

Volatile Components of Some Selected Turkish Liverworts

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The distribution of liverworts and hornworts in Turkey has been described. The volatile components of the ether extracts of some selected Turkish, Spanish and French liverwort species, *Corsinia coriandrina*, *Lejeunea lamacerina*, *Lunularia cruciata*, *Marchantia polymorpha*, *Metzgeria furcata*, *Pellia neesiana*, *Porella cordaana*, *Porella platyphylla* and *Scapania undulata* were analysed by GC-MS. *C. coriandrina* produces isothiocyanates and *S,S*-dimethyliminodithiocarbonates similar to those isolated from the marine hydroid, *Tridentata marginata*. The species also produces the characteristic 2-arylbenzofurans. *L. lamacerina* is closely related chemically to the Japanese *Lejeunea aquatica*, *L. flava* and *L. japonica* since it elaborates the same cuparene sesquiterpenoids as those found in the latter three species.

Keywords: Turkish liverworts, Volatile components, *Corsinia coriandrina*, Isothiocyanates, Iminodithiocarbonates, Stilbenes, Bibenzyls.

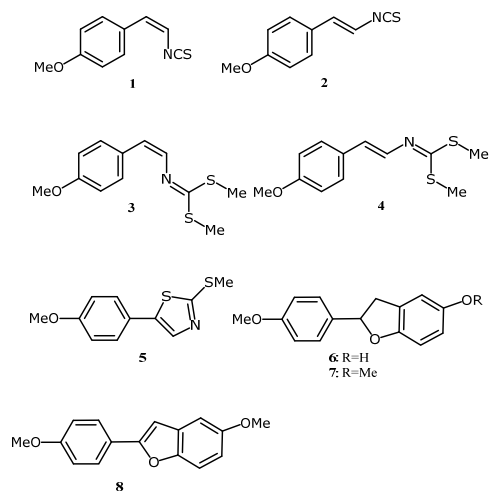
The bryophytes which comprise more than 24,000 species, worldwide are taxonomically placed between algae and pteridophytes. Among the bryophytes liverworts are rich sources of lipophilic terpenoids and aromatic compounds, several of which show interesting biological activities [1a-h]. Such secondary metabolites are very important for classification of liverworts [1a-g]. Turkey is one of the rich countries of bryophytes and totally 887 species recognized among which 163 are the liverworts [2].

Distribution of liverworts and hornworts in Turkey

Among the collected liverworts species in Turkey, *Conocephalum conicum*, *Frullania tamarisci*, *Lunularia cruciata*, *Marchantia polymorpha*, *Metzgeria furcata*, *Pellia endiviifolia* and *Porella platyphylla* are most widely distributed. The second predominant population is *Frullania dilatata*, *Lejeunea cavifolia*, *Lophocolea bidentata*, *L. heterophylla*, *Radula complanata*, *Reboulia hemisphaerica* and *Targionia hypophylla*. The distribution of hornworts in Turkey is rare as seen in Japan and the other countries [1f, g]. The distribution profile of Turkish liverworts is closely related to that of Europe [3]. Here we report the volatile components of 10 Turkish liverwort species and French and Spanish liverwort, *Corsinia coriandrina*.

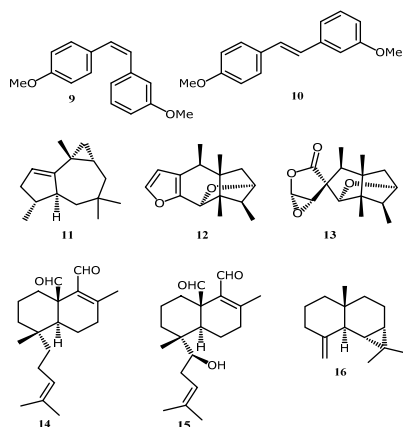
Volatile components of some selected Turkish liverworts

Each specimen was extracted with ether and the crude green oil after evaporation of the solvent was analysed by TLC and GC-MS. The identification of each component appeared on GC-MS was carried out by comparison of the retention times of authentic samples, the Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) Library Ver. 2, Mass Finder 2.3 [4] and our own MS library. The absolute configuration of each terpenoids identified has tentatively been shown according to that of references [1a-g].

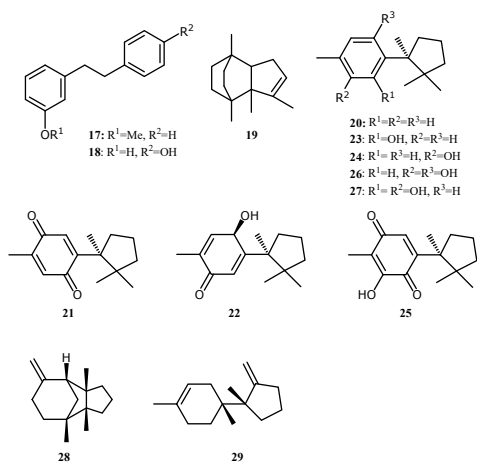
***Corsinia coriandrina* (Corsiaceae)**

The Mediterranean species *Corsinia coriandrina* belongs to the Corsiaceae (Marchantiales). The fresh species shows faint smell like sulphur-containing substances when it is crushed by hand.

It is chemically interesting because *C. coriandrina* produces very specific nitrogen and sulphur-containing compounds showing isothiocyanates, (*Z*)-coriandrin (**1**) and (*E*)-coriandrin (**2**) and *S,S*-dimethyliminodithiocarbonates, (*Z*)-*O*-methyltridentatol A (**3**) and (*E*)-*O*-methyltridentatol B (**4**), moieties attached to (*E*)- or (*Z*)-configured 2-(4-methoxyphenyl)ethen-1-yl skeleton [5a,b]. Naturally occurring nitrogen- and/or sulphur-containing compounds are very rare in liverworts. Only prenylindoles and benzyl and β -phenylethyl (*E*)-methyl thioacrylates have been found in *Riccardia*, *Isotachis* and *Balantiopsis* species, respectively [1c-g].



Isothiocyanates (**1**, **2**) as well as the related dimethyliminodithiocarbonates (**3**, **4**) have also been detected in the French and Spanish specimens, the latter of which also contains 5-(4-methoxyphenyl)-2-methylthiothiazol (=O-methyltridentatol C) (**5**), as a minor constituent, unknown isothiocyanates and S-methylthiocarbonates [5a]. The free phenols and O-sulfates corresponding to **1**, **4** and **5** have already been isolated from a marine hydroid, *Tridentata marginata*, Sertulariidae, and called tridentatol A-C [6a,b].



Another major constituent of the Turkish *C. coriandrina* is corsifuran A, 5-methoxy-2-(4-methoxyphenyl)-2,3-dihydrobenzofuran (**6**), along with a minor amount of the related corsifuran C (**8**). The Spanish specimen also contains corsifuran B (**7**) as well as (*Z*)- (**9**) and (*E*)-3,4'-dimethoxystilbene (**10**) as minor constituents, thus suggesting that compounds **6-8** originated from stilbenoids (**9**, **10**) found in the same species [5c]. In addition to the above components, α -pinene, (*Z*)-nerolidol and drimenol, and 4-methoxybenzaldehyde were detected. α -Pinene, β -caryophyllene and (*E*)-nerolidol were identified in the Spanish specimen [5a, b]. Generally, liverworts are rich sources of terpenoids, however, the present *C. coriandrina* produces only a few terpenoids as a small amount and very rare nitrogen/sulphur-containing aromatic compounds. Thus *C. coriandrina* is chemically very different from any other liverwort that we examined so far.

Porella platyphylla (Porellaceae)

Previously we investigated secondary metabolites of the European *Porella platyphylla* and isolated several pinguisane-type sesquiterpenoids [1c]. The Turkish species elaborated two pinguisane-type sesquiterpenes, pinguisanin (**12**) and isopinguisanolid (**13**) and unidentified pinguisanes as the major components along with an African-type sesquiterpene hydrocarbon,

swartzianin A (**11**) and two sacculatane-type diterpene dialdehydes (**14**) and (**15**) as minor products which are quite rare natural products. The sacculatane diterpenoids have been distributed in the liverworts, *Fossombronia*, *Lepidozia*, *Makinoa*, *Marchantia*, *Pallavicinia*, *Paraschistochila*, *Pellia*, *Plagiochila*, *Symphogyna* and *Trichocoleopsis* [1c, g, h]. The GC-MS pattern of the present Turkish *P. platyphylla* is close to that of European same species.

Porella cordaeana (Porellaceae)

Porella cordaeana, collected in Europe, have been analysed by our group and many drimane- and pinguisane-type sesquiterpenoids isolated and their structures elucidated [1c]. The pinguisane-type sesquiterpenoids, pinguisanin (**12**) and isopinguisanolid (**13**) which were found in *P. platyphylla* were detected in the present species together with γ -maaliene (**16**). The major component of the Turkish specimen was 3-methoxybibenzyl (**17a**) which was not previously identified in this species. The bibenzyl and prenyl bibenzyl are the most important chemical markers of the liverwort *Radula* (Radulaceae) whose morphology is quite different from that of the genus *Porella* [1c, 1g, h].

Pellia neesiana (Pelliaceae)

In *Pellia neesiana* only an aromadendrane-type sesquiterpene alcohol, spathulenol, has been detected. Previously, sacculatal, a pungent diterpene dialdehyde, the structure of which resembles that of perrottetianal (**14**) has been isolated from the Japanese *P. neesiana* [1c]. The poor content of secondary metabolites of the present specimen is due to completely dry material used for the extraction.

Lunularia cruciata (Lunulariaceae)

Lunularia cruciata is one of the popular liverworts in Europe. Earlier, the Japanese specimen was chemically analysed [1c] and two acyclic bis-bibenzyl derivatives, perrottetin F and its dimer, cruciatin were isolated and their structures elucidated [1c]. The Turkish species contained α -pinene and a few ubiquitous sesquiterpenoids, β -elemene, bicyclosquiphellandrene, α -gurjunene, (*Z*)-nerolidol, together with tetradecanoic and oleic acids, and three fatty acid methyl esters, methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate, methyl (5*Z*,11*Z*,14*Z*,17*Z*)-eicosatetraenoate and methyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapentaenoate, and three phytosterols, campe-stigma- and sitosterols.

Lunularin (**18**), a bibenzyl which is a significant precursor of bis-bibenzyls [7a, b] widely distributed in many different liverworts [1c, g, h, 8], was detected in this species.

Lejeunea lamacerina (Lejeuneaceae)

The Lejeuneaceae is the largest family of the liverworts, with almost 1000 species in 91 currently accepted genera [1g]. *Lejeunea lamacerina* is chemically interesting since it contained cyclobazzanene (**19**) and various cuparene-type sesquiterpenoids (**20-27**) all of which are known as the natural products [1a, c, g], together with lunularin (**18**) and tetradecanoic acid. The Turkish specimen is closely related chemically to the Japanese *Lejeunea aquatica*, *L. flava* and *L. japonica* since the latter three produced the same cuparenes (**21,23, 25-27**) [9] as those found in the Turkish *L. lamacerina*.

Scapania undulata (Scapaniaceae)

Scapania undulata has chemically studied in detail [1a, c, 10] and a number of sesqui- and diterpenoids have been isolated. The major components of the present species are β -barbatene (**28**), (*E*)- β -farnesene, bazzanene (**29**), and tetradecanoic, hexadecanoic, linoleic acid and squalene which are ubiquitous secondary metabolites in liverworts.

Metzgeria furcata (Metzgeriaceae)

Metzgeria furcata belongs to the order Metzgeriales and morphologically very tiny thalloid liverwort. It does not produce any terpenoids, and only heptadecane and isovanillin has been detected in this species.

Marchantia polymorpha (Marchantiaceae)

Marchantia polymorpha is one of the most popular liverworts worldwide. Many terpenoids and bis-bibenzyl derivatives have been isolated and their structures as well as biological activity reported in detail [1c, g, h]. In fact, the Turkish variety also contained several ubiquitous sesquiterpenoids as major components, such as α -gurjunene, α -eudesmol together with β -barbatene (28), aromadendrene, α -selinene, and β -eudesmol. Surprisingly, no cuparene-type sesquiterpenoids have been identified, in the Turkish specimen, although the Japanese and European *M. polymorpha* contained a large amount of cuparene type sesquiterpenoids (20, 24) and their derivatives as the major components [1a,c,g,h].

Only several Turkish liverworts were analysed by GC-MS. Among them, the chemical constituents of *Corsinia coriandrina* are very different from the other liverwort species since it produces characteristic isothiocyanates and S,S-dimethyliminodithiocarbonates which have not yet been found in any liverworts, except for the presence in the marine organism [6a,6b]. GC/MS analysis of many characteristic scent volatiles from *Asterella*, *Lophocolea*, *Mannia* and *Scapania* and isolation and structural elucidation of potent hot-tasting components from *Porella*, *Pellia*, and *Chiloscyphus* and very strong bitter substances from the Lophoziaaceae species listed in the experimental section is in progress.

Experimental

Plant materials: Each species was collected in the whole Turkish regions in 2007 and identified by Gokler and Ozenoglu. Voucher specimens were deposited at the Herbarium of the Faculty of Pharmacy (ESSE), Anadolu University, Turkey.

Class, Family and species names of the hornworts collected in Turkey**Anthocerotaceae (hornworts):**

Anthoceros punctatus, *Phaeoceros bulbiculosus*, *P. laevis*.

Marchantiopsida (liverworts):

Sphaerocarpaceae: *Sphaerocarpos michelii*, *S. texanus*.

Targioniaceae: *Targionia hypophylla*, *Targionia lorbeeriana*.

Aytoniaceae: *Plagiochasma rupestre*, *Reboulia hemisphaerica*, *Mannia androgyna*, *Asterella gracilis*, *A. lindenbergiana*.

Conocephalaceae: *Conocephalum conicum*.

Lunulariaceae: *Lunularia cruciata*, *Clevea rousseliana*, *Athalamia hyaline*.

Marchantiaceae: *Marchantia alpestris*, *M. paleacea*, *M. polymorpha*.

Corsiniaceae: *Corsinia coriandrina*.

Oxymitraceae: *Oxymitra paleacea*.

Ricciaceae: *Ricciocarpos natans*, *Riccia bicarinata*, *R. bifurca*, *R. ciliata*, *R. ciliifera*, *crystallina*, *R. fluitans*, *R. frostii*, *R. glauca*, *R. aciliifera*, *R. gougetiana*, *R. macrocarpa*, *R. michelii*, *R. rhenana*, *R. sorocarpa*.

Metzgeriaceae: *Metzgeria conjugata*, *M. furcata*.

Aneuraceae: *Aneura pinguis*, *Riccardia chamedoryfolia*, *R. palmata*, *R. multifida*.

Pelliaceae: *Pellia endiviifolia*, *P. epiphylla*, *P. neesiana*.

Blasiaceae: *B. pusilla*.

Codoniaceae: *F. angulosa*, *F. caespitiformis*, *F. pusilla*, *Petalophyllum ralfsii*.

Lophoziaaceae: *Barbilophozia attenuate*, *B. barbata*, *B. hatcheri*, *Lophozia bicrenata*, *L. collaris*, *L. excise*, *L. incisa*, *L. longidens*, *L. longiflora*, *L. sudetica*, *L. turbinata*, *L. ventricosa*, *Gymnocolea acutiloba*, *Tritomaria exsecta*, *T. exsectiformis*, *T. quinquedentata*.

Jungermanniaceae: *Mylia taylorii*, *Jungermannia atrovirens*, *J. caucasica*, *J. gracillima*, *J. handelii*, *J. hyaline*, *J. lignicola*, *J. obovata*, *J. sphaerocarpa*, *J. subtilissima*, *Nardia compressa*.

Gymnomitriaceae: *Marsupella emarginata*, *M. funckii*, *Gymnomitrium concinnatum*.

Arnelliaceae: *Gongylanthus ericetorum*, *Southbya nigrella*, *S. tophacea*.

Plagiochilaceae: *Plagiochila asplenoides*, *P. porelloides*.

Geocalyceae: *Lophocolea bidentata*, *L. heterophylla*, *L. minor*, *Chiloscyphus pallescens*, *C. polyanthos*.

Scapaniaceae: *Diplophyllum albicans*, *Scapania aequiloba*, *S. aspera*, *S. compacta*, *S. curta*, *S. irrigua*, *S. umbrosa*, *S. undulata*, *S. verrucosa*.

Cephalozellaceae: *Cephalozella baumgartneri*, *C. divaricate*, *C. hampeana*, *C. stellulifera*.

Cephaloziaaceae: *Cephalozia bicuspidate*, *C. catenulate*, *C. pleniceps*, *Nowellia curvifolia*, *Cladopodiella fluitans*.

Antheliaceae: *Anthelia julacea*.

Lepidoziaaceae: *Lepidozia reptans*, *Bazzania tricrenata*, *B. trilobata*, **Calypogeiaaceae:** *Calypogeia argute*, *C. azurea*, *C. fissa*, *Carypogeia muellerana*, *C. sphagnicola*.

Pseudolepicoleaceae: *Blepharostoma tricophyllum*.

Radulaceae: *Radula complanata*, *R. lindenbergiana*.

Porellaceae: *Porella arboris-vitae*, *P. baueri*, *P. cordaeana*, *P. obtusata*, *P. pinnata*, *P. platyphylla*.

Frullaniaceae: *Frullania dilatata*, *F. jackii*, *F. tamarisci*.

Jubliaceae: *Jubula hutchinsiae*.

Lejeuneaceae: *Lejeunea cavifolia*, *L. lamacerina*, *L. patens*, *Cololejeunea calcarea*, *C. rosettiana*.

Extraction and GC-MS analysis: Half-dried Turkish liverworts (1-20 g) was mechanically crushed to give powder which were extracted with ether for 3 days. Each extract was filtered through a small column packed with Celite and the solvent evaporated at room temperature yielding a green oil. The crude oil was analysed by TLC (SiO₂, *n*-hexane/EtOAc 4:1 and 1:1). Spots were visualized by UV light (254 nm and 350 nm) and by spraying Godin' reagent and heating on a hot-plate at 120°C. A small amount of each crude extract was analysed by GC-MS. The condition of GC/MS analyses were as follows: Temperature programming was carried out from 50°C isothermal for 3 min., then 50-230°C at 3°C/min., and finally isothermal at 230°C for 20 min. Injection temperature was 250°C. A fused silica capillary column coated with β -DEX120 (30 m x 0.25 mm i.d., film thickness 0.25 mm) was used. Identifications were based on comparison of the retention times of authentic samples, the Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) Library Ver. 2, the data bank Mass Finder 2.3 [4] and our own MS library.

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