Brown Algae (Phaeophyceae) from the Coast of Madagascar: preliminary Bioactivity Studies and Isolation of Natural Products

Marie Pascaline Rahelivao · Margit Gruner ·
Hanta Andriamanantoanina · Ingmar Bauer ·
Hans-Joachim Knölker

Received: 5 August 2015 / Accepted: 18 August 2015 / Published online: 10 September 2015
© The Author(s) 2015. This article is published with open access at Springerlink.com

Abstract Eight species of brown algae (Phaeophyceae) from the coast of Madagascar have been investigated for their chemical constituents. Fucosterol (3) was obtained as the most abundant compound. The brown alga Sargassum ilicifolium was the source for the first isolation of the terpenoid C_{27}-alcohol 1,1',2-trinorsqualenol (1) from marine sources. From S. incisifolium we isolated the highly unsaturated glycolipid 1-O-palmitoyl-2-O-stearidonoyl-3-O-β-D-galactopyranosylglycerol (4) and we report the first full assignment of its 1H and 13C NMR data. Apo-9′-fucoxanthinone (8) along with 24-ketocholesterol (5), (22E)-3β-hydroxycholesta-5,22-dien-24-one (6), and saringosterol (7) were obtained from Turbinaria ornata. The crude extracts of all eight species of brown algae exhibited a pronounced antimicrobial activity against the Gram-positive bacteria Bacillus cereus, Staphylococcus aureus, and Streptococcus pneumoniae.

Graphical Abstract

Keywords Brown algae · Steroids · Terpenoids · NMR spectroscopy · Antimicrobial activity

1 Introduction

Marine algae have come more and more into focus as promising sources of novel and potentially bioactive primary and secondary metabolites [1–8]. The coastal waters of Madagascar, the fourth largest island in the world with nearly 5000 km of coastline, are inhabited by a wealth of marine organisms. Recently, we published a study on the chemical composition of various red algae collected at
diverse places at the coast of Madagascar [9]. Among others, brominated indols, A-ring contracted steroidal and debilone have been isolated from these species. To date, only few representatives of brown algae from Madagascar have been investigated for their chemical constituents. For the brown algae *Spatoglossum* sp., *Sargassum* sp. 1, *Sargassum* sp. 4, *Zonaria* sp., *Chnoospora* sp., and *Spatoglossum* sp. Andriamanantoina and Rinaudo identified alginate polymers which are composed of (1→4)-β-D-mannuronic acid (M) and (1→4)-α-L-guluronic acid (G) units [10, 11]. They examined the influence of the block distribution (MM and GG) along the alginate chain on their gel forming ability under acidic conditions. Recently, Andriamanantoina and coworkers investigated the polysaccharide fraction of *Sargassum* sp., *Hormophysa* sp., and *Turbinaria* sp. and identified gel forming alginites [12]. The present study aims at the investigation of the chemical constituents of the nonpolar fractions from brown algae collected at the coast of Madagascar.

### 2 Results and Discussion

#### 2.1 *Sargassum ilicifolium*

Samples of brown seaweeds of *Sargassum ilicifolium* were extracted with methanol. Bioassay-guided fractionation of the crude methanol extract of *S. ilicifolium* led to the isolation of 1,1’,2-trinorsqualenol (1) along with stigmasta-5,28-dien-3β-ol (2) and fucosterol (3) (Fig. 1). The latter has long been known as the predominant sterol of Phaeophyceae [13, 14]. Compound 1 was isolated as colorless oil. The molecular mass of 386 was obtained from EI (m/z = 386 [M+]¹) and ESI mass spectra (m/z = 404.4 [M + NH₄]⁺). This information combined with ¹H and ³C NMR data supported the molecular formula C₂₇H₄₆O. The ¹H NMR spectrum displayed signals for 46 protons including five vinylic protons at δ = 5.09–5.15 ppm (m, 5 H, C=CH).

The ³C NMR and DEPT data of 1 revealed the presence of six methyl groups (C-22, C-23, C-24, C-25, C-26, C-27), five quaternary olefinic C-atoms (C-4, C-8, C-13, C-17, C-21), five olefinic CH moieties (C-5, C-9, C-12, C-16, C-20), one HO–CH₂ group (C-1), and ten CH₂ groups (C-2, C-3, C-6, C-7, C-10, C-11, C-14, C-15, C-18, C-19). A proton to carbon assignment could be achieved by an HSQC experiment. The location of the methyl groups and the olefinic moieties could be assigned on the basis of COSY, HMBC, and NOESY correlations. The ⁴E configuration of the double bonds in positions 4, 8, 12, and 16 was deduced from NOESY experiments by correlation of the methyl groups (H₃-23, H₃-24, H₃-25, H₃-26) with the respective allylic protons and absence of a correlation with the vinylic protons. The data obtained from 1D and 2D NMR experiments unambiguously led to the elucidation of compound 1 as (4E,8E,12E,16E)-4,8,13,17,21-pentamethyl docosa-4,8,12,16,20-penta-1,0-ol, which is also known as 1,1’,2-trinorsqualenol, with full assignment of all ¹H and ¹³C NMR signals. Our ¹H and ¹³C NMR data are in good agreement with those of the synthetic product described by Gref and coworkers [15] whereas the original data of Prestwich et al. [16] deviate slightly (Tables 1, 2).

1,1’,2-Trinorsqualenol (1) was first obtained as ³H-labeled isotopomer by Corey et al. from 2,3:22,23-dioxisosqualene [17]. Prestwich and coworkers synthesized the parent compound from 2,3-oxidosqualene by periodic acid cleavage of the epoxide and subsequent reduction of the aldehyde with sodium borohydride [16]. 1,1’,2-Trinorsqualenol (1) was found to be an active squalene oxidase inhibitor with an IC₅₀ value of 4 μM [16]. This finding induced several studies on the squalene oxidase inhibiting activity of 1,1’,2-trinorsqualenol (1) and congeners (e.g. Ref. [18, 19]). 1,1’,2-Trinorsqualenol (1) is readily available by synthesis from 2,3-oxidosqualene. However, there is only one previous report from 2010 by Li et al. who detected 1,1’,2-trinorsqualenol (1) as a natural product by GC–MS analysis of *Zanthoxylum* oil [20]. In the present work, we describe the first isolation of 1,1’,2-trinorsqualenol (1) from a marine organism and its full spectroscopic characterization.

Our sample of stigmasta-5,28-dien-3β-ol (2) from *S. ilicifolium* was obtained as an amorphous solid with a melting point of 119–120 °C. ¹H and ¹³C NMR data are in agreement with those of the synthetic sample reported by Djerassi et al. [21] and slightly deviate from those of the product isolated from *Saxifraga montana* H [22]. The ¹H NMR spectrum shows two sets of signals for H₃-26, H₃-27, and H-28 which indicates the presence of a mixture of C-24 epimers in a ratio of 1:1. This also accounts for the lower melting point of 119–120 °C of 2 obtained from *S. ilicifolium* compared to the pure epimers [(24R)-isomer: mp = 132–133 °C [21], (24S)-isomer: mp = 141–142 °C [21]]. Stigmasta-5,28-dien-3β-ol (2) was first obtained by Ikekawa via reduction of the corresponding propargylic...
alcohol [23]. Djerassi and coworkers synthesized both C-24 epimers of stigmasta-5,28-dien-3β-ol (2) and proved that the sample of Ikekawa was a 1:1 mixture of both isomers [21]. Isolations from living organisms were reported from the marine sponge Haliclona sp. [24], from the perennial herbaceous plant Saxifraga montana H. [22] and others. In 2014, stigmasta-5,28-dien-3β-ol (2) was isolated from the Chinese brown alga Sargassum thunbergii by the group of Guo [25]. The present report describes the first isolation of stigmasta-5,28-dien-3β-ol (2) from the brown alga S. ilicifolium.

In an antimicrobial agar diffusion test, the crude extract of S. ilicifolium was found to be very active against the Gram-negative bacteria Shigella boydii and Klebsiella oxytoca, the Gram-positive bacteria Streptococcus pneumoniae and Staphylococcus aureus, and the yeasts Candida membranaefaciens, Trichosporon macoides, and Cryptococcus neoformans (Table 3). Significant activity was also observed against the Gram-negative bacterium Enterobacter cloacae and the Gram-positive bacterium Bacillus cereus. No activity was found against the Gram-negative bacteria Pseudomonas aeruginosa, Escherichia coli, Salmonella enteridis, and the yeast Candida albicans.

### 2.2 Sargassum incisifolium

The brown alga Sargassum incisifolium was extracted with ethyl acetate. The ethyl acetate extract was adsorbed on silica gel and subjected to flash chromatography with diethyl ether and then methanol. Purification of the diethyl ether fraction by column chromatography afforded fucosterol (3). The methanol fraction was subjected to normal-phase column chromatography to give two fractions. Fraction 2 afforded a mixture of inseparable monogalactosyldiacylglycerols bearing fatty acid side chains with only few olefinic groups according to the intensity of vinylic signals in the 1H NMR spectrum. Fraction 1 was further purified by reversed-phase HPLC using THF–H2O (50:50–80:20) as mobile phase to afford 1-O-palmitoyl-2-O-stearidonoyl-3-O-b-D-galactopyranosylglycerol (4) (Fig. 2) as an amorphous gum. The [M – NH4]+ peak at m/z = 768.7 and the [M + NH4 + Na – H]+ peak at m/z = 790.6 in the ESI-mass spectrum of 4 in combination with the number and intensity of 1H and 13C NMR signals led to the molecular formula C43H74O10. According to ESI mass spectrometry minor amounts of several other monogalactosyldiacylglycerols bearing fatty acids with a lower degree of unsaturation are present in the mixture. This corresponds to findings of Marcolongo et al. who investigated the monogalactosyldiacylglycerol fraction isolated from the thermophilic blue-green alga Phormidium sp. ETS-05 and identified palmitic acid and stearidonic acid as main acyl components during their fatty acid analysis by GC [26].

The IR spectrum of 4 exhibited absorption bands corresponding to hydroxyl (3391 cm−1) and carboxylic ester

### Table 1

| Position | From Sargassum ilicifolium δH (600 MHz, CDCl3) | Synthetic product [15] δH (400 MHz, CDCl3)a | Synthetic product [16] δH (300 MHz, CDCl3)a |
|----------|-----------------------------------------------|---------------------------------------------|---------------------------------------------|
| 5        | 5.15 (m, 1 H)b                                | 5.03–5.23 (m, 5 H, C=CH)                    | 5.06 (br m, 5 H, C=CH)                      |
| 9        | 5.13 (m, 1 H)b                                |                                             |                                             |
| 12, 16   | 5.11 (m, 2 H)b                                |                                             |                                             |
| 20       | 5.09 (m, 1 H)b                                |                                             |                                             |
| 1        | 3.62 (t, J = 6.6 Hz, 2 H)                     | 3.63 (t, J = 6.4 Hz, 2 H, OCH3)             | 3.48 (t, J = 6.5 Hz, 2 H, OCH3)             |
| 6        | 2.07 (m, 2 H)b                                | 1.90–2.14 (m, 18 H, 9 CH3)                  | 1.92 (br m, 18 H, C=CH2)                    |
| 19       | 2.06 (m, 2 H)b                                |                                             |                                             |
| 3, 14    | 2.05 (m, 4 H)b                                |                                             |                                             |
| 10, 11   | 2.01 (m, 4 H)b                                |                                             |                                             |
| 7        | 1.98 (m, 2 H)b                                |                                             |                                             |
| 15, 18   | 1.97–1.95 (m, 4 H)b                           |                                             |                                             |
| 27       | 1.67 (s, 3 H)                                 | 1.68 (s, 3 H, CH3)                          |                                             |
| 2        | 1.66 (m, 2 H)b                                | 1.52–1.73 (m, 17 H, 6 CH3, CH2) [15]        | 1.58 (br s, 6 H, C-2 CH3, C-22 CH3)         |
| 23       | 1.60 (m, 3 H)b                                |                                             |                                             |
| 22, 24, 25, 26 | 1.59 (s, 12 H)b                             | 1.55, 1.51 (br s, 15 H, C-23, C-24, C-25, C-26, C-27 CH3) | 2.91 (br s, 1 H, OH) |

OH

**Table 1** 1H NMR spectroscopic data of 1

- **Position**: The chemical position in the molecule
- **From Sargassum ilicifolium δH (600 MHz, CDCl3)**: The chemical shifts for the sample from Sargassum ilicifolium in ppm
- **Synthetic product [15] δH (400 MHz, CDCl3)a**: The chemical shifts for the synthetic product in ppm
- **Synthetic product [16] δH (300 MHz, CDCl3)a**: The chemical shifts for another synthetic product in ppm

a Assignment based on comparison with our data from Sargassum ilicifolium. Original assignment in parentheses
b Chemical shift determined from the HSQC spectrum
(1738 cm\(^{-1}\)) groups. The 1D and 2D NMR spectra of 4 displayed signals for a carbon chain bearing four methylene-interrupted double bonds, a saturated carbon chain, a sugar moiety, a glycerol unit, and two carbonyl groups. The sugar residue could be identified as \(\beta\)-D-galactose by comparison of the \(^{13}\)C NMR shifts with those of various sugars listed in Ref. [27] and the typical proton coupling pattern. Most characteristic is the small coupling constant \((J = 3.0\) Hz) observed for the equatorial proton H-4 at \(\delta = 4.03\) ppm which indicates that both neighboring protons are in axial positions (cis relationship). The sugar moiety is attached to the glycerol core at the terminal position C-3 based on the HMBC correlations of the protons at C-3 at \(\delta = 3.76\) (dd, \(J = 11.1, 6.2, 1\) H, H-3b) and 3.92 ppm (dd, \(J = 11.3, 5.6\) Hz, 1 H, H-3a) with the carbon C-1 resonating at \(\delta = 103.98\) ppm (see Fig. S2a, center). This is also confirmed by NOESY correlations of the protons H-3a and H-3b with H-1 (see Fig. S4, S4b).

One of the fatty acid chains was identified as stearidonic acid (C18:4) containing four skipped double bonds. This was proven by the presence of \(^1\)H NMR signals for six methylene protons at \(\delta = 2.77–2.84\) ppm (m, 6 H, H\(_2\) and H\(_{14}\)) located between the double bonds. Correlations between the methylene and olefinic protons in the C18:4 acyl chain were observed in the COSY and NOESY spectra. The methylene protons at \(\delta = 2.34\) ppm (t, \(J = 7.7\) Hz, 2 H, H\(_{2}\) and H\(_{14}\)) in the C18:4 chain display a cross-peak with the carbonyl group at \(\delta = 173.50\) ppm (C-1) in the HMBC spectrum (Fig. S2a, bottom). The attachment of the stearidonoyl group at the central C-2 atom of the glycerol core was established by HMBC correlations of the protons at \(\delta = 5.29–5.44\) ppm (H-2 and

### Table 2 \(^{13}\)C NMR spectroscopic data of 1

| Position | From *Sargassum ilicifolium* \(\delta C (150\) MHz, CDCl\(_3\)) | Synthetic product [15] \(\delta C (100\) MHz, CDCl\(_3\))^a | Synthetic product [16] \(\delta C (75\) MHz, CDCl\(_3\))^a |
|----------|---------------------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| 13       | 135.14                                                        | 135.1 (C)                                       | 134.67                                           |
| 17       | 134.95                                                        | 134.9 (2 C)                                     | 134.58                                           |
| 8        | 134.92                                                        |                                                 | 134.48                                           |
| 4        | 134.58                                                        | 134.5 (2 C)                                     | 134.19 (C-4, C-8, C-13, C-17)                    |
| 21       | 131.26                                                        | 131.2 (C)                                       | 130.70 (C-21)                                    |
| 5        | 124.84                                                        | 124.8 (CH)                                      | 124.44                                           |
| 9        | 124.43                                                        | 124.4 (2 CH)                                    | 124.27                                           |
| 20       | 124.39                                                        |                                                 | 124.22                                           |
| 12, 16   | 124.26, 124.25                                                | 124.2 (2 CH)                                    | 124.12 (C-5, C-9, C-12, C-16, C-20)              |
| 1        | 62.84                                                         | 62.8 (CH\(_2\), CH\(_2\)O)                     | 62.12 (C-1)                                      |
| 11       | 39.74                                                         | 39.7 (CH\(_2\))                                 | See position 10                                  |
| 15       | 39.72                                                         |                                                 | See positions 6, 19                              |
| 18       |                                                              |                                                 | 39.56                                            |
| 7        | 39.65                                                         | 39.6 (CH\(_2\))                                 | 35.77                                            |
| 3        | 36.00                                                         | 35.9 (CH\(_2\))                                 | 31.44                                            |
| 2        | 30.69                                                         | 30.7 (CH\(_2\))                                 | 30.66                                            |
| 10       | 28.25                                                         | 28.2 (2 CH\(_2\))                               | 28.08 (C-10, C-11)                               |
| 14       | 26.76                                                         | 26.7 (CH\(_2\))                                 | See positions 2, 3, 7, 18                         |
| 19       | 26.65                                                         | 26.6 (CH\(_2\))                                 | 26.60, 26.42, 25.43 (C-6, C-15, C-19)            |
| 6        | 26.55                                                         | 26.5 (CH\(_2\))                                 |                                                 |
| 27       | 25.69                                                         | 25.6 (2 CH\(_3\))                               | See positions 24, 25, 26                         |
| 22       | 17.67                                                         | 17.6 (CH\(_3\))                                 | 22.50 (C-22)                                     |
| 24       | 15.99                                                         | 15.9 (2 CH\(_3\))                               | 15.74, 15.61, 13.88                             |
| 25, 26   | 15.84                                                         | 15.8 (CH\(_3\))                                 | (C-24, C-25, C-26, C-27)                        |

^a Assignment based on comparison with our data from *Sargassum ilicifolium*. Original assignment in parentheses
Table 3  Antimicrobial activities of the crude extracts of brown algae by agar diffusion testa

| Microbes                     | Hormophysea cuneiformis | Sargassum ilicifolium | Sargassum incisifolium | Sargassum polycystum | Sargassum sp. | Turbinaria conoides | Turbinaria decurrens | Turbinaria ornata |
|------------------------------|--------------------------|------------------------|------------------------|----------------------|---------------|---------------------|----------------------|-------------------|
| Gram-negative bacteria       |                          |                        |                        |                      |               |                     |                      |                   |
| Enterobacter cloacae ATCC 700323 | 6.5                      | 8                      | 6                      | 6.5                  | 6.5           | 6                   | 8                    | 9                 |
| Klebsiella oxytoca ATCC 8724 | 6.5                      | 9                      | 6.5                    | 6.5                  | 6             | 6.5                 | 9                    | 9                 |
| Shigella boydii ATCC 9204    | nt                       | 11                     | nt                     | nt                   | nt            | nt                  | nt                   | 12                |
| Pseudomonas aeruginosa ATCC 9027 | nt                       | 6                      | nt                     | nt                   | nt            | nt                  | nt                   | 6                 |
| Escherichia coli             | 6.5                      | 6                      | 6.5                    | 6                    | 6             | 6                   | 6                    | 6                 |
| Salmonella enteridis         | 6                        | 6                      | 6                      | 7                    | 6             | 6                   | 6                    | 6                 |
| Gram-positive bacteria       |                          |                        |                        |                      |               |                     |                      |                   |
| Bacillus cereus ATCC 13061   | 7.5                      | 8                      | 10                     | 9                    | 8.5           | 9                   | 9                    | 11                |
| Staphylococcus aureus ATCC 11632 | 12                      | 11                     | 13                     | 12.5                 | 13            | 12                  | 12                   | 13.5              |
| Streptococcus pneumoniae ATCC 6301 | 13                      | 13.5                   | 15                     | 13                   | 16            | 14                  | 13                   | 15                |
| Yeasts                       |                          |                        |                        |                      |               |                     |                      |                   |
| Candida albicans             | 6                        | 6                      | 6                      | 6                    | 6             | 6                   | 6                    | 6.5               |
| Candida membranaefaciens ATCC 201377 | nt                      | 13                     | nt                     | nt                   | nt            | nt                  | nt                   | 13                |
| Cryptococcus neoformans ATCC 76484 | nt                      | 9                      | nt                     | nt                   | nt            | nt                  | nt                   | 9                 |
| Trichosporon mucoides ATCC 204094 | nt                      | 10                     | nt                     | nt                   | nt            | nt                  | nt                   | 9                 |

a Each test was run in triplicate and the mean values are given; the solvent (methanol) was used for negative control
b Concentration of crude methanol extract: 1 mg/mL, 10 μL solution/6 mm disc; Ø < 7 mm: inactive, 7 mm ≤ Ø < 8 mm: slightly active, 8 mm ≤ Ø < 9 mm: significantly active, Ø ≥ 9 mm: very active; nt not tested
Monogalactosyldiacylglycerols (MGDG) and digalactosyldiacylglycerols are widely spread in nature particularly in chloroplast membranes [28–31]. Accordingly, they have also been found in brown algae. For example, Kim et al. isolated four monogalactosyldiacylglycerols from the brown alga _S. thunbergii_ collected at the coastal areas of Korea [32]. A series of monogalactosyldiacylglycerols has been identified by Liu and coworkers in _Sargassum horneri_ [33]. 1-O-Palmitoyl-2-O-stearidonoyl-3-O-β-D-galactopyranosyglycerol (4) has been mentioned as a trace component in a mixture of monogalactolipids obtained from _Sctyonema_ sp. as derived from enzymatic hydrolysis, HRMS, and EI-MS fragmentation studies [34]. A monogalactosyldiacylglycerol (MGDG) fraction that should contain considerable amounts of glycolipid 4 according to their fatty acid analysis by GC has been isolated by the group of Marcolongo from the thermophile blue-green alga _Phormidium_ sp. ETS-05 [26]. The presence of the stearidonoyl side chain, however, was not supported by their 1H and 13C NMR data. The MGDG fraction displayed anti-inflammatory activity in croton-oil-induced ear edema and carrageenan-induced paw edema in mice [35], and on human articular cartilage [36, 37]. Herein, we describe the first isolation of 1-O-palmitoyl-2-O-stearidonoyl-3-O-β-D-galactopyranosyglycerol (4) from a brown alga ( _Sargassum incisifolium_ ) with full assignment of the 1H and 13C NMR signals.

In the agar diffusion test, the crude extract of _Sargassum incisifolium_ exhibited very strong antimicrobial activity against the Gram-positive bacteria _S. pneumoniae_, _S. auratus_, and _B. cereus_ (Table 3). No activity was found against the Gram-negative bacteria, _Enterobacter cloacae_, _Klebsiella oxytoca_, _E. coli_, _S. enteridis_, and the yeast _C. albicans_. Moreover, the crude extract of _Sargassum incisifolium_ shows antimalarial activity with an IC50 value of 57.80 ± 1.91 μg/mL for inhibition of the FCM29 strain of _Plasmodium falciparum_.

2.3 Turbinaria ornata

The methanol extract of the marine brown alga _T. ornata_ was extracted with dichloromethane. After column chromatography on silica gel, the four known steroids fucosterol (3) [13, 14] (Fig. 1), 24-ketocholesterol (5) (first preparation: Ref. [38], first isolation from an alga: Ref. [39]) (Fig. 3), (22E)-3β-hydroxysterola-5,22-dien-24-one (6) (first isolation: Ref. [40], preparation: Ref. [41]), and saringosterol (7) (first isolations: Ref. [42, 43], synthesis: e.g., Ref. [21, 44, 45]) were isolated together with apo-9'-fucoxanthinone (8) (first report: Ref. [45], first isolation: Ref. [47]).

The mixture of 24-ketocholesterol (5) and enone 6 was obtained as a colorless, amorphous powder. The two components could be detected by GC–MS (5/6 = 3:1). The respective EI mass spectra showed molecular ion peaks at m/z = 400 and at m/z = 398 which could be assigned to the molecular formulae _C27H44O2_ and _C27H42O2_, respectively. In the 1H and 13C NMR spectra the signals for the steroidal backbone are identical. Only the signals for the
side chain could be differentiated and assigned to 5 or 6, respectively. The most significant differences are the signals for C-22/C-23 and H-22/H-23. Compound 6 displayed the typical chemical shifts for olefinic protons and carbon atoms at these positions (δ_H = 6.08 (d, J = 15.4 Hz, 1 H, H-23), 6.73 (dd, J = 15.4, 8.7 Hz, 1 H, H-22); δ_C = 126.2 (C-23), 152.5 (C-22)). Comparison of the 1H and 13C NMR data with those reported in the literature (5: Ref. [48], 6: Ref. [40, 49]) confirmed the identity of compounds 5 and 6. While numerous papers are available on the isolation of 5 from various kinds of organisms, enone 6 has been mentioned less frequently and has been found only in marine organisms.

Saringosterol (7) was obtained as a colorless, amorphous solid. Beside the signals for the steroidal backbone including the methyl groups, the 1H NMR spectrum revealed the presence of an additional double bond by three additional signals for olefinic protons. Signal doubling of the olefinic protons H-29 [δ_H = 5.12 (dd, J = 10.9, 1.5 Hz, 1 H, H-29a), 5.13 (dd, J = 10.9, 1.5 Hz, H-29a), 5.17 (dd, J = 17.3, 1.5 Hz, H-29b), 5.18 (dd, J = 17.3, 1.5 Hz, H-29b)] and the carbon atoms C-28 (δ_C = 142.55, 142.46) and C-29 (δ_C = 112.93, 112.84) indicated the presence of a 1:1 mixture of C-24 epimers. The 1H NMR data of the side chain are in agreement with those reported by Djerassi et al. [21]. The respective 13C NMR shifts have been compared with those published by the groups of Ayyad [45] and Wang [50]. In general a good agreement has been observed. The signals for C-28 and C-29 reported by the group of Ayyad are about 4–5 ppm upfield shifted compared to the values of Wang and us.

Saringosterol (7) has been isolated from diverse organisms, in particular brown algae (for examples see Ref. [42, 43, 50–54]). A variety of bioactivities has been disclosed for this compound including antitrypanosomal activity [54], selective LXRβ agonist activity (potential cholesterol reducing agent) [55], and inhibition of Mycobacterium tuberculosis growth [56].

Compound 8 was isolated as an amorphous solid with a melting point of 84–85 °C and a specific optical rotation of [α]_D^20 = −18.0 (c = 0.05, MeOH). The molecular formula was determined as C_{18}H_{22}O_{4} from the [M + NH_4]^+ ion at m/z = 284 in the ESI–MS in combination with the number and intensities of the 1H and 13C NMR signals. Compound 8 shows a characteristic IR absorption band at ν = 1937 cm⁻¹ indicating the presence of an allene group. The 1H NMR spectrum displayed signals for 21 protons which could be assigned to five methyl, two methylene and two methine groups according to the DEPT data. The 13C NMR spectrum displayed resonances for 15 carbon atoms including two carbonyl groups at δ_C = 196.63 and 170.34 ppm in CDCl_3 and at δ_C = 200.86 and 172.41 ppm in CD_3OD and two oxygenated carbon atoms at δ_C = 72.05 and 67.37 ppm in CDCl_3 and 72.38 and 69.13 ppm in CD_3OD (Table 4). The 13C NMR (CDCl_3) signals for the carbon atoms of the allene moiety appear at δ_C = 118.45 (C-6), 209.47 (C-7), and 101.41 ppm (C-8). Comparison of the NMR data with those reported for apo-9'-

### Table 4 1H and 13C NMR spectroscopic data of apo-9’-fucoxanthinone (8) in CD_3OD

| Position | Turbinaria ornata | Amphidinium sp. [47] | Turbinaria ornata | Amphidinium sp. [47] |
|----------|------------------|----------------------|------------------|----------------------|
|          | δ_H (600 MHz)    | J (Hz)               | δ_H (600 MHz)    | J (Hz)               |
| 1        | 1.56 dd          | 12.8, 11.6           | 1.60 dd          | 12.7, 11.5           |
| 2 ax     | 2.00 ddd         | 12.8, 4.1, 2.2       | 2.04 ddd         | 12.7, 4.1, 2.2       |
| eq       | 5.38 tt          | 11.6, 4.1            | 5.42 tt          | 11.5, 4.1            |
| 4 ax     | 1.46 br t        | 12.1                 | 1.49 dd          | 12.7, 11.5           |
| eq       | 2.23 ddd         | 12.8, 4.1, 2.2       | 2.27 ddd         | 12.7, 4.1, 2.2       |
| 5        | 8.56 s           | 9.0 s                | 101.41           | 101.5                |
| 9        | 200.86           |                     | 200.86           |                     |
| 10       | 2.20 s           | 2.24 s               | 26.81            | 26.9                 |
| 11       | 1.43 s           | 1.46 s               | 29.40            | 29.5                 |
| 12       | 1.17 s           | 1.20 s               | 32.22            | 32.3                 |
| 13       | 1.39 s           | 1.43 s               | 30.74            | 30.8                 |
| 14       |                  | 172.41               | 172.5            |                     |
| 15       | 2.03 s           | 2.07 s               | 21.34            | 21.4                 |
fucoxanthinone (8) by Kobayashi et al. confirmed the identity of the compound (Table 4) [47]. Apo-9′-fucoxanthinone (8) was first reported as an oxidative degradation product of fucoxanthin [46, 57]. The structure and the absolute configuration of 8 were established by synthesis and X-ray crystallographic analysis [58]. Apo-9′-fucoxanthinone (8) has been isolated, for example, from the cultured marine dinoflagellate Amphidinium sp. [46] and from the brown algae Scytophron lomentaria [59], S. thunbergii [60], and Cladophorophus sp. f. verticillatus [61]. This is the first report on the isolation of 8 from T. ornata. It has been disclosed previously that apo-9′-fucoxanthinone (8) exhibits cytotoxicity against murine lymphoma L-1210 and human epidermoid carcinoma KB cells in vitro with IC50 values of 0.29 and 0.24 μg/mL, respectively [47].

In the agar diffusion test the crude extract of T. ornata showed very strong antimicrobial activity against the Gram-negative bacteria Shigella boydii, Enterobacter cloaceae, and Klebsiella oxytoca and the Gram-positive bacteria S. pneumoniae, S. aureus, and B. cereus (Table 3). An inhibiting activity was also detected against the yeasts Candida membranaefaciens, Trichosporon mucoides, and Cryptococcus neoformans.

2.4 Sargassum polycystum, Sargassum sp. (S. sect. Binderiana), Turbinaria decurrens, Turbinaria conoides, and Hornophyusa cuneiformis

Bioassay-guided fractionation of the methanol extracts of Sargassum polycystum, Turbinaria decurrens, Turbinaria conoides, and Hornophyusa cuneiformis led in all cases to the isolation of fucosterol (3). Also the crude extract of Sargassum sp. (S. sect. Binderiana) contained fucosterol (3) as major component, but in this case it was obtained as an inseparable mixture with β-sitosterol.

The crude methanol extracts of S. polycystum and Sargassum sp. (S. sect. Binderiana) exhibited very strong antimicrobial activities against Gram-positive bacteria, in particular against S. pneumoniae and S. aureus, and less pronounced against B. cereus (Table 3). The crude methanol extract of T. decurrens was very active against the Gram-negative bacterium Klebsiella oxytoca, the Gram-positive bacteria S. pneumoniae, S. aureus, and B. cereus, and against the yeasts C. membranaefaciens, Trichosporon mucoides, and Cryptococcus neoformans. Moreover, significant activity was observed against Enterobacter cloaceae. Kumar et al. reported various antimicrobial and cytotoxic activities of different extracts of T. conoides (J. Agardh) Kutting [62]. In our study, the crude methanol extract of T. conoides exhibited very strong activity against S. pneumoniae, S. aureus, and B. cereus. The crude methanol extract of H. cuneiformis, was very active against the Gram-positive bacteria S. pneumoniae and S. aureus and only slightly active against B. cereus.

Obviously, the crude methanol extracts of all species of brown algae investigated in the present study show a similar activity against various pathogenic microbes. In particular, in all cases a very strong activity against the Gram-positive bacteria S. pneumoniae and S. aureus was observed. This may be ascribed partly to the content of phytosterols, which have been isolated from all species of brown algae. The antibacterial activity of β-sitosterol, stigmasterol, and their acetates has been described earlier by Sharma [63]. Fucosterol (3) has been reported to show anti-oxidant and hepatoprotective activities in rats [64]. In addition, antihistaminic, anticholinergic, and antiviral activities have been described for fucosterol isolated from T. conoides (J. Agardh) Kutting [65]. Saringosterol (7) has been identified as active principle in the extract of Lessonia nigrescens for the inhibition of Mycobacterium tuberculosis [56].

3 Experimental Section

3.1 Plant Material

Samples of the brown algae H. cuneiformis, T. ornata, and T. conoides, were collected in October in Tamatave on the east coast of Madagascar. The marine algae of the species S. polycystum (BOL 169761), S. ilicifolium, Sargassum sp. (S. sect. Binderiana) (BOL 169760), and T. decurrens were collected in October in Beravy, located in Tuléar on the south-west coast of Madagascar. The brown alga Sargassum incisifolium (BOL 169752) was collected in May in Evatra, a small village north of Fort-Dauphin located in the south-east of Madagascar.

The brown algae have been identified by Dr. Lydiane Mattio and Professor Robert J. Anderson, Biological Sciences Department and Marine Research Institute, University of Cape Town, South Africa. Voucher specimens of three species of brown algae investigated in this study have been deposited at the Bolus herbarium (BOL) of the University of Cape Town, South Africa. The corresponding BOL accession numbers are given in brackets after the name of the algae.

3.2 Extraction and Isolation

General: The fresh seaweed was washed under tap water, rinsed with distilled water, subsequently dried at 48–50 °C using a universal drying oven (Binder FD 53, Germany), and then finely powdered in an Ultra Turrax Janke–Kunkel T25 S1 homogenizer (IKA, Germany) with a stitch of 1 mm. In all cases, except Sargassum incisifolium
(extraction with ethyl acetate), we extracted the dried and crushed samples of the algae with methanol. When checking the methanol extract by TLC, we obtained different results concerning the polarity of the compounds. Depending on whether the compounds were less polar (best eluted with diethyl ether) or more polar (best eluted with dichloromethane), we performed a second extraction with either diethyl ether or dichloromethane, respectively. Following this procedure, we obtained the non-polar compounds of the methanol extract that were purified by column chromatography.

The crushed and dried material of *S. ilicifolium* (500 g) was extracted with methanol. After removal of the solvent in vacuo, the methanol extract (11 g) was further extracted with different solvents of increasing polarity, namely diethyl ether, dichloromethane, and ethyl acetate. The successive extractions were carried out under magnetic stirring at room temperature. After removal of the solvent in vacuo, the diethyl ether extract (1.6 g) was subjected to flash chromatography on silica gel using pentane–diethyl ether (3:2) as eluent. A fraction of 10 mg of **1,1,2-trinorsqualenol** (**1**), **5,28-dien-3β-ol** (**2**), and **7β-carotene** (**3**) was further purified by column chromatography using pentane–diethyl ether (7:3) as eluent to afford 180 mg of fucosterol (**3**). The fraction eluting with methanol (1.0 g) was subjected to flash chromatography on silica gel with ethyl acetate–methanol (10:1) to obtain 500 mg of a product mixture. Subsequent column chromatography on silica gel with ethyl acetate as eluent afforded two fractions of 40 mg (first fraction) and 70 mg (second fraction). Both fractions were subjected to column chromatography on silica gel using ethyl acetate–methanol (20:1) as mobile phase. The first fraction (20 mg) was further purified by preparative HPLC (column: Vydac 208TP1030, reversed-phase C8, 30 × 250 mm; flow rate: 20 mL min⁻¹; eluent A: H₂O + 0.1 % TFA, eluent B: THF + 0.1 % TFA; gradient from 50 to 80 % B in 20 min) to yield 6 mg of the monogalactosyldiacylglycerol **4**. The second fraction afforded 5 mg of a mixture of monogalactosyldiacylglycerols.

The crushed material of *T. ornata* (680 g) was extracted with methanol. The methanol extract was concentrated under reduced pressure and the residue (17 g) was further extracted with dichloromethane. After removal of the solvent in vacuo, the residue (9.0 g) was subjected to flash chromatography on silica gel using dichloromethane as eluent. The resulting mixture (260 mg) was further purified by column chromatography on silica gel eluting with pentane–diethyl ether (3:2) to obtain four fractions. Fraction 1 (19 mg) was identified as fucosterol (**3**). Fraction 2 (5.0 mg) showed one spot on the TLC but was identified as a mixture of the two steroids **5** and **6** in a ratio of about 3:1 according to GC–MS and ¹H NMR spectroscopy. Fraction 3 (8.0 mg) was identified as saringosterol (**7**) and fraction 4 (19 mg) as apo-9′-fucoxanthinone (**8**).

The crushed and dried *S. polycystum* (3.4 g) was extracted with methanol. The organic extract was evaporated to dryness and a dark oily residue (85 mg) was obtained. The crude extract (85 mg) was then further extracted with dichloromethane and purified by column chromatography on silica gel using pentane–ethyl acetate (10:1) as mobile phase to afford 12 mg of fucosterol (**3**).

The crushed and dried plant material of *Sargassum* sp. (S. sect. Binderiana) (16 g) was minced and extracted exhaustively with methanol. After filtration, the organic extract was evaporated to dryness, and a dark oily residue was obtained. The crude extract (85 mg) was then further extracted with dichloromethane and purified by column chromatography on silica gel using pentane–ethyl acetate (10:1) as mobile phase to afford 12 mg of fucosterol (**3**).

The crushed and dried *Sargassum incrifolium* (80 g) was exhaustively extracted with ethyl acetate to afford 2.0 g of a crude product. The ethyl acetate extract (2.0 g) was subjected to flash chromatography on silica gel using diethyl ether and subsequently methanol as eluents. The fraction eluting with diethyl ether (1.0 g) was separated by column chromatography on silica gel using pentane–diethyl ether (3:1) to afford a main fraction of 310 mg which was further purified by column chromatography using pentane–diethyl ether (7:3) as eluent to afford 180 mg of fucosterol (**3**). The fraction eluting with methanol (1.0 g) was subjected to flash chromatography on silica gel with ethyl acetate–methanol (10:1) to obtain 500 mg of a product mixture. Subsequent column chromatography on silica gel with ethyl acetate as eluent afforded two fractions of 40 mg (first fraction) and 70 mg (second fraction). Both fractions were subjected to column chromatography on silica gel using ethyl acetate–methanol (20:1) as mobile phase. The first fraction (20 mg) was further purified by preparative HPLC (column: Vydac 208TP1030, reversed-phase C8, 30 × 250 mm; flow rate: 20 mL min⁻¹; eluent A: H₂O + 0.1 % TFA, eluent B: THF + 0.1 % TFA; gradient from 50 to 80 % B in 20 min) to yield 6 mg of the monogalactosyldiacylglycerol **4**. The second fraction afforded 5 mg of a mixture of monogalactosyldiacylglycerols.
The crushed material of *T. decurrens* (12 g) was repeatedly extracted with methanol and the combined extracts were concentrated under reduced pressure. The crude extract (300 mg) was subjected to column chromatography on silica gel with a mixture of pentane-diethyl ether (4:1) to give 19 mg of a product mixture which was further purified by a second column chromatography using the same conditions to afford 13 mg of fucosterol (3).

The crude extract of *T. conoides* was obtained after extraction of the crushed material (40 g) with methanol. After removal of the solvent, the residue (960 mg) was extracted with dichloromethane at room temperature. The dichloromethane extract (890 mg) was subjected to flash chromatography on silica gel with diethyl ether. The diethyl ether extract (890 mg) was subjected to flash chromatography on silica gel using pentane-diethyl ether fraction (190 mg) was separated by column chromatography on silica gel with diethyl ether. The diethyl ether extract (4:1) to give 19 mg of a product mixture which was separated by column chromatography on silica gel with pentane-diethyl ether (4:1) as mobile phase afforded 21 mg of fucosterol (3).

The dried plant material (9.4 g) of *H. cuneiformis* was minced and extracted exhaustively with methanol. After evaporation of the solvent, a dark oily residue (84 mg) was obtained. The crude extract (84 mg) was then repeatedly extracted with diethyl ether, and the combined diethyl ether extracts were concentrated under reduced pressure to give a residue of 65 mg. The diethyl ether extract (65 mg) was separated by column chromatography on silica gel using a mixture of pentane-ethyl acetate (4:1) as mobile phase afforded 1 mg of fucosterol (3).

### 3.3 Spectroscopic Characterization

**General:** Optical rotations were determined on a Perkin Elmer 341 polarimeter at a wavelength of 589 nm (sodium D line) using a 1.0-decimeter cell with a total volume of 1.0 mL. UV spectra were measured on a Perkin Elmer Lambda 25 UV–Vis spectrometer. Fluorescence spectra were measured on a Varian Cary Eclipse IR spectra were recorded on a Thermo Nicolet Avatar 360 E. S. P. FT-IR spectrometer using the ATR technique (attenuated total reflectance). NMR spectra were recorded on a Bruker AVANCE III 600 spectrometer. The chemical shifts δ are reported in ppm using the solvent signal as internal standard. Assignment of the 1H NMR and 13C NMR signals was achieved using the following 2D NMR experiments: COSY, HSQC, HMBC, NOESY, and HSQC-TOCSY. The mass spectra were measured by GC–MS coupling with an Agilent Technologies 6890 N GC system equipped with a 5973 N Mass Selective Detector (electron impact, 70 eV). ESI–MS were recorded on a Bruker–Esquire mass spectrometer with an ion trap detector; positive and negative ions were detected. Thin layer chromatography was performed on aluminum plates coated with silica gel 60-F254 (Merck). For visualization, the plates were analyzed under UV light or treated with a solution of 0.5 g vanillin dissolved in 100 mL of 80/20 (v/v) sulfuric acid/ethanol and subsequently heated. Analytical HPLC was carried out on an Agilent 1100 device equipped with a G1315B UV-DAD (detection at 215, 260 and 560 nm), G1321A fluorescence and an evaporative light scattering detector (ELS 1000, Polymer Laboratories) using a Vydac 208TP104 column (reversed-phase C8, 4.6 x 250 mm) under the following conditions: flow rate: 1.0 mL min⁻¹; eluent A: H₂O + 0.1 % TFA; eluent B: MeCN + 0.1 % TFA; gradient from 20 to 90 % B in 35 min. Preparative HPLC was carried out using a Varian ProStar Model 320 UV and an evaporative light scattering detector (ELS 1000, Polymer Laboratories) connected via a Sunchrom Quick-Split splitter.

### 4E,8E,12E,16E)-4,8,13,17,21-Pentamethyldecosadien-1-ol (139)

Stigmasta-5,28-dien-3β-ol (2) (1:1 mixture of C-24-epimers): Amorphous solid; mp = 119–120 °C (lit., 1:1 mixture of C-24-epimers: mp = 123–127 °C [23], (24R)-epimer: mp = 132–133 °C [21], (24S)-epimer: mp = 141–142 °C [21]); 1H NMR (600 MHz, CDCl₃, selected signals): δ (ppm) = 0.69 (s, 3 H, H-18), 0.85–0.91 (m, 6 H, H₂-26, H₃-27, two epimers), 1.02 (s, 3 H, H₃-19), 3.50–3.57 (m, 1 H, H-3), 5.17 (dd, J = 17.9, 1.4 Hz, 1 H, H-29a), 5.29 (ddd, J = 11.3, 2.6, 1.4 Hz, 1 H, H-29b), 5.35–5.38 (m, 1 H, H-6), 5.72–5.79 (m, 1 H, H-28, two epimers); 13C NMR (150 MHz, CDCl₃, selected signals): δ (ppm) = 121.50 (C-6), 136.86, 136.95 (C-28, two epimers), 140.90 (C-3), 156.69 (C-12), 136 (14), 121 (18), 95 (98), 81 (63), 69 (100), 55 (20), 41 (29); ESI–MS (25 V): m/z = 404.4 [M + Na]⁺.
Brown Algae (Phaeophyceae) from the Coast of Madagascar: Preliminary Bioactivity Studies 233

$J = 11.3, 5.6 \text{ Hz}, 1 \text{ H, H-3b}, 4.00 (dd, J = 12.2, 6.2 \text{ Hz}, 1 \text{ H, H-6''b}), 4.03 (d, J = 3.0 \text{ Hz}, 1 \text{ H, H-4''}), 4.22 (dd, J = 12.0, 6.4 \text{ Hz}, 1 \text{ H, H-1a}), 4.29 (dd, J = 7.5, 1.1 \text{ Hz}, 1 \text{ H, H-1''}), 4.40 (dd, J = 12.0, 3.4 \text{ Hz}, 1 \text{ H, H-1b}), 5.29-5.44 (m, 9 \text{ H, H-2, H-6', H-7', H-9', H-10', H-12', H-13', H-15', H-16'}),^{13}\text{C} \text{ NMR and DEPT (150 MHz, CDCl}_3\):} \delta (\text{ppm}) = 14.12 (C-16'), 14.27 (C-18'), 20.55 (C-17'), 22.69 (C-15'), 24.48 (C-3'), 24.87 (C-3''), 25.52, 25.61 (2 \text{ C} (C-8', C-11', C-14')), 26.82 (C-5'), 28.99 (C-4'), 29.05-29.76 (C-4''-C-13'), 31.92 (C-14'), 34.14 (C-2'), 34.28 (C-2'), 62.68 (C-1), 63.01 (C-6''), 68.46 (C-3), 69.55 (C-4''), 70.18 (C-2), 71.74 (C-2''), 73.44 (C-3''), 74.51 (C-5''), 103.98 (C-1''), 127.10 (C-15'), 127.89, 128.08, 128.16, 128.23, 128.30 (C-7', C-9', C-10', C-12', C-13'), 129.52 (C-6'), 131.97 (C-16), 137.50 (C-1'), 173.78 (C-1''); ESI–MS (+25 \text{ V}) \text{ of signals due to the presence of two epimers.}

Apo-9'-fucoxanthone (8): Amorphous solid; mp = 84–85 °C (lit., mp = 76.0–78.5 °C [66]); [\text{M}]^2_\text{D} = -18.0 (c = 0.05, MeOH) [lit., [\text{M}]^2_\text{D} = -23.0 (c = 0.04, MeOH) [67], [\text{M}]^2_\text{D} = -29.7 (EtOH) [68], [\text{M}]^2_\text{D} = -36.0 (c = 0.6) [66], [\text{M}]^2_\text{D} = -284 (c = 0.1, MeOH) [47], [\text{M}]^2_\text{D} = -25.8 (c = 0.1, CHCl}_3) [69]); CD \Delta \varepsilon \text{ MeOH} (\lambda \text{ nm}: -3.32 (206), +3.88 (229), -0.37 (257) [lit., CD \Delta \varepsilon \text{ EtOH} (\lambda \text{ nm}: -3.27 (207), +3.31 (230), -3.13 (257)] [47]); UV (MeOH): \lambda_{\text{max}} (228 \text{ nm}) = 228; fluorescence (MeOH): \lambda_{\text{max}} (238 nm) = 307 nm; IR (ATR): \nu (\text{cm}^{-1}) = 3433 (br), 2960, 2930, 2866, 2056, 1937, 1734, 1675, 1454, 1367, 1242, 1165, 1073, 1030, 958, 858, 818; \text{H NMR (600 MHz, CDCl}_3\):} \delta (\text{ppm}) = 1.15 (s, 3 \text{ H, H-2'), 1.40–1.46 (m, 1 \text{ H, H-2ax}, 1.78 (C-1')}; ESI–MS (+25 \text{ V}) \text{ of signals due to the presence of two epimers.}

24-Oxocholest-5-en-3β-ol (24-ketocholesterol) (5) (3:1 mixture with 6): Colorless amorphous solid; \text{H NMR (600 MHz, CDCl}_3\), selected signals): \delta (\text{ppm}) = 0.93 (d, \text{J = 6.8 Hz}, 3 \text{ H, H-21}), 1.10 (d, \text{J = 6.8 Hz}, 6 \text{ H, H-26, H-27}), 1.24–1.28' (m, 1 \text{ H, H-22a}), 1.37–1.43'' (m, 1 \text{ H, H-20}), 1.70–1.78' (m, 1 \text{ H, H-22b}), 2.36–2.40 (m, 1 \text{ H, H-23a}), 2.44–2.50 (m, 1 \text{ H, H-23b}), 2.64 (sept, \text{J = 7.1 Hz, 1 \text{ H, H-25'}}, \text{signals determined from HSQC spectrum};^{13}\text{C} \text{ NMR (150 MHz, CDCl}_3\), selected signals): \δ (\text{ppm}) = 18.31, 18.38 (C-26, C-27), 18.51 (C-21), 29.84 (C-22), 35.39 (C-20), 37.24 (C-23), 40.83 (C-25), 215.48 (C-24); GC–MS (EI, 70 \text{ eV}): \text{m/z} (%) = 400 (50), 382 (41), 314 (33), 271 (33), 255 (35), 213 (58), 145 (47), 105 (52), 43 (100).

Antimicrobial assay: The antimicrobial activities were determined using the agar diffusion technique in Petri dishes. The crude brown algal extracts were tested for their antimicrobial activity against three Gram-positive bacteria: \text{B. cereus (ATCC 13061)}, \text{S. aureus (ATCC 11632)}, and \text{S. pneumoniae (ATCC 6301)}; six Gram-negative bacteria: \text{Enterobacter cloacae (ATCC 700323), Klebsiella oxytoca (ATCC 8724)}, \text{Shigella boydii (ATCC 9204)}, \text{E. coli, S. enteridis, P. aeruginosa (ATCC 9027)}; and against four yeast strains: \text{C. albicans, C. membranaefaciens (ATCC 201377), Cryptococcus neoformans (ATCC 65207)}, and \text{Trichosporon mucoides (ATCC 204094)}. The pathogens were supplied by the Laboratoire de Microbiologie de l’Environnement (LME), Centre National de Recherche sur l’Environnement (CNRE), Antananarivo, Madagascar. The crude extracts were dissolved in methanol with a concentration of 1 mg/mL. A sample (10 \text{ µL}) of each solution was
added via a pipette onto a sterile antibiotic filter disc of 6 mm diameter and oven dried at 40–50 °C. The discs were placed on Müller–Hinton agar plates which had been inoculated with the microorganisms mentioned above. The plates were incubated for 24 h at 37 °C for the bacteria and for 48 h at 25 °C for the yeast. The diameters of the inhibition zones generated around the discs were measured (Ø in mm). The tests were performed in triplicate and the mean values were determined. Methanol, used to dissolve the extracts, was checked for the absence of antimicrobial activity. The diameters of the halos of inhibition can be rationalized on a qualitative basis as follows: Ø < 7 mm: inactive, 7 mm ≤ Ø < 8 mm: slightly active, 8 mm ≤ Ø < 9 mm: significantly active, Ø ≥ 9 mm: very active.

Antimalaria test: The antiplasmodial activity against the FCM29 strain of Plasmodium falciparum was determined by using the microfluorimetric assay previously reported [70]. The result is given as an IC₅₀ value in μg/mL.

4 Conclusions

We present one of the first extensive studies on the non-polar chemical constituents of brown algae from the coast of Madagascar. Our results confirm the early reports that fucosterol (3) is the major sterol of brown algae (Phaeophyceae) [14]. Moreover, the sterols stigmasta-5,28-dien-3β-ol (2), 24-ketocholesterol (5), (22E)-3β-hydroxycholesta-5,22-dien-24-one (6), and saringosterol (7) have been isolated from S. ilicifolium and T. ornata, respectively. The allenic norterpenoid apo-9'-fucoxanthinone (8) was obtained from T. ornata. For the first time, 1,1',2-trinorsqualenol (1) has been obtained as a pure compound from natural sources and moreover, its isolation from a marine organism (S. ilicifolium) is unprecedented. The glycolipid 1-O-palmitoyl-2-O-stearidonyl-3-O-β-D-galactopyranosylglycerol (4) was obtained from the brown alga Sargassum incisifolium and for the first time, we report the complete assignment of its 1H NMR and 13C NMR data. Antimicrobial tests of the crude extracts of the various brown alga revealed a strong activity against Gram-positive bacteria, in particular against S. aureus and Streptococcus pneumonia, which may ascribed to the content of phytosterols.

Acknowledgments Marie Pascaline Rahelivao is grateful to the European Commission (Erasmus Mundus Programme), the Gesellschaft von Freunden und Förderern der TU Dresden (GFF TU Dresden), and the TUD Graduiertenakademie for providing scholarships. We would like to thank Dr. Lydiane Matto and Professor Robert J. Anderson, Biological Sciences Department and Marine Research Institute, University of Cape Town, South Africa, for the identification of the brown algae. We are indebted to Mrs. Herivony Onja Andriambeloson, Laboratoire de Microbiologie de l’Environnement (LME), Centre National de Recherche sur l’Environnement (CNRE), Antananarivo, Madagascar, for the antimicrobial activity tests and Mr. Michel Ratsimbason, Centre National d’Application de Recherche Pharmaceutique (CNARP), Antananarivo, Madagascar, for providing the chloroquine-resistant FCM29 strain of Plasmodium falciparum.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. D.J. Faulkner, Nat. Prod. Rep. 19, 1–49 (2002)
2. A.J. Smit, J. Appl. Phycol. 16, 245–262 (2004)
3. R.K. Jha, X. Zi-rong, Mar. Drugs 2, 123–146 (2004)
4. K.H.M. Cardozo, T. Guaratini, M.P. Barros, V.R. Falcão, A.P. Tonon, N.P. Lopes, S. Campos, M.A. Torres, A.O. Souza, P. Colepicolo, E. Pinto, Comp. Biochem. Physiol. C: Toxicol Pharmacol. 146, 60–78 (2007)
5. L. O’Sullivan, B. Murphy, P. McLoughlin, P. Duggan, P.G. Lawlor, H. Hughes, G.E. Gardiner, Mar. Drugs 8, 2038–2064 (2010)
6. A.A. El Gamal, Saudi Pharm. J. 18, 1–25 (2010)
7. N. Sithranga Boopathy, K. Kathiresan, J. Oncol. 2010, Article ID 214186, doi: 10.1155/2010/214186
8. C.L.F. de Almeida, H.S. de Falcão, G.R.M. de Lima, C.A. de Montenegro, N.S. Lira, P.F. de Athayde-Filho, L.C. Rodrigues, M.D.F.V. de Souza, J.M. Barbosa-Filho, L.M. Batista, Int. J. Mol. Sci. 12, 4550–4573 (2011)
9. M.P. Rahelivao, M. Gruner, H. Andriamanantoanina, B. Andriamihaja, I. Bauer, H.-J. Könller, Mar. Drugs 13, 4197–4216 (2015)
10. H. Andriamanantoanina, M. Rinaudo, Polym. Int. 59, 1531–1541 (2010)
11. H. Andriamanantoanina, M. Rinaudo, Carbohydr. Polym. 82, 555–560 (2010)
12. M.P. Rahelivao, H. Andriamanantoanina, A. Heyraud, M. Rinaudo, Food Hydrocoll. 32, 143–146 (2013)
13. P.W. Carter, I.M. Heilbron, B. Lythgoe, Proc. R. Soc. London. Ser. B 128, 82–109 (1939)
14. G.W. Patterson, Lipids 6, 120–127 (1971)
15. F. Bekkara-Aounallah, R. Gref, M. Othman, L.H. Reddy, B. Pili, V. Allain, C. Bourgaux, H. Hillaireau, S. Lepeˆtre-Mouelhi, D. Desmaële, J. Nicolas, N. Chafi, P. Couvreur, Adv. Funct. Mater. 18, 3715–3725 (2008)
16. S.E. Sen, G.D. Prestwich, J. Am. Chem. Soc. 111, 1508–1510 (1989)
17. E.J. Corey, K. Lin, M. Jautelat, J. Am. Chem. Soc. 90, 2724–2726 (1968)
18. I. Abe, Y. Kashiwagi, H. Noguchi, Bioorg. Med. Chem. Lett. 10, 2525–2528 (2000)
19. G. Grosa, F. Viola, M. Ceruti, P. Brusa, L. Delprino, F. Dosio, L. Cattel, Eur. J. Med. Chem. 29, 17–23 (1994)
20. Y.C. Hu, M.Y. Li, Zhongguo Tiaowei pin 35, 93–116 (2010)
21. C.A.N. Catalan, W.C.M.C. Kokke, C. Duque, C. Djerassi, J. Org. Chem. 48, 5207–5214 (1983)
22. J.X. Liu, D.-L. Di, Y.-P. Shi, J. Chin. Chem. Soc. 55, 863–870 (2008)
23. Y. Fujimoto, M. Morisaki, N. Ikekawa, J. Chem. Soc. Perkin Trans. 1, 2302–2307 (1975)
24. L.M. Zeng, Z. Zeng, J.Y. Su, Chem. Res. Chin. Univ. 11, 174–177 (1995)
25. W.F. He, L.G. Yao, H.L. Liu, Y.W. Guo, J. Asian Nat. Prod. Res. 16, 685–689 (2014)
26. G. Marcolongo, F. de Appolonia, A. Venzo, C.P. Berrie, T. Carofiglio, C. Ceschi Berrini, Nat. Prod. Res. 20, 766–774 (2006)
27. H.O. Kalinowski, S. Berger, S. Braun, 13C-NMR-Spektroskopie (Georg Thieme, Stuttgart, 1984)
28. M. Kates, in Advances in lipid research, vol. 8, ed. by P. Rodolfo, K. David (Academic Press, New York, 1970), pp. 225–265
29. N. Murata, P.A. Siegenthaler, in Lipids in photosynthesis: Structure, Function and Genetics, ed. by P.A. Siegenthaler, N. Murata (Kluwer, Dordrecht, 1998), pp. 1–20
30. H. Wada, N. Murata, Lipids in photosynthesis: essential and regulatory functions (Springer, Dordrecht, 2009)
31. S. Amara, N. Barouh, J. Lecomte, D. Lafont, S. Robert, P. Villeneuve, A. de Caro, F. Carrière, Biochim. Biophys. Acta 1801, 508–516 (2010)
32. Y.H. Kim, E.H. Kim, C. Lee, M.H. Kim, J.R. Rho, Lipids 42, 395–399 (2007)
33. A.C. Ma, Z. Chen, T. Wang, N. Song, Q. Yan, Y.C. Fang, H.S. Guan, H.B. Liu, J. Agric. Food Chem. 62, 11157–11162 (2014)
34. V. Reshef, E. Mizrachi, T. Maretzki, C. Silberstein, S. Loya, A. Hizi, S. Carmeli, J. Nat. Prod. 60, 1251–1260 (1997)
35. A. Bruno, C. Rossi, G. Marcolongo, A. Di Lena, A. Venzo, C.P. Berrie, D. Corda, Eur. J. Pharmacol. 524, 159–168 (2005)
36. M. Lentì, C. Gentili, A. Pianezi, G. Marcolongo, A. Lalli, R. Cancetta, F.D. Cancetta, Nat. Prod. Res. 23, 754–762 (2009)
37. V. Ulivi, M. Lentì, C. Gentili, G. Marcolongo, R. Cancetta, F. Descalzi Cancetta, Arthritis Res. Ther. 13, R92 (2011)
38. B. Riegel, I.A. Kaye, J. Am. Chem. Soc. 66, 723–724 (1944)
39. A.M. Motzfeldt, Acta Chem. Scand. 24, 1846–1847 (1970)
40. P. Koch, C. Djerassi, V. Lakshmi, F.J. Schmitz, Helv. Chim. Acta 66, 2431–2436 (1983)
41. P. Koch, Y. Nakatani, B. Luu, G. Ourisson, Bull. Soc. Chim. Fr. 189–194 (1983)
42. N. Ikekawa, K. Tsuda, N. Morisaki, Chem. Ind. (London) 1179–1180 (1966)
43. N. Ikekawa, N. Morisaki, K. Tsuda, T. Yoshida, Steroids 12, 41–48 (1968)
44. W. Sucrow, B. Raddich, Chem. Ber. 103, 2711–2717 (1970)
45. S.E.N. Ayyyad, S.Z.A. Sowellim, M.S. El-Hosini, A. Abo-Atia, Z. Naturforsch. 58c, 333–336 (2003)
46. A. Jensen, Acta Chem. Scand. 18, 2005–2007 (1964)
47. Y. Doi, M. Ishibashi, N. Yamaguchi, J. Kobayashi, J. Nat. Prod. 58, 1097–1099 (1995)
48. S. Ogawa, G. Kakiyama, A. Muto, A. Hosoda, K. Mitamura, S. Ikegawa, A.F. Hofmann, T. Iida, Steroids 74, 81–87 (2009)
49. F. de Riccardis, L. Minale, M. Iorizzi, C. Debitus, C. Levi, J. Nat. Prod. 56, 282–287 (1993)
50. Z.H. Wu, T. Liu, C.X. Gu, C.L. Shao, J. Zhou, C.Y. Wang, Chem. Nat. Compd. 48, 158–160 (2012)
51. K. Tsuda, R. Hayatsu, Y. Kishida, S. Akagi, J. Am. Chem. Soc. 80, 921–925 (1958)
52. J.D. Newburger, J.J. Uebel, M. Ikawa, K.K. Andersen, R.B. Gagosian, Phytochemistry 20, 2042–2043 (1979)
53. T. Milkova, G. Talev, R. Christov, S. Dimitrova-Konakliova, S. Popov, Phytochemistry 45, 93–95 (1997)
54. S. Hoet, L. Pieters, G.G. Muccioli, J.-L. Habib-Jiwan, F.R. Opperdoes, J. Quetin-Leclercq, J. Nat. Prod. 70, 1360–1363 (2007)
55. Z. Chen, J. Liu, Z. Fu, C. Ye, R. Zhang, Y. Song, Y. Zhang, H. Li, H. Ying, H. Liu, J. Agric. Food Chem. 62, 6130–6137 (2014)
56. G.A. Wächter, S.G. Franzblau, G. Montenegro, J.J. Hoffmann, W.M. Maiese, B.N. Timmermann, J. Nat. Prod. 64, 1463–1464 (2001)
57. R. Bonnett, A.K. Mallams, A.A. Spark, J.L. Tee, B.C.L. Weedon, A. McCormick, J. Chem. Soc. C 3, 429–454 (1969)
58. J.R. Hlubucek, J. Hora, S.W. Russell, T.P. Toube, B.C.L. Wee- don, J. Chem. Soc. Perkin Trans. 1, 848–852 (1974)
59. K. Mori, T. Ooi, M. Hiraoka, N. Oka, H. Hamada, M. Tamura, T. Kusumi, Mar. Drugs 2, 63–72 (2004)
60. K.E. Park, Y.A. Kim, H.A. Jung, H.J. Lee, J.W. Ahn, B.J. Lee, Y. Seo, J. Korean Chem. Soc. 48, 394–398 (2004)
61. M. El Hattab, G. Culioli, R. Valls, M. Richou, L. Piovetti, Biochem. Syst. Ecol. 36, 447–451 (2008)
62. S.S. Kumar, Y. Kumar, M.S.Y. Khan, V. Gupta, E. De Clercq, Iran. J. Pharm. Res. 9, 411–416 (2010)
63. R.K. Sharma, Bioorg. Chem. 21, 49–60 (1993)
64. S. Lee, Y.S. Lee, S.H. Jung, S.S. Kang, K.H. Shin, Arch. Pharm. Res. 26, 719–722 (2003)
65. S.S. Kumar, Y. Kumar, M.S.Y. Khan, J. Anbu, E. De Clercq, Pharmacologyonline 1, 1104–1112 (2009)
66. T. Hashimoto, M. Tori, Y. Asakawa, Phytochemistry 30, 2927–2931 (1991)
67. H. Zhang, X. Xiao, M.M. Conte, Z. Khalil, R.J. Capon, Org. Biomol. Chem. 10, 9671–9676 (2012)
68. A. Baumeler, W. Brade, A. Haag, C.H. Eugster, Helv. Chim. Acta 73, 700–715 (1990)
69. P.B. Shinde, M.A. Kim, B.W. Son, C.O. Lee, J.H. Jung, Nat. Prod. Sci. 13, 365–368 (2007)
70. Y. Corbett, L. Herrera, J. Gonzalez, L. Cubilla, T.L. Capson, P.D. Coley, T.A. Kursar, L.I. Romero, E. Ortega-Barria, Am. J. Trop. Med. Hyg. 70, 119–124 (2004)