%) and hexahydrofarnesyl acetone (9.2%) sesquiterpenes in *A.rupicola* var. *rupicola* volatile oil content. Because the only species is *A. creticum* consisting α-farnesene (95%) as its chamber odours as its taxonomical feature, it can be a distinguishable character for *A. rupicola* var. *rupicola* being a flagy and odourless species between the other *Arum* species like *Arum creticum*.[16] Other essential oils in *A. rupicola* Boiss. var. *rupicola* were mentioned as p-cresol, as benzoids; unidentified sesquiterpenoids, methyl butyrate, and 6-methyl heptan-2-one as fatty acids;[16] by headspace and GC-MS.

In our research results, 2-pentylfuran (amylfuran) found in *A. rupicola* var. *rupicola* essential oil composition is an odor described like as green, earthy beany with vegetable like nuances,[26] also hexanoic acid (palmitic acid) with a sweat, pungent, cheesy, goat-like, rancid smell. Nonanal is an aldehyde also present (17.7%) in our essential oil with fatty, citrus, green, gravy, fruity, floral, waxy, sweet, and lavender odour. Nonanal (12%) and decanal (33%) were also primarily found in *A. cylindraceum*, a Cretan *Arum lilies* growing on the island of Crete, producing a very weak odour (to a human nose).[27] Emitting a urine-like smell is a very known characteristics of the flowers of *Arum* species to prevent themselves from many insects (flies and beetles) to oviposit as being their brood-site-mimicking pollination system. Because the insect communication are based on chemical signals, the ability have evolved by other organisms by emitting a dummy floral scent so equal chemically to sexual pheromones of insects to copulate.[28-30]

The family Araceae has aworldwide distribution from Arctic-Alpine to xerophytic and also in the tropical regions, as an early Crataeceous family.[31] The exciting feature of Araceae is thermogenesis emitting volatile compounds to attract pollinators.[32] The pollinators have been attracted not by the faeces/urine odour but by the stigma exudes they fed on.[33] It is very important taxonomical classifications.

Farnesyl acetone (5%) and hexahydrofarnesyl acetone (9.2%) was a newly found sesquiterpenes in *A. rupicola* var. *rupicola* volatile oil. Because the only species is *A. creticum* consisting α-farnesene (95%) as its chamber odours as its taxonomical feature, it can be a distinguishable character for *A. rupicola* var. *rupicola* being a flagy and odourless species between the other *Arum* species like *Arum creticum*.[16] Other essential oils in *A. rupicola* Boiss. var. *rupicola* were mentioned as p-cresol, as benzoids; unidentified sesquiterpenoids, methyl butyrate, and 6-methyl heptan-2-one as fatty acids;[16] by headspace and GC-MS.

Conclusion

Farnesyl acetone (5%) and hexahydrofarnesyl acetone (9.2%) sesquiterpenes were new records for *A. rupicola* var. *rupicola* volatile oil content. Because the only species is *Arum creticum* consisting α-farnesene (95%) as its chamber odours as its taxonomical feature, it can be a distinguishable character for *A. rupicola* var. *rupicola* being a flagy and odourless species between the other *Arum* species like *A. creticum*.

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There is no conflict of interest in the study.

Author Contributions

Hatice Demiray: Idea, analysis and comment, source search, article writing

Betül Demirci: Design, data collection and processing, analysis and comment, critisizm

Hasan Yıldırım: Collection and identification of plant material, comment, article writing

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