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RESEARCH ARTICLE

Sterols in *Laurencia obtusa* var. *pyramidata* (Hudson) J. V. Bory ex. J. Agardh

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Abstract

The sterol content of *Laurencia obtusa* var. *pyramidata* (Hudson) J. V. Bory ex. J. Agardh collected from Igneada, in the western part of the Black Sea coast of Turkey, was analyzed. They were cholest-5-en-3-ol (cholesterol), *β*-sitosterol, ergosta-5,7,22-trien-3-ol (ergosterol), 24-norcholesta-5,22*(E)*-dien-3*β*-ol, cholest-5,22*(E)*-dien-3*β*-ol, ergosta-5,24(28)-dien-3*β*-ol, 27-norergosta-5,22*(E)*-dien-3*β*-ol, ergosta-5, 22*(E)*-dien-3-ol, 23*(S)*-methylcholesterol, 23(*S*)-ethylcholesterol. The latter four were found in *Laurencia* species for the first time.

Keywords: Sterols, GC-MS, *Laurencia pyramidalis*, Black Sea

Introduction

Sterols are derived from steroids containing an alcohol and an alkyl chain. Carbon numbers of sterols are 26-29. They are found in general in animals and the predominant sterol is cholesterol. There have been several studies on sterol content of marine algae. The main sterol, cholesterol, has been found as the major sterol in red algae (Tsuda *et al*. 1957) and fucosterol has been found in brown algae (Heilborn *et al*. 1934). These sterols were also isolated from various algae species (Guven and Bergisadi 1973; Guven *et al*. 1975; Guven and Bergisadi, 1975; Guven and Hakyemez 1975; Guven and Guler, 1979; Guven *et al*. 1980).

Marine red algae of the genus *Laurencia* occur worldwide and also in Turkish coasts (Fritsch 1899; Guven 1970). Several papers have dealt with the chemical

and pharmacological contents but investigation of sterol content of this species is rare. The researches on *Laurencia obtusa* concentrated on halogenated terpene compounds. *Laurencia* species in which several sterols were found are *L. pinnatifida* (Gibbons *et al*. 1967; Quinoa *et al*. 1988), *L. pinnata* (Faux *et al*. 1969; Fukuzawa *et al*. 1981; 1986), *L. pyramidalis* (Ahmad *et al*. 1992), *L.* obtusa (Kobayashi *et al*. 1992a; 1992b), *L. karlae* (Zhong *et al*. 1996), *L. cartilaginosa* (Yang *et al*. 2005), *L. trishticha* (Sun *et al*. 2007), *L. papillosa* (Lisboa *et al*. 1982; Kamenarska *et al*. 2006; Al-lihaibi *et al*. 2010; Alarif *et al*. 2011) and *L. coronopus* (Kamenarska *et al*. 2006). In addition, HHCP pollution has been studied in *L. obtusa* in the Turkish coasts (Guven *et al*. 2013).

This work aims to analyze and report the sterol content of *L. obtusa* found in the Turkish coasts.

Materials and Methods

The material of *Laurencia obtusa* var. *pyramidata* (Hudson) J. V. Bory ex. J. Agardh was collected in 2007 from Igneada in the western part of the Black Sea coast of Turkey.

All solvents used were Merck products (Darmstadt, Germany). Sodium sulphate was supplied by BASF (Baden, Germany).

General procedure: The collected alga was air-dried and milled to coarse powder. The extraction method was applied according to Reiner *et al*. (1962). Alga (60g) was extracted with dichloromethane (DCM) in soxhlet apparatus for 4 h. The extract was distilled at 35 ^{o}C, then it was saponified with alcoholic solution of KOH (1%) under a reflux condenser for 1 h. After saponification, water was added and the extraction in a separatory funnel with DCM was done. The DCM phase was separated and distilled at 35 ^{o}C. The residue was taken with hexane and applied to GC/MS analyses.

Detection Method: The gas chromatography mass spectrometer (HP 6890 Series GC system; Hewlett Packard, Wilmington) was fitted with an electronic pressure control and a mass selective detector (HP 5972 A; ionization energy: 70 eV; HP-PONA capillary column (50m 0.25 µm film thickness). The chromatographic conditions were: sample size 2 µl, injection port temperature 280 ^{o}C, configured for split injection; initial oven temperature 40 ^{o}C rising to 280 ^{o}C at 8 ^{o}C/min, final hold of 20 min. helium was used as carrier gas (1 ml/min).

The sterols were identified by comparing the retention times and spectrum with HP memory.

Results and Discussion

The sterols identified by GC/MS in *L. obtusa* are listed and shown in Table 1 and Figure 1. The only sterol that was not found in our study is *L.* an oxidation product of 2*a*-oxa-2-oxo-5*α*-hydroxy-3,4-di-norcholestane reported by Kobayashi *et al*. (1992). The sterols reported in *Laurencia* spp. which are common in our study are cholesterol in *L. pinnatifida* (Quinoa *et al*. 1988), *L. pyramidalis* (Ahmad *et al*. 1992), *L. karlae* (Zhong *et al*. 1996), *L. trishticha* (Sun *et al*. 2007), *L. papillosa* (Lisboa *et al*. 1982; Kamenarska *et al*. 2006; Sun *et al*. 2007), 24-norcholesta-5,22-dien-3*β*-ol in *L. coronopus*, and cholesta-5,22(*E*)-dien-3*β*-ol in *L. papillosa* (Kamenarska *et al*. 2006).

Ergosterol was found in a number of green algae (Lopes *et al*. 2011). Ergosta-5,24(28)-dien-3*β*-ol (6) is another sterol reported in marine brown algae (Fleury *et al*. 1994). *β*-sitosterol was found in many species of red algae (Shameel *et al*. 2013).

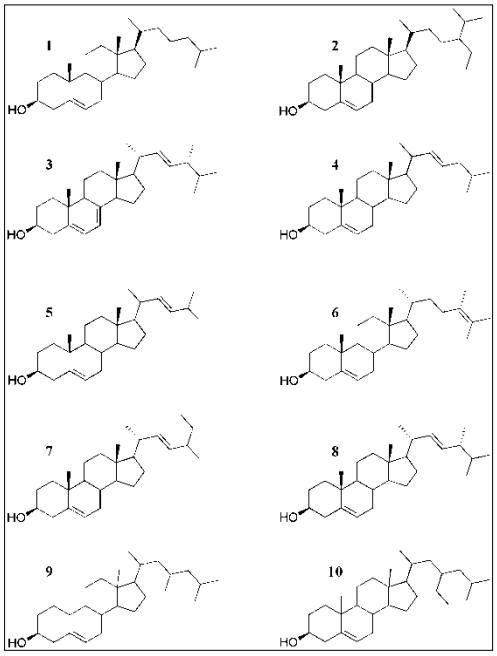

Figure 1. Chemical structures of the identified sterols: cholesterol (1), *β*-sitosterol (2), ergosterol (3), cholest-5,22*(E)*-dien-3*β*-ol (4), 24-norcholesta-5,22*(E)*-dien-3*β*-ol (5), ergosta-5,24-dien-3*β*-ol (6), 27-norergosta-5,22*(E)*-dien-3*β*-ol (7), ergosta-5,22*(E)*-dien-3*β*-ol (8), 23(*S*)-methylcholesterol (9), 23(*S*)-ethylcholesterol (10).

Table 1. GC/MS analysis results of sterol content of *Laurencia obtusa* var. *pyramidata* (Hudson) J. V. Bory ex. J. Agardh.

Ret. Time	Compounds (Numbers in parentheses refer to Figure 1)	Molecular Formula	M^+ and fragments
31.221	24-norcholesta-5,22*(E)*-dien-3β-ol (5)	$C_{26}H_{42}O$	370^+, 355, 337, 322, 300, 271, 255, 213, 159, 81, 69, 97, 55
32.626	Cholest-5,22*(E)*-dien-3β-ol (4)	$C_{27}H_{44}O$	384^+, 366, 300, 271, 255, 213, 159, 145, 111, 105, 69, 55
32.823	27-norergosta-5,22*(E)*-dien-3β-ol (7)	$C_{27}H_{44}O$	384^+, 366, 300, 271, 255, 213, 159, 145, 111, 105, 69, 55
33.405	Cholesterol (1)	$C_{27}H_{46}O$	386^+, 368, 301, 275, 255, 213, 159, 145, 105, 91, 79, 55
34.140	Ergosta-5,22*(E)*-dien-3β-ol (8)	$C_{28}H_{46}O$	398^+, 386, 271, 255, 213, 159, 145, 133, 107, 69, 55
34.678	Ergosterol (3)	$C_{28}H_{44}O$	396^+, 378, 363, 337, 271, 253, 237, 227, 211, 199, 157, 143, 119, 91, 81, 69, 55
34.897	Ergosta-5,24-dien-3β-ol (6)	$C_{28}H_{46}O$	398^+, 383, 314, 299, 271, 229, 213, 159, 145, 105, 83, 55
35.007	23(*S*)-methylcholesterol (9)	$C_{28}H_{48}O$	400^+, 382, 367, 315, 289, 255, 213, 159, 145, 105, 91, 55
36.631	23(*S*)-ethylcholesterol (10)	$C_{29}H_{50}O$	414^+, 396, 381, 354, 329, 314, 303, 273, 255, 231, 213
36.642	β-sitosterol (2)	$C_{29}H_{50}O$	414^+, 396, 381, 354, 329, 314, 273, 255, 231, 213, 199, 187, 173, 159, 145, 133, 119, 107, 91, 81, 69, 55

Conclusion

This study confirmed that *Laurencia obtusa* var. *pyramidata* (Hudson) J. V. Bory ex. J. Agardh, a common red alga species on the Turkish Black Sea coasts, contains a C26, three C27, four C28 and two C29 sterols. Three of them were found in other *Laurencia* species and three of them were previously found in the other algae species. However, four of the sterols, namely, 27-norergosta-5,22(*E*)-dien-3*β*-ol, ergosta-5,22*(E)*-dien-3*β*-ol, 23(*S*)-methylcholesterol and 23(*S*)-ethylcholesterol were found in genus *Laurencia* for the first time.

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