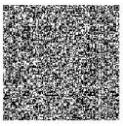

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Sterol Composition of the Indian Green Lipped Mussel *Perna virdis*

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Abstract

Sterol composition of green lipped mussel *Perna virdis* was analysed using GCMS. Cholesterol was found to be the dominant sterol (54.162% of the total sterol content). Other sterols such as Cholesta-5,22-dien-3-ol (3β), ergosta-5,22-dien-3-ol (3 β,22E, 24S), 26,26-dimethyl-5,24 (28)-Ergostadien-3β-ol, 26-nor-5cholesten- 3β-ol, stigmasterol and γ sitosterol were also detected. Presence of phytosterols like stigmasterol and γ sitosterol underlines high nutritional potential and food value of this bivalve mollusk.

Keywords: *Perna virdis*, Kerala coast, Sterols, Food value

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1. Introduction

The green mussel *Perna virdis*, commonly known as the Indian green mussel is a native of the IndoPacific region, primarily distributed along the Indian and south east Asian coasts. They generally inhabit marine intertidal, sub tidal and estuarine environments with high salinity. *Perna virdis* is a characteristic species of the fauna of mid littorial and sub littorial zones. The species is regarded as an important edible commodity. It is a remarkable species in terms of its ability to reach very high biomass level, to withstand environmental pollutants, to colonise artificial marine habitats and to invade new geographic territories. Extensive studies have been carried out on this particular species and it was proved to be an excellent source of carbohydrates, proteins, lipids and essential vitamins. Bivalves as sea foods are considered important next to fish and prawns, from nutritional point of view. They have been consumed for thousands of years. So the earlier investigations were limited to the biological, nutritional and medicinal aspects of this edible bivalve [1]. Studies on the protein content on selected organs of Perna virdis at Tranquebar coastal waters, Tamilnadu [2], work on the neutraceutical property of Perna [3], evaluation of the seasonal variation in nutritional values of Perna virdis by Wafar et al 1976[4] and the antibacterial activity of the extracts of green Mussel (Perna virdis) [5] are worth discussing in this context.

Despite the interest as a dietary source, little detailed information exists on the steroidal composition of Mussel. Even though attempts were made by Duoli , 2007 [6] to study the steroidal composition of *Perna virdis*, collected from China, the sterols were identified by comparison with mixtures of sterols from the NewZealand Green Lipped mussel, which had been previously identified by GC-MS [7]. This study endeavours to investigate the steroids present in the marine bivalve mollusc *Perna virdis* and here in we provide the first GCMS data of the sterol composition of previously unsampled *Perna virdis* from southern coast of India.

2. Experimental

The specimens were collected from Chellanam of Cochin during 2012 March. The live organisms after collection was thoroughly cleaned in seawater and stored in glass containers kept frozen until the start of the extraction work. The organism was macerated and further extracted with fresh methanol. The extracts were combined; filtered and excess alcohol was evaporated invacuo below 40˚C. The concentrated crude methanolic extract was then evaporated and extracted with ethyl acetate, to acquire the lipophilic fraction. The extract was evaporated and the residues were purified by column chromatography. The residue of the ethyl acetate extract was column chromatographed over silica gel (60-120 mesh) and eluted with hexane with increasing concentration of ethylacetate, to get the semi pure fractions and analysed by GCMS.

GCMS analyses of the samples were done using PerkinElmer Clarus 680 GCMS equipped with head space sampler. The oven temperature was programmed from 35˚C to 280˚C at the rate of 10˚per minute and is hold for 10 minutes. The column used for the analysis was a 30mx0.25mm i.d. capillary column coated with a film of dimethylpolysiloxane containing 5% diphenylpolysiloxane (Elite 5 MS Column) An aliquot of 0.5µl of the sample was injected into the GC–MS system in the split mode (split ratio1 : 50). Helium was used as the carrier gas with a flow rate of 1mLmin–1. The injector and temperature was optimized at 280˚C. The MS operating parameters were as follows: ionization energy -70 eV; ion source temperature-200˚C, solvent delay 4 min and scan range 40–600 u. Identification of the components was based on direct comparison of mass spectral data with NIST library version 2.1.

3. Results and Discussion

Our investigation of *Perna virdis* provided us with seven steroids, cholesta-5,22-dien-3-ol(3β), cholesterol, ergosta-5,22-dien-3-ol (3 β,22E, 24S), 26,26-dimethyl-5,24 (28)-ergostadien-3β-ol, 26-nor-5cholesten- 3β-ol, stigmasterol and γ sitosterol. The total ion chromatogram and the percentage composition are given in Figure 1 and table 1 respectively. The structures of the sterols identified and the mass spectrum corresponding to each peak are

given in Figure 2 and 3. The steroids were found to present in the order cholesterol> ergosta-5,22-dien-3-ol (3 β,22E, 24S)> Cholesta-5,22-dien-3-ol (3β)> 26,26-dimethyl-5,24 (28)-Ergostadien-3β-ol = 26-nor-5cholesten- 3β-ol> γ sitosterol> stigmasterol.

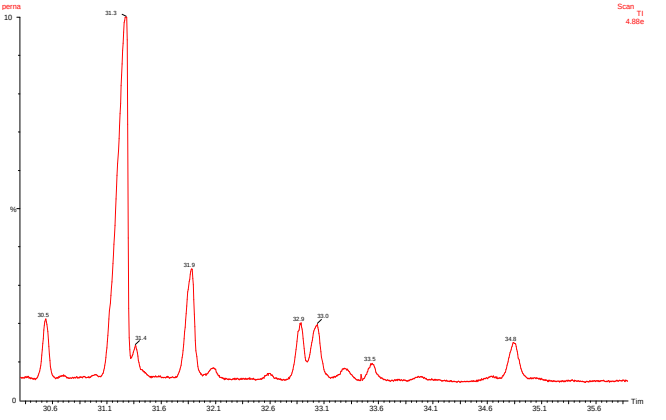
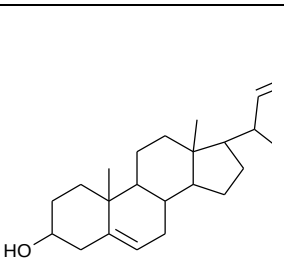


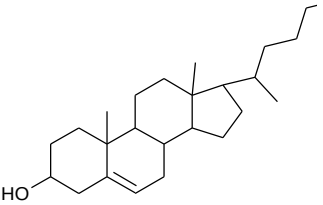
Figure 1: Total Ion Chromatogram of the sterols identified

RT	**Sterols Identified**	**% sterols/total sterol content**
30.58	Cholesta-5,22-dien-3-ol (3β)	5.938
31.32	Cholesterol	54.162
31.92	ergosta-5,22-dien-3-ol (3 β,22E, 24S)	13.480
32.93	26,26-dimethyl-5,24 (28)-Ergostadien-3β-ol	7.036
33.08	26-nor-5cholesten- 3β-ol	8.434
33.59	Stigmasterol	2.668
34.88	γ sitosterol	8.279

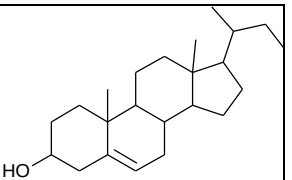
Table 1: Percentage composition of sterols



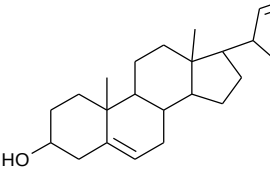
Cholesta-5,22-dien-3-ol (3β)



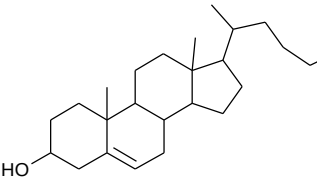
cholesterol



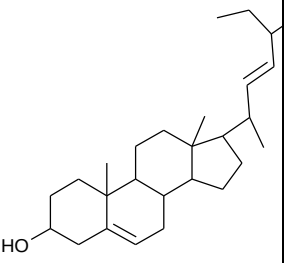
26-nor-5cholesten-3β-ol



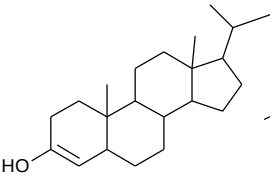
ergosta-5,22-dien-3-ol (3 β,22E, 24S)



26,26-dimethyl-5,24 (28)-Ergostadien-3β-ol



stigmasterol



γ sitosterol

Figure 2: Structure of sterols identified

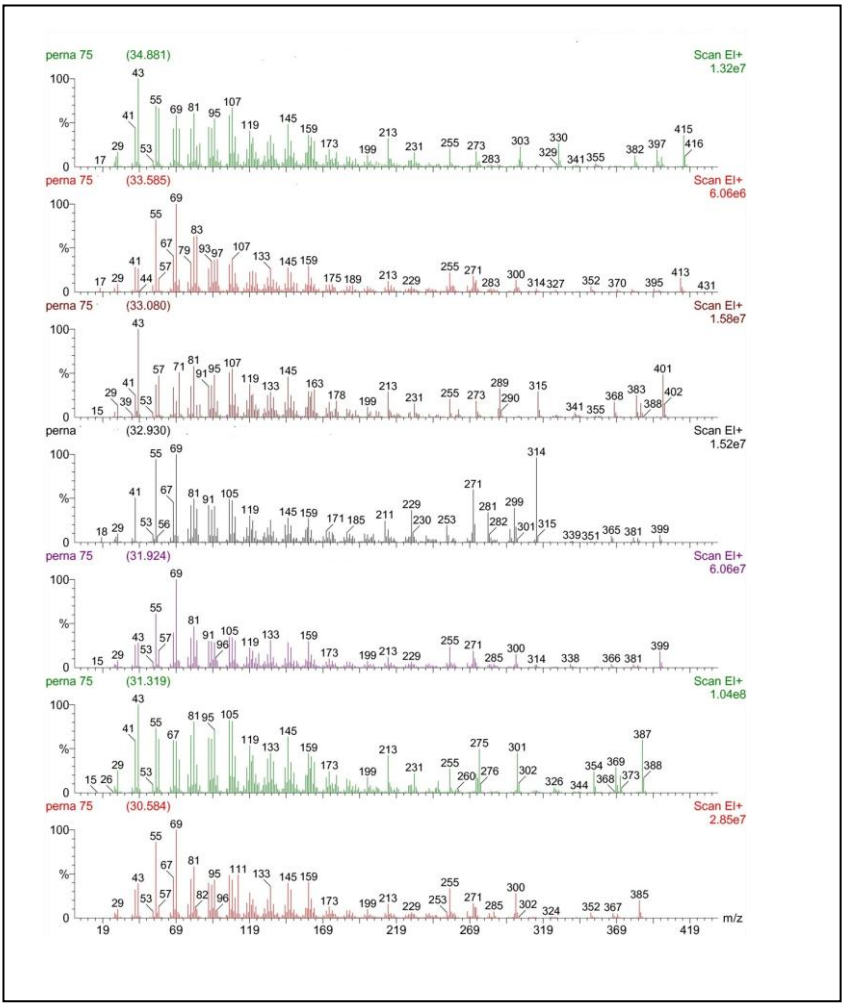


Figure 3: Mass fragmentation of the sterols identified from *Perna virdis*

Investigations on De-novo sterol synthesis in marine mollusks have assumed that cholesterol is the principal sterol formed from acetate and Mevalonate pathway [8, 9]. Composition of sterols other than cholesterol occurring in molluscan tissues may be affected by the dietary sources of sterols [10]. Also Khan and Goad (1983)[11] have mentioned three possible sources of sterols in mollusks 1. Denovo synthesis by usual Δ^5 sterol biosynthesis route, 2. Accumulation of dietary sterols, 3. Interconversion of dietary sterols to sterols in body of organisms. Compared with other marine invertibrates, marine bivalve mollusks are unique as they contain a wide range of

sterols, besides cholesterol[12]. Plant sterols such as stigmasterol and sitosterol was identified in species studied. It was established that the sterol profile of the lipids in the marine mollusks reflects the sterol profile of the food source (zooplankton, dinoflagellates and algae) [7].

4. Conclusion

Perna virdis is a found to be a proven source for a variety of rare sterols. Previous studies have proved that, bivalves are low in steroids (50mg/g wet weight) when compared to the traditional protein sources such as beef (100mg/g wet weight) [12]. Also it was previously proved that non-cholesterol sterols derived from plants could reduce blood serum cholesterol in humans [13]. We could conclude that this bivalve under study, exhibited high conversion efficiency and hence high food value, with respect to the steroidal profile considered in this study. Also the accomplishment of the biological activity studies of these isolated steroids would certainly result in lead molecules for the development of promising drugs in future.

Acknowledgments

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