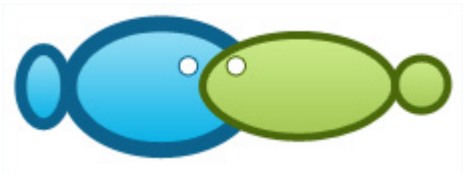

Corallina officinalis chemical compounds obtained by supercritical fluid extraction

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Abstract. *Corallina officinalis* chemical compounds were obtained by supercritical fluid extraction at 30 MPa and 40°C with the CO_2 flow rate of 2.0 kg/h. Extraction yield was 1.09%. The extract obtained was analyzed by gas chromatography and gas chromatography-mass spectrometry. Chemical compounds identified and quantified were: acyclic alkanes (14.92%), branched alkanes (2.06%), alkenes (5.44%), organobromine compounds (2.88%), organosulfur compound (0.6%), aromatic compound (0.6%), monoterpenes (8.42%), sesquiterpenes (2.02%), diterpenes (4.32%), triterpene (13.64%) and not identified compounds (25.16%). The most abundant compound present in *C. officinalis* extract obtained by supercritical fluid extraction was a triperpen squalen which was present in a percentage of 13.64%.
Key Words: red algae, organic compounds, GC-MS, triperpen squalen.

Introduction. In South-eastern Adriatic Sea there is over 300 algal species. The Rhodophyta comprise 202 taxa (66.5%), followed by Phaeophyceae (60 taxa, 19.7%) and Chlorophyceae (42 taxa, 13.8%) (Roganovic et al 2010). The pigment composition gives algae their wide colors variety and for several algal groups, their common names are: brown, red and green algae.

At the depth of 268 m, dark purple red algae exist where light is faintly blue-green and its intensity is only 0.0005% of surface light (Barsanti & Gualtieri 2006; Littler et al 1985). At this depth the red part of the sunlight spectrum is not sufficient for photosynthesis. These algae possess accessory pigments that absorb light in spectral regions different from those of chlorophyll *a* and channel this absorbed light energy to chlorophyll *a*, which is the molecule that converts sunlight energy into a chemical compound (Barsanti & Gualtieri 2006). Algae that live in high irradiance habitat have pigments that protect them against the photodamages caused by singlet oxygen (Barsanti & Gualtieri 2006). Algal pigments are chlorophyll *a* which is present in the thylakoid membrane together with β-carotene and lutein (Barsanti & Gualtieri 2006). Chlorophyll *a* and phycobiliproteins are organized into phycobilisomes (Barsanti & Gualtieri 2006). All red algae thylakoids have phycobilisomes tied to the stromal surface, which contain the accessory phycobiliprotein pigments: allophycocyanin, phycocyanin and phycoerythrin in its five forms (Barsanti & Gualtieri 2006).

Coralline red algae (Corallinophycidae, Rhodophyta) are important components of marine ecosystems. They are impregnated with calcium carbonate (Lewin 1962; Goreau 1963; Foster 2001; Steneck 1986; Bosence & Wilson 2003; Basso 2012). Coralligenous structures are present in circalittoral zone and can develop in infralittoral zone. This habitat type was recorded on several South-eastern Adriatic littoral sites (Gamulin-Brida 1983; Karaman & Gamulin-Brida 1971; Lovric & Rac 2006; Macic et al 2010; Pulevic 1980). Coralligenous biocenosis inhabits solid bottom in circalittoral zone and its main characteristic is less amount of light than in infralittoral zone which induced the presence of numerous sciafillic species, especially those with carbonate shells or talus (Family Corallinaceae).

The red algal phylum (Rhodophyta) have ca 7,100 living species and Corallinales are the third most abundant species group, with 725 described living taxa (Johansen 1981). Red algae contain many secondary metabolites (Faulkner 2000; Faulkner 2001;

Faulkner 2002; Blunt et al 2009). The red algae contain secondary metabolites, although "in practice there is little difficulty in distinguishing what is or is not a red alga" (Ragan & Gutell 1995). Marine algae produce a wide variety of natural compounds, referred to the name secondary metabolites because not being involved in the basic biochemical processes (Cimino & Ghiselin 2001). Secondary metabolites represent a small fraction compared to the whole organism complete biomass (Cannell 1998; Ianora et al 2006). The term "secondary metabolite" does not have the inferior meaning to "primary metabolite". Secondary metabolites also contribute to growth, reproduction and defense playing a primary role for the organism. In marine algae, many secondary metabolites are halogenated, reflecting the availability of chloride and bromide ions in seawater. Bromide is more frequently used by algae for organohalogen production, although chlorine occurs in higher concentrations than bromine in seawater. Marine halogenated compounds comprise a wide variety of compounds, starting from peptides, terpenes, polyketides, indoles, acetogenins and phenols to volatile halogenated hydrocarbons (Butler & Sandy 2009). The chlorine and bromine appear to be the main halogens used to increase secondary metabolites biological activity, whereas iodine and fluorine remain within the chemical structures (Neumann et al 2008). Iodination is more frequent in brown algae than in red and green algae metabolites (Kupper et al 1998). Red and green algae secondary metabolite compounds contain bromine or chlorine as much as 90% and 7%, respectively (Harper et al 2001).

Plant pigments present in red algae are: chlorophyll *a,* β-carotene, lutein and accessory red/blue phycobilin pigments, predominantly the red-colored phycoerythrin in stalked phycobilisomes on thylakoids (van den Hoek et al 1995). Red algae are the main producers of halogenated compounds (Faulkner 2001; Wright et al 2003). The chemical components found in *Corallina officinalis* are aliphatic hydrocarbons, cyclic hydrocarbons, monoterpenes, diterpenes, aldehydes, phenols, alcohols, ketones and esters (Borik 2014). The sterol composition was investigated in *C. officinalis* L., *Corallina granifera*, *Corallina mediterranea* and *Corallina elongata* (Palermo et al 1990; Sallam et al 1982). Seasonal *C. officinalis* lipid compounds content was investigated (Awad et al 2003). Also amino acids (Madgwick et al 1970) and polysaccharides (Cases et al 1994) were determined in *C. officinalis*.

The aim of the present paper was to use a well known method, the supercritical carbon dioxide extraction to obtain chemical compounds present in *C.officinalis* and to identify and quantify them. The chemical compounds present in *C. officinalis* extract obtained by supercritical fluid extraction were not reported, up to now.

Material and Method

The red algae *C. officinalis* was collected in August, 2016 at South-eastern Adriatic coast, Nišice, Dobre vode, Montenegro. The collected algae were air dried for a month. After drying the water content was 7.45±0.12%. The algae were grounded in a rotating blade coffee grinder Bosch MKM 6003. Grinded plant material was extracted with CO_2 on a lab-made supercritical fluid extraction apparatus. Laboratory unit for supercritical extraction consisted of: CO_2 reservoir, cooling bath (ethylene-glycol/ethanol), air compressor, air-driven CO_2 pump (Haskel® MS-71), heating bath, extraction cell, separator vessel and flow meter (Matheson FM-1050, E800) (Martinez & Vance 2008). Temperature was controlled using proportional-integral-derivative (PID) controller. Pressure controllers were two WIKA manometers (model 212.20) 60 MPa for the pressure control in the extraction cell and one WIKA manometer (model 212.20) 4 MPa for the pressure control in separator.

Grinded algae (50 g), in a powder form, were poured in the extractor vessel and the extract was collected in a separator with the glass tube with previously determined glass tubes mass. The pressure was 30 MPa at 40°C. Extraction yield was 1.09%. The mass of dried material in the extractor and CO_2 mass flow rate were kept constant during the experiment. The CO_2 flow rate (2.0 kg/h) was measured by a Matheson FM-1050 (E800) flow meter. Extraction lasted for 4 hours.

Obtained extract was dissolved in hexane to obtain ~1% sample solution which was subjected to further analyses. The characterization and quantification of compounds present in the extract was performed by analytical gas chromatography (GC) with flame ionization detector (FID) and gas chromatography-mass spectrometry (GC-MS) instrument. The Agilent 7890 A GC equipped with Agilent 5975 MSD has been used. GC-MS was fitted with HP-5MS (Agilent J&W 19091S-433) column (30m x 0.25 mm ID, 0.25 µm). The temperature of injection port was 250ºC, splitless injection. The starting oven temperature was set at 60ºC with the temperature linear increase of 4ºC/min till 150ºC, then the temperature increase was 9ºC/min till 280ºC. The carrier gas was helium (He) with a 0.7 mL/min flow. Ionization energy was 70 eV. Injection volume was 1 µL. MS conditions were: scan (45 to 450 amu), threshold 100MS quad at 150ºC, MS source at 250ºC. The identification of components was carried out based on computer matching with NIST 2008 MS library. Quantitative analysis has been provided based on calibration curves. Standard compounds were dissolved in *n*-hexane. Standard compounds were prepared in six different concentrations. The R^2 for each calibration curve was 0.999. All analyses were performed in triplicate. The results are expressed as compound content in % (w/w).

Results. The *C. officinalis* extract yield obtained by supercritical fluid extraction was 1.09% by weight. The qualitative and quantitative extract composition is given in Table 1. The compounds determined in the extract were: acyclic alkanes, branched alkanes, alkenes, organobromine compounds, organosulfur compound, aromatic compound, oxygen containing compounds, alcohols, aldehyde, esters, monoterpenes, sesquiterpenes, diterpenes and triterpene. There were eight acyclic alkanes present in the extract with the total of 14.92%. The most predominant acyclic alkane was eicosane. In the *C. officinalis* extract obtained by headspace extraction, *n*-hexadecane was detected in 0.11%, in extracts obtained by Soxhlet apparatus the quantity varied by season 0%, 0.09% and 0.15%, while the quantity in the extract obtained by supercritical fluid extraction was 2.00% (Borik 2014; Awad et al 2003). The most abundant acyclic alkane in the extract obtained by headspace extraction was *n*-pentacosane with 7.85%, in the extract obtained by Soxhlet apparatus the *n*-pentacosane quantity depended on season and was present in 0.08%, 0.10% or 0.28% (Borik 2014; Awad et al 2003). In supercritical fluid extract it was present in 0.64%. Three branched alkanes were detected in 2.06%. Two alkenes were present in the extract with the percentage of 5.54%. There were two organobromine compounds detected: 2-bromooctadecanal, which is also oxygen containing compound, in a percentage of 1.34% and 1-bromotriacontane, brominated triterpene, in a percent of 1.54%. Few brominated triterpenes are known. Those that have been identified have structural complexity and are secondary metabolites most probably derived from squalen (Gribble 1999). Organosulfur compound was detected in a percentage of 0.60%. In the *C. officinalis* extract obtained by headspace extraction organosulfur compounds detected were 3,5-dimethyl-1,2,4-trithiolane (0.11%) and 5,6-dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine (0.19%) (Borik 2014). The aromatic compound present in the supercritical fluid extract was 2,3,6-trimethyl-anisole, in a percentage of 0.60%. Oxygen containing compounds detected were: 4-hydroxy-2-butanone and 1,7-dimethyl-2-oxo-7-(4'-formyl-butyl)-norbornane. Two monoterpene cyclic ethers were detected: eucalyptol and car-3-en-2-one. The car-3-en-2-one was second most abundant oxygenated compound present in the extract with 8%. Two sesquiterpene alcohols were present in the extract: glaucyl alcohol (4.36%) and cedrol (0.44%). The oxygenated diterpene phytol isomer was present in 3.68%.

Table 1

Qualitative and quantitative *Corallina officinalis* extract content obtained

Compound	*Compound content (%)*
Acyclic alkanes	
Dodecane	2.00
Tetradecane	0.72
Hexadecane	0.56
Octadecane	3.20
Eicosane	4.18
Heneicosane	0.66
Docosane	2.96
Pentacosane	0.64
Branched alkanes	
Isononane	0.54
4-Methyltridecane	0.54
3-Methylhenicosane	0.98
Alkenes	
1-Nonadecene	1.02
1-Octadecene	4.42
Organobromine compounds	
2-Bromooctadecanal	1.34
1-Bromotriacontane	1.54
Organosulfur compound	
di-tert-dodecyl disulfide	0.60
Oxygen containing compounds	
2-Butanone, 4-hydroxy-	0.66
1,7-dimethyl-2-oxo-7-(4'-formyl-butyl)-norbornane	0.36
Alcohols	
4,4-dimethyl-11-methylene-undecanol	2.00
Glaucyl alcohol	4.36
1-Octadecanol	0.96
(3Z,13Z)-2-methyloctadeca-3,13-dien-1-ol	0.52
Aldehyde	
E-15-Heptadecenal	1.46
Aromatic compound	
Anisole, 2,3,6-trimethyl-	0.60
Esters	
Linalyl acetate	0.52
Ethyl 6,9,12-hexadecatrienoate	0.28
Methyl elaidate	0.54
14α-Cheilanth-12-enic Methyl ester	8.64
Monoterpenes	
Car-3-en-2-one	8.00
Eucalyptol	0.42
Sesquiterpenes	
Cedrol	0.44
Hexahydrofarnesyl acetone	1.58
Diterpenes	
Neophytadiene	0.64
Phytol isomer	3.68
Triterpene	
Squalene	13.64
Not identified	25.16

In the extract obtained by Soxhlet apparatus the phytol quantity varied by season and was: 12.60%, 0.09% and 0.08%, while in extract obtained by headspace extraction phytol was present in 0.17% (Awad et al 2003; Borik 2014). Oxygen containing compounds in percentage higher than 1% were: the alcohol 4,4-dimethyl-11-methylene-undecanol (2.00%), hexahydrofarnesyl acetone (1.58%) and the aldehyde E-15-heptadecenal (1.46%). Other oxygen containing compounds were present in less than 1%. Two diterpenes present in the extract were neophytadiene (0.64%) and oxygenated diterpene phytol isomer (3.68%). The most abundant compound in the extract was triterpene squalene which made 13.64% of the total extract composition.

Conclusions. In *C. officinalis* extract obtained by supercritical carbon dioxide extraction, 74.84% compounds were identified, while 25.16% were compounds that were not identified. The most abundant compound was a triterpene squalene (13.64%). The acyclic alkanes were present in the extract in 14.92%, branched alkanes in 2.06% and alkenes in 5.44%. Organobromine compounds were identified and their content in the extract was 2.88%. Organosulfur and aromatic compound identified had the same quantity (0.60%). Oxygen containing compounds were present in 30.38%, along with 2-bromooctadecanal (1.34%) and phytol isomer (3.68%) which were placed to organobromine compounds and diterpenes, respectively. Diterpenes were present in the extract in 4.32%. The triterpene squalene was present in 13.64%. *C. officinalis* extract obtained by supercritical carbon dioxide extraction can be used for the isolation of squalene.

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