Marine Sponges of the Genus Callyspongia

Subjects: Chemistry, Organic
Contributor: Lucas Sousa

In this entry, information about the common metabolites of the Callyspongia genus were grouped, as well as studies of the biological activity of these compounds. Through NMR data, 212 metabolites were identified from genus Callyspongia (15 species and Callyspongia sp.), belonging to classes such as polyacetylenes, terpenoids, steroids, alkaloids, polyketides, simple phenols, phenylpropanoids, nucleosides, cyclic peptides, and cyclic depsipep-tides. A total of 109 molecules have been reported with bioactive activity, mainly cytotoxic and antimicrobial (antibacterial and antifungal) action.

Keywords: Callyspongia ; demosponges ; polyacetylenes ; anticancer action

1. Introduction

The genus *Callyspongia* (Callyspongiidae) encompasses a group of demosponges including 261 described species, of which approximately 180 have been accepted after taxonomic reviews. The marine organisms of *Callyspongia* are distributed in tropical ecosystems, especially in the central and western Pacific, but also in the regions of the Indian, the West Atlantic, and the East Pacific Oceans. The reason for the interest in the genus *Callyspongia* is related to its potential production of bioactive compounds. In this review, we group the chemical information about the metabolites isolated from the genus *Callyspongia*, as well as studies of the biological activity of these compounds. Through NMR data, 212 metabolites were identified from genus *Callyspongia* (15 species and *Callyspongia* sp.), belonging to classes such as polyacetylenes, terpenoids, steroids, alkaloids, polyketides, simple phenols, phenylpropanoids, nucleosides, cyclic peptides, and cyclic depsipeptides. A total of 109 molecules have been reported with bioactive activity, mainly cytotoxic and antimicrobial (antibacterial and antifungal) action.

2. Chemical Aspects of *Callyspongia* species

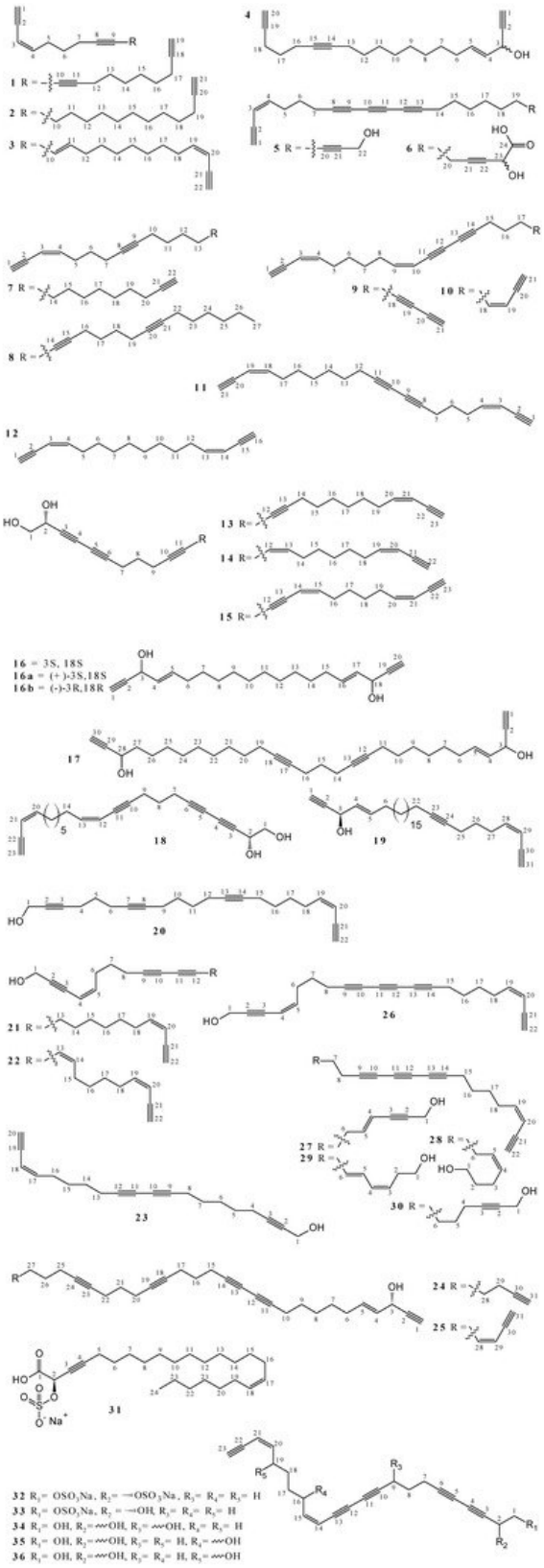
NMR spectroscopy-based studies on *Callyspongia* unidentified species (*Callyspongia* sp.) along with other 15 identified species (*C. abnormis*, *C. aerizusa*, *C. bilamellata*, *C. californica*, *C. diffusa*, *C. fibrosa*, *C. fistularis*, *C. flammea*, *C. implexa*, *C. lindgreni*, *C. pseudoreticulata*, *C. siphonella*, *C. spinosissima*, *C. truncata* and *C. vaginalis*) resulted in the structural characterization of 212 isolated metabolites from different classes: polyacetylenes; terpenoids and steroids; alkaloids; simple phenols and phenylpropanoids; nucleosides; cyclic peptides and cyclic depsipeptides; polyketides; and miscellaneous.

These substances were described according to the extract used in the isolation, relevant structural characteristics, and the elucidation data based on NMR data. This information is presented in together with additional information such as chemical formula, type of metabolite, one-dimensional NMR data, geographic location, and references related to the compound obtention in *Callyspongia* species. Regarding the 1D NMR data, the chemical shifts, the solvent and frequency used in process, and the coupling constant of all compounds, were investigated. In addition, although NMR was the only spectroscopic information reported in this study, mainly due to the large volume of data, other techniques were used in the primary studies to support structural identification and elucidation, such as: specific rotation, X-ray crystallography, Thin-Layer Chromatography (TLC), melting point, two-dimensional NMR spectroscopy, Mass Spectrometry (EM), and spectroscopy in the infrared (IR) and ultraviolet (UV) regions.

2.1. Polyacetylenes

The polyacetylenes aikupikanynes A (**1**), B (**2**) and C (**3**), D (**4**), E (**5**) and F (**6**) and octahydrosiphonochalyne (**7**) were isolated from methanol (MeOH) extract of *Callyspongia* sp., a red sea sponge [1]. Other metabolites were also isolated: callimplexen A (**8**) from *Callyspongia implexa* (MeOH/Dichloromethane (CH_2Cl_2) 1:1 extract) [2]; callyberynes A (**9**), B (**10**) and C (**11**) from *Callyspongia* sp. (MeOH/CH_2Cl_2 3:1 extract) [3]; **9** and **11** from *Callyspongia truncata* (MeOH extract) [4]; and the diacetylene Callydiyne (**12**) from *Callyspongia flammea* (MeOH extract) [5]. Polyacetylenes **1**–**12** (**Figure 1**) were elucidated by ^{1}H and ^{13}C NMR and have unsaturated hydrocarbon moieties associated with olefinic and alkynyl double

and triple bonds, respectively. The only symmetrical compound is **12** and structures **4**, **5** and **6** have characteristics of fatty acyls.



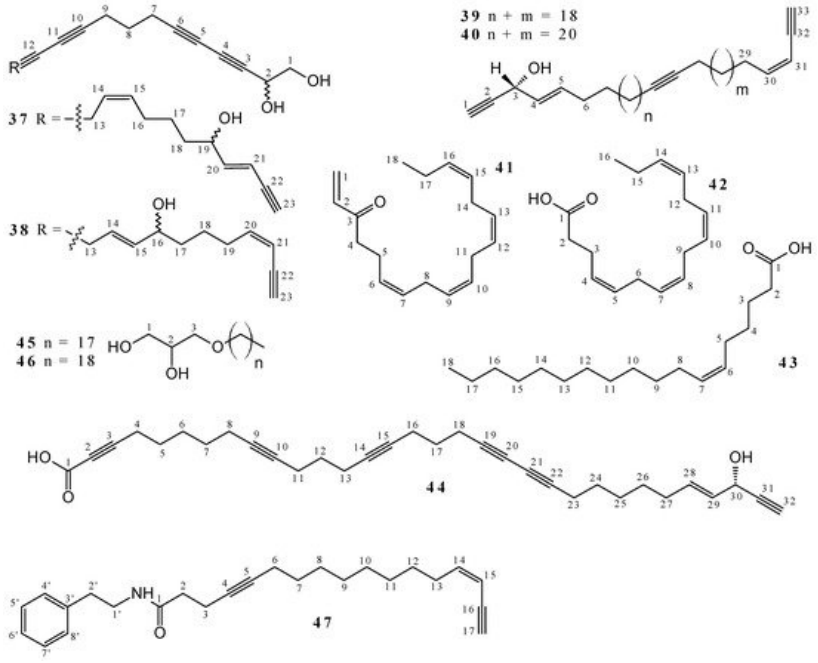


Figure 1. Structures of polyacetylenes isolated from *Callyspongia* species.

Six polyacetylene diols were obtained from studies based on *Callyspongia* genus. 14,15-dihydrosiphonodiol (**13**), Callyspongidiol (**14**) and siphonodiol (**15**) were isolated from Ethyl acetate (EtOAc) extract of *Callyspongia* sp. [6]; **13** and **15** from ethanol (EtOH) extract of *Callyspongia lindgreni* [7]; from these later, only **15** from *Callyspongia lindgreni* (CH_2Cl_2 extract) [8] and *Callyspongia truncata* (MeOH extract) [4]. Two isomeric structures were isolated from *Callyspongia* sp. (EtOH extract): (3*S*,18*S*,4*E*,16*E*)-eicosa-1,19-diyne-3,18-diol-4,16-diene (**16a**) and (−)-(4*E*,16*E*)-icosa-4,16-diene-1,19-diyne-3,18-diol (**16b**). Compound **16a** has also been identified in *Callyspongia pseudoreticulata* (MeOH extract) [9][10]. In addition, callyspongendiol (**17**) was isolated from *Callyspongia siphonella* (CH_2Cl_2/MeOH 1:1 extract) [11][12], and Tetrahydrosiphonodiol (**18**) from *Callyspongia lindgreni* (EtOH extract) [7]. Polyacetylene Diols **13**–**18** are open chain unsaturated hydrocarbons (**Figure 1**) that have their structures elucidated by ^{1}H and ^{13}C NMR. The regiochemistry patterns for the two hydroxyls in the structures vary considerably depending on the metabolite, having close proximity in **13**, **14**, **15** and **18**. Isomers **16a** and **16b** are the only structures with symmetric atom connectivity; they differ from each other according to the configuration of stereogenic centers.

A total of 12 polyacetylene alcohols were obtained from *Callyspongia* species: (3*R*,4*E*,28*Z*)-hentriacont-4,28-diene-1,23,30-triyn-3-ol (**19**), Callyspongenols A (**20**), B (**21**), C (**22**) and D (**23**), Callysponynes A (**24**) and B (**25**), dehydroisophonochalynol (**26**), siphonellanols A (**27**), B (**28**) and C (**29**) and siphonchalynol (**30**) (**Figure 1**). Studies involving *Callyspongia* sp. afforded different metabolites depending on the solvent used in the extraction: acetone (**19**) [13], MeOH/CH_2Cl_2 1:1 (**20**–**22** and **26**) [14] and EtOAc (**24** and **25**) [15] extracts; while those related to *Callyspongia siphonella* were obtained from MeOH/CH_2Cl_2 1:1 (**23** and **26**) [11][12] and MeOH (**26**–**30**) [16] extracts. The polyacetylene alcohols were elucidated by ^{1}H and ^{13}C NMR, but only **19**–**29** present elucidative data.

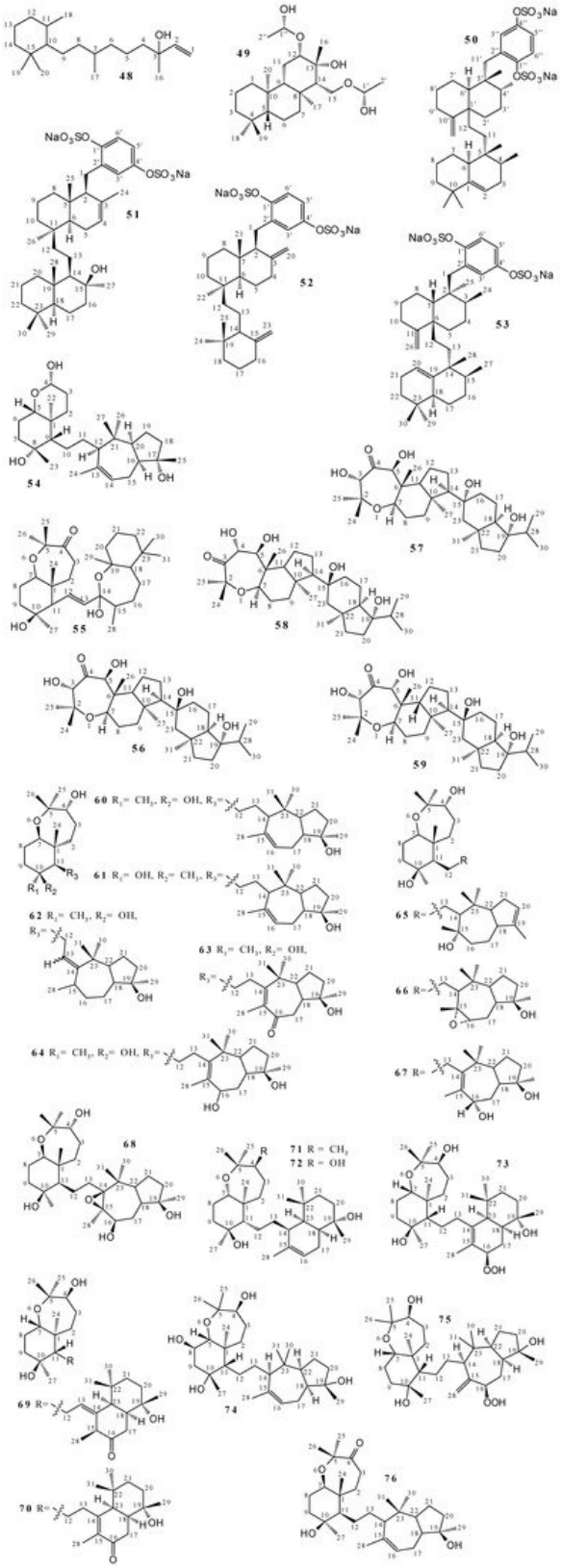
Studies involving *Callyspongia truncata* resulted in obtaining the acetylenic sulfate fatty acid callysponginol sulfate A (**31**) from a mixture of H_2O, MeOH, $CHCl_3$, and EtOAc extracts [17]. The methanolic extract provided callyspongins A (**32**) and B (**33**) [4][18], as well as callytriols A (**34**), B (**35**), C (**36**), D (**37**), and E (**38**) [4]. The polyacetylene lipids callyspongynes A (**39**) and B (**40**) were also isolated from an ethanolic extract of *Callyspongia* sp. [19]. The metabolites **32**–**40** were elucidated by ^{1}H and ^{13}C NMR and have an oxygenated and unsaturated aliphatic structure with double and triple bonds (**Figure 1**). Compounds **32** and **33** are derived from siphonodiol and along with **31** are classified as sulfated compounds. Metabolites **34**–**38** have three hydroxyls, while **39** and **40** are simple monoalcohol.

Four metabolites were isolated from ethanolic extracts from different species: (6*Z*,9*Z*,12*Z*,15*Z*)-1,6,9,12,15-octadecapenten-3-one (**41**) (*Callyspongia sp.*) [20], (4*Z*,7*Z*,10*Z*,13*Z*)-4,7,10,13-hexadecatetraenoic acid (**42**) (*Callyspongia sp.*) [20], petroselenic acid (**43**) (*Callyspongia siphonella*) [21], and callyspongynic Acid (**44**) (*Callyspongia truncata*) [22]. In addition, glycerolipid 3-octadecyloxy-propane-1,2-diol (**45**) was obtained from 95% EtOH + MeOH/CH_2Cl_2 1:1 extracts [23], and batyl alcohol (**46**) from methanolic extract, both from *Callyspongia fibrosa* [24]; the polyacetylenic amide callyspongamide A (**47**) was isolated from *Callyspongia fistularis* (MeOH/CH_2Cl_2 1:1 extract) [25][26][27]. Among the elucidated compounds, only **41**, **44**, **45**, and **47** have ^{1}H and ^{13}C NMR data reported. Compound **46** was characterized by ^{1}H NMR only, while **41** and **44**–**47** present the spectroscopic data. The metabolites are structurally distinct, but some similarities are visible (**Figure 1**). Substance **41** has a conjugated ketone system, while **42**–**44** have carboxyl groups,

among which **44** also has a hydroxyl unit. Glycerolipids **45** and **46** are the only saturated compounds having hydroxyls and ether oxygen, with the only structural difference between them being the presence of an additional methylene unit in **45**. Double and triple bonds, an aromatic unit, and an amide form compound **47**.

2.2. Terpenoids and Steroids

The diterpenes callyspinol (**48**) and isocopalanol (**49**) were isolated, respectively, from *Callyspongia spinosissima* (MeOH extract) [28] and *Callyspongia sp.* (acetone extract) [29]. Compounds **48** and **49** were elucidated by ^{1}H and ^{13}C NMR and are structurally different (**Figure 2**): **48** has only one ring and a double bond, and is monooxygenated, while **49** has a three-membered ring and is saturated and polyoxygenated. Four *Callyspongia* sp. triterpenes were also isolated: akaterpin (**50**) from an acetone extract [30] and ilhabelanol (**51**), ilhabrene (**52**), and isoakaterpin (**53**) from an extraction with EtOH followed by MeOH [31]. The molecules **50**–**53** (**Figure 2**) were characterized by ^{1}H and ^{13}C NMR and they are oxygenated, sulfated, and formed by cyclic and aromatic units.



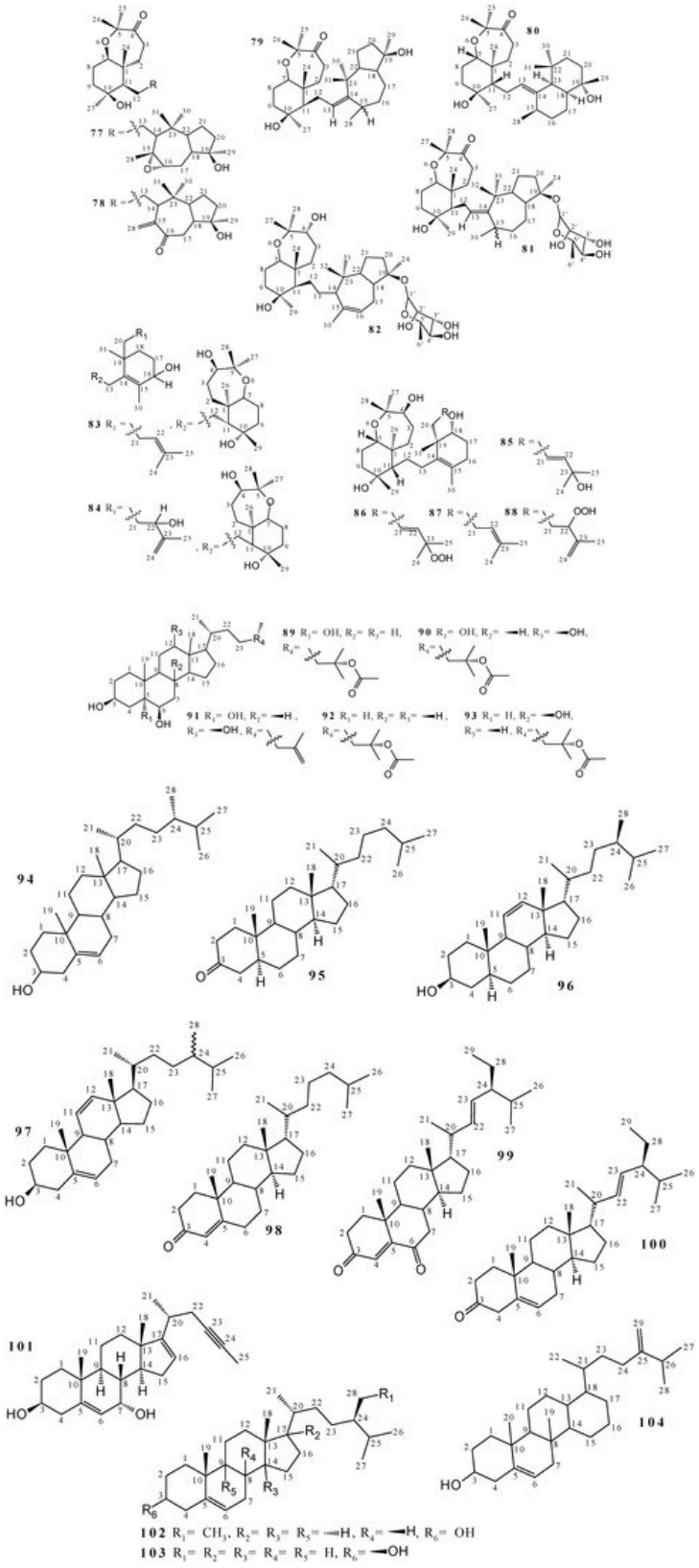


Figure 2. Structures of terpenoids and steroids from *Callyspongia* species.

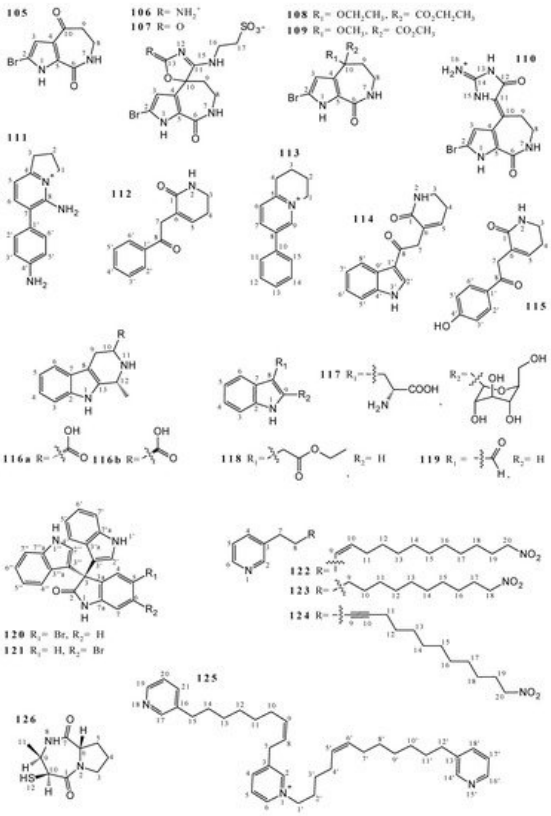
A total of 38 sipholane triterpenoids were isolated from *Callyspongia sipholena* (*Siphonochalina Siphonela*): (2*S*,4a*S*,5*S*,6*R*,8a*S*)-5-(2-((1*S*,3a*S*,5*R*,8a*S*,*Z*)-1-hydroxy-1,4,4,6-tetramethyl-1,2,3,3a,4,5,8,8a-octahydroazulen-5-yl)-ethyl)-4a,6-dimethyloctahydro-2H-chromene-2,6-diol (**54**) [32]; dahabinone A (**55**) [33]; neviotives A (**56**) [34][35][36][37], B (**57**) [33], C (**58**) [35], and D (**59**) [37]; sipholenols A (**60**) [21][11][38][35][36][37][39][40][41][42], B (**61**) [42], C (**62**) [42], D (**63**) [42], E (**64**) [42], F (**65**) [33], G (**66**) [33], H (**67**) [33], I (**68**) [40], J (**69**) [32], K (**70**) [32], L (**71**) [35], L (**72**) [11][32][36], M (**73**) [32], N (**74**) [37], and O (**75**) [37]; sipholenones A (**76**) [21][11][38][35][36][39][40][41][42], B (**77**) [42], C (**78**) [42], D (**79**) [33], and E (**80**) [32]; sipholenosides A (**81**) [33] and B (**82**) [33]; siphonellinol (**83**) [43] and siphonellinols B (**84**) [33], C (**85**) [40], C-23-hydroperoxide (**86**) [32], D (**87**) [32][37], and E (**88**) [32]. The extracts studied were: EtOAc (**54**, **60**, **69**, **70**, **72**, **73**, **76**, **80**, and **86**–**88**), EtOAc/MeOH 1:1 (**55**, **57**, **65**–**67**, **79**, **81**–**82**, and **84**), petroleum ether (**60**–**64**, **76**–**78**, and **83**), chloroform (**56**), CH_2Cl_2/MeOH 1:1 (**56**, **58**, **60**, **71**, **72**, and **76**), MeOH (**60**, **68**, **76**, and **85**), EtOH (**56**, **59**, **60**, **74**–**76**, and **87**) and EtOH 70% (**56**, **60**, **72**, and **76**) extracts. Molecules **63** and **67** present elucidating ^{1}H NMR data, and the other metabolites are fully characterized by both

^{1}H and ^{13}C NMR. Sipholane triterpenoids have distinct structures (**Figure 2**), which are composed of monocyclic and polycyclic rings, unsaturation, epoxy oxygen, ether, alcohol, and carbonyls.

Fifteen sterols were isolated from *Callyspongia* species: 24*S*-24-methyl-cholestane-3*β*,5*α*,6*β*,25-tetraol-25-mono acetate (**89**), 24*S*-24-methyl chelestane-3*β*,5*α*,6*β*,12*β*,25-pentaol-25-*O*-acetate (**90**), 24*S*-24-methyl cholest-25-ene-3*β*,5*α*,6*β*,12*β*-tetrol (**91**), 24*S*-24-methyl cholestane-3*β*,6*β*,25-triol-25-*O*-acetate (**92**), 24S-24-methyl cholestane-3*β*,6*β*,8*β*,25-tetraol-25-*O*-acetate (**93**) and 24*S*-24-methylcholesterol (**94**), *5*α-cholestanone (**95**), callysterol (**96** and **97**) or ergosta-5,11-dien-3*β*-ol (**97**), cholestenone (**98**), Stigmasta-4,22-dien-3,6-dione (**99**), stigmasterone (**100**), gelliusterol E (**101**), *β*-sitosterol (**102**), siphonocholin (**103**), and ergosta-5,24(28)-dien-3*β*-ol (**104**). The obtainment of these metabolites is associated with the following extracts: **89**–**94** to MeOH extract from *Callyspongia fibrosa* [24]; **95**, **96** [21], **98**–**100** [21], and **103** [44][45] to EtOH extract from *Callyspongia siphonella*; **97** [46] and **104** [11] to MeOH/CH_2Cl_2 1:1 extract from *Callyspongia siphonella* and, **101**, and **102** to MeOH/CH_2Cl_2 1:1 extract from *Callyspongia implexa* [2]. Compounds **89**–**94**, **97**, and **101** were elucidated by ^{1}H and ^{13}C NMR, while remaining compounds of this set do not present NMR data, but are compared with information from other studies. These compounds are four-ring sterols (**Figure 2**), with **89**–**103** being formed by three six-membered rings and one of five, while in **104** a four six-membered ring system is present.

2.3. Alkaloids

Several alkaloids were isolated and properly characterized from *Callyspongia* species. The bromopyrrole alkaloids 2-bromoaldisine (**105**), callyspongisines A (**106**), B (**107**), C (**108**), and D (**109**) and hymenialdisine (**110**) were obtained from the hydroalcoholic extract from *Callyspongia* sp. [47]. The bicyclic structures of compounds **105**–**110** were elucidated by ^{1}H and ^{13}C NMR and are formed by a seven-membered cyclic amide and a pyrrole attached to a bromine atom (**Figure 3**).



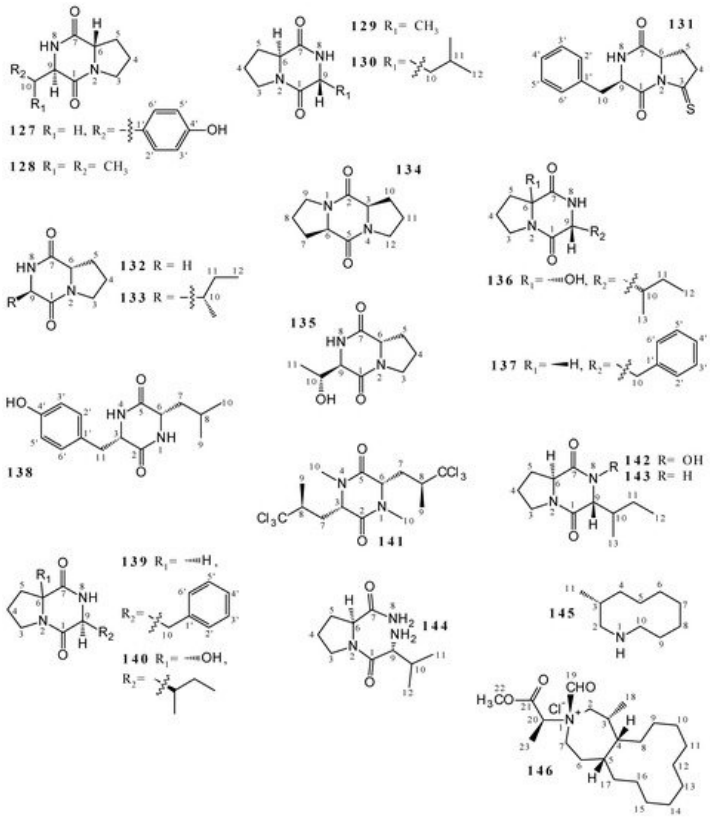


Figure 3. Structures of alkaloids isolated from *Callyspongia* species.

Some alkaloids were obtained from EtOH 95% extract of *Callyspongia* sp.: callyimine A (**111**) [48], callylactam A (**112**) [48], clathryimine B (**113**) [48], 3-(2-(1*H*-indol-3-yl)-2-oxoethyl)-5,6-dihydropyridin-2(1*H*)-one (**114**) [48], 3-(2-(4-hydroxyphenyl)-2-oxoethyl)-5,6-dihydropyridin-2(1*H*)-one (**115**) [48], (1*R*,3*R*)-1-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indole-3-carboxylic acid (**116a**) [49], (1*R*,3*S*)-1-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indole-3-carboxylic acid (**116b**) [49], C^2-*α*-*D*-mannosylpyranosyl-tryptophan (**117**) [49], Ethyl 2-(1*H*-indol-3-yl) acetate (**118**) [50], and the indol derivative 1*H*-indole-3-carbaldehyde (**119**) [50] (**Figure 3**). Molecules **111** and **113** are structurally similar due to the presence of aromatic rings and nitrogen as a heteroatom, while **112** and **115** are only differentiated by the presence of a hydroxyl group in **115**; and the structures **114** and **116a**-**119** are formed by an indol heterocycle. Metabolites **111**–**119** not present NMR data, but compare with information from others studies.

The isomers 5-bromo trisindoline (**120**) and 6-bromo trisindoline (**121**) were isolated from the ethanolic extract of *Callyspongia siphonella* [21], and they are differentiated by the position of bromine in the aromatic ring of the indole unit of the molecules. In addition, from *Callyspongia* sp. were isolated the untenines A (**122**), B (**123**), and C (**124**), from the methanolic extract [51], and niphatoxin C (**125**), from the mixture of CH_2Cl_2/MeOH 4:1 and MeOH extracts [52]. The **122**–**125** structures have the pyridine group in the molecule. Metabolites **120**–**125** (**Figure 3**) were determined by ^1H and ^{13}C NMR.

Studies of some sponges *Callyspongia* sp. resulted in the isolation of Callysponine (**126**), cyclo-(*S*-Pro-*R*-Tyr) (**127**), cyclo-(*S*-Pro-*R*-Val) (**128**), cyclo-(*S*-Pro-*R*-Ala) (**129**), cyclo-(*S*-Pro-*R*-Leu) (**130**), callysponine A (**131**), cyclo-(Gly-Pro) (**132**), cyclo-(Ile-Pro) (**133**), cyclo-(Pro-Pro) (**134**), cyclo-(Thr-Pro) (**135**), cyclo-(*R*-Pro-6-hydroxyl-*R*-Ile) (**136**), cyclo-(*R*-Pro-*R*-Phe) (**137**), cyclo-(*R*-Tyr-*R*-Phe) (**138**), cyclo-(*S*-Pro-*S*-Phe) (**139**), Staphyloamide A (**140**), dysamide A (**141**), callyspongidipeptide A (**142**), cyclo-((*S*)-Pro-(*R*)-Ile) (**143**), seco-((*S*)-Pro-(*R*)-Val) (**144**), (3*R*)-methylazacyclodecane (**145**), and callyazepin (**146**) (**Figure 3**). The analyzed metabolites were obtained from the following extracts: EtOH for **126–130** [53] and **141** [54], EtOH 95% for **129** and **130** [49][55], **136**–**140** [49] and **142**–**144** [55], EtOH/H_2O 9:1 for **131**–**135** [56][57][58][59][60][61][62][63], and MeOH + CH_2Cl_2 for **145** and **146** [64]. Only **126**, **130**, **131**, **136**, **141**, **142**, and **144**–**146** present ^1H and ^{13}C NMR data. The structures of **138**, **141**, **144**, and **145** are monocyclic, while **126**–**137**, **139**, **140**, **142**, **143**, and **146** are bicyclic.

3. Biological Aspects of Metabolites Isolated in *Callyspongia* species

3.1. Polyacetylenes

The aikupikanynes E (**5**) and F (**6**) from *Callyspongia* sp. showed moderate activity (with IC_{50} values of 5 and 10 µg/mL) against the cancer cell lines studied [1]. Other polyacetylenes obtained from *Callyspongia truncata* showed a potent

metamorphosis-inducing activity in the ascidian *Halocynthia roretzi* larvae (with ED_{100} values of 0.13–1.3 μg/mL) for **9**, **11**, **15**, and **32**–**38**, and antifouling activity against the barnacle *Balanus amphitrite* larvae (with ED_{50} values of 0.24–4.5 μg/mL) for **15** and **32**–**38** [4]. In addition, the inhibitory effect of the fertilization of starfish gametes of **32** and **33** in concentrations of 6.3 and 50 μM, respectively, [18].

Three polyacetylene diols were isolated from *Callyspongia* sp. and have driving Th1 polarization and antiproliferative effect against HL-60 (IC_{50} values: 6.5 μg/mL for **13**,**14** and 2.8 μg/mL for **15**) and HCT-15 (IC_{50} values: 21 μg/mL for **13**, 22 μg/mL for **14** and 34 μg/mL for **15**) [6]. **13**, **15** and **18** exhibited strong inhibitory activity against gastric H,K-ATPase (IC_{50} 1.0×10^{-5} M) [7][65]. The **16a** and **16b** isomers are weakly cytotoxic, with IC_{50} values of 0.47 for **16a** natural, 1.5 (± 0.29) for **16a** synthetic, 0.11 for **16b** natural and 0.35 (± 0.13) for **16b** synthetic against TR-LE and 1.8 (± 5.0) for **16a** and 5.3 (± 1.1) for **16b** synthetics against HeLa [10]. Other activities have been attributed to siphonodiol (**15**): medium antibacterial effect against *S. aureus* (MIC 12.5 μg/mL) and *S. pyrogenes* C-203 (MIC 6.2 μg/mL), and weak antifungal activity against *T. asteroids* (MIC 25.0 μg/mL) [8][65].

The metabolites **17** and **23** from *Callyspongia siphonella* proved to be weakly cytotoxic active against HCT-116. In addition, **17** and **26** were found to be weak cytotoxic against cells of MCF-7 with IC_{50} values of 65.7 and 73.6 μM, respectively, while **23** (IC_{50}: 11.7 μM) presented greater activities [12].

The compound (3*R*,4*E*,28*Z*)-hentriacont-4,28-diene-1,23,30-triyn-3-ol (**19**) has been reported to be cytotoxic against the NBT-II cell line at concentrations of 5 and 10 μg/mL [13]. The metabolites **20**–**22** and **26** are moderately cytotoxic against the P388 cell lines (IC_{50} values in μg/mL: 2.2 for **20**, **22**, and **26** and 10.0 for **21**) and HeLa (IC_{50} values in μg/mL: 4.5 for **20**, 10.0 for **21**, 3.9 for **22**, and 5.1 for **26**) [14]. Cytotoxic compounds **26**–**30** also have moderate activity against HeLa (IC_{50} values 23.9–26.5 μM), MCF-7 (IC_{50} values 54.9–69.2 μM), and A549 (IC_{50} values 58.5–63.4 μM) cell lines [16]. In vitro cytotoxicity activities of compounds **24** and **25** were evaluated and verified to fight MOLT-4 cell lines (IC_{50} values: 1.9 μM for both), K-562 (IC_{50} values 5.6–6.1 μM), and HCT 116 (IC_{50} values 5.4–7.0 μM), only **24** against T-47D (IC_{50} value: 8.9 μM) and **25** against MDA-MB-231 (IC_{50} value: 9.9 μM) [15].

Two interesting compounds were isolated from *Callyspongia truncata*, the Callysponginol sulfate A (**31**), which was found to inhibit MT1-MMP with an IC_{50} of 15.0 μg/mL [17], and Callyspongynic Acid (**44**), a α-glucosidase inhibitor with an IC_{50} of 0.25 μg/mL [22]. The glycerolipid Batyl alcohol **46** showed biofilm inhibition capacity for *Alteromona macleodii*, *Ochrobactrum pseudogrignonense*, *Vibrio harveyi*, and *Staphylococcus aureus* at 0.5 and 0.025 mg/mL [66]. The polyacetylenic amide callyspongamide A (**47**) was shown to be moderately cytotoxic against HeLa (IC_{50} of 4.1 μg/mL) [25].

3.2. Terpenoids and Steroids

The metabolites **60**, **72**, **76**, and **104**, from *Callyspongia siphonella*, proved to be weakly cytotoxic active against HCT-116, but **60**, **72**, and **76** were found to have moderate activity (at the respective IC_{50} values of 14.8 ± 2.33, 19.8 ± 3.78, and 95.8 ± 1.34 μM) [11]. In addition, **60** presented high cytotocix activity against cells of MCF-7 with IC_{50} values of 8.8 μM [12]. The effects on Reversing P-gp-Mediated MDR to colchicine involving the KB-3-1 cell lines were also tested (IC_{50} values in μM: 5.6 ± 0.5 for **54**, 4.8 ± 0.1 for **60**, 5.1 ± 0.3 for **72**, 4.7 ± 0.3 for **73**, 4.7 ± 0.4 for **80**, 4.2 ± 0.1 for **87** and 4.6 ± 0.6 for **88**) and KB-C2 (IC_{50} values in μM: 390 ± 40 for **54**, 140 ± 30 for **60**, 150 ± 10 for **72**, 780 ± 60 for **73**, 62 ± 11 for **80**, 180 ± 10 for **87** and 560 ± 50 for **88**) [32].

The isocopalanol (**49**) showed inhibition ability for the PANC-1 cell line with an IC_{50} of 0.1 μg/mL [29]. akaterpin (**50**) has been proven to inhibit PI-PLC (IC_{50} of 0.5 μg/mL) and neural sphingomyelinase (IC_{50} of 30 μg/mL) [30]. The sulfated meroterpenoids **51–53** are inhibitors of L-APRT at IC_{50} of 0.7, 0.7 and 1.05 μM, respectively, [31].

The metabolites **56**, **58**, **60**, and **71** showed activity against PC-3 (IC_{50} 7.9 ± 0.12–71.2 ± 0.34 μM) and A549 (IC_{50} 8.9 ± 0.01–87.2 ± 1.34 μM) cell lines, with compound **60** being the most active [35]. The cell lines MCF-7 (IC_{50} 3.0 ± 0.4–19.2 ± 0.6 μM) and HepG-2 (IC_{50} 2.8 ± 0.4–18.7 ± 0.9 μM) were tested for **56**, **60**, **71**, and **76**, and **76** had the most significant effect [36] (also obtained MCF-7 IC_{50} values of 1.162 for **60** and 0.9 μM for **76** [39]). In the same study, antiviral activity against HAV-10 was also weak for **56** and **71** (which also showed weak effectiveness against HSV-1) and moderate for **60** [36] (**60** is an inhibitor of P-gp too) [67]. In addition, the antimicrobial activities of **56** and **71** were measured , in which **56** obtained the greater result (12.7 ± 0.58–17.2 ± 0.58 mm) and **71** obtained a moderate one against gram positive bacteria only (12.3 ± 0.72–14.5 ± 0.72 mm) [36]. Compounds **56** and **59** also strongly inhibit RANKL-induced osteoclastogenesis with IC_{50} values of 32.8 and 12.8 μM, respectively, [37].

Sipholenol A (**60**) and sipholenone A (**76**) exhibited antiproliferative activity against +SA mouse mammary epithelial cells. While compound **76** was found to be a potential inhibitor (IC_{50} 20–30 μM), **60** had lower activity (IC_{50} 70 μM) [39].

Substances **60** and **76**, in addition to **85**, showed Reversal effects for KB-C2 [40], and **76** had both anti-angiogenic activity in CAM assay (0.026 μM per pellet) [39] and antibacterial activity [36]. In another study, substances **89**–**92** were associated with moderate antimalarial activity against *Plasmodium falciparum* [24], in which **89** showed the best result. Callysterol (**97**) showed an anti-inflammatory effect [46] and cholestenone (**98**) had an anti-metastatic effect on lung adenocarcinoma [67] [68]. Gelliusterol E (**101**) inhibited the formation and growth of *chlamydial trachomatis* (IC_{50} value 2.3 μM) [2], and siphonocholin (**103**) inhibited the production of violacein, being an Anti-QS and Anti-biofilm compound [44]. β-Sitosterol (**102**) was found to exhibit anthelminthic [69], antimutagenic (at 0.5 mg/kg inhibited the mutagenicity of tetracycline) [69], angiogenic [70], antibacterial [71][72][73], antifungal against *Fusarium* spp. [73], antidiabetic [71][74], analgesic [69][75], antipyretic [76], anti-inflammatory [69][75][76][77][78][79][80][81][82][83], cytotoxic [77][78][79][80][81][82][83], hypocholesterolemic [84], and immunomodulatory activities [85].

References

1. Youssef, D.T.A.; Yoshida, W.Y.; Kelly, M.; Scheuer, P.J. Polyacetylenes from a red sea sponge Callyspongia species. J. Nat. Prod. 2000, 63, 1406–1410.
2. Abdelmohsen, U.R.; Cheng, C.; Reimer, A.; Kozjak-Pavlovic, V.; Ibrahim, A.K.; Rudel, T.; Hentschel, U.; Edrada-Ebel, R.; Ahmed, S.A. Antichlamydial sterol from the red sea sponge Callyspongia aff implexa. Planta Med. 2015, 81, 382–387.
3. Umeyama, A.; Nagano, C.; Arihara, S. Three novel C21 polyacetylenes from the marine sponge Callyspongia sp. J. Nat. Prod. 1997, 60, 131–133.
4. Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. Seven new polyacetylene derivatives, showing both potent metamorphosis-inducing activity in ascidian larvae and antifouling activity against barnacle larvae, from the marine Sponge Callyspongia truncata. J. Nat. Prod. 1997, 60, 126–130.
5. Miao, S.; Andersen, R.J. Callydiyne, a new diacetylenic hydrocarbon from the sponge Callyspongla flammea. J. Nat. Prod. 1991, 54, 1433–1434.
6. Umeyama, A.; Matsuoka, N.; Mine, R.; Nakata, A.; Arimoto, E.; Matsui, M.; Shoji, N.; Arihara, S.; Takei, M.; Hashimoto, T. Polyacetylene diols with antiproliferative and driving Th1 polarization effects from the marine sponge Callyspongia sp. J. Nat. Med. 2010, 64, 93–97.
7. Fusetani, N.; Sugano, M.; Matsunaga, S.; Hashimoto, K. H,K-atpase inhibitors from the marine sponge Siphonochalina truncata: Absolute configuration of siphonodiol and two related metabolites. Tetrahedron Lett. 1987, 28, 4311–4312.
8. Tada, H.; Yasuda, F. Siphonodiol, a new polyacetylenic metabolite from the sponge Siphonochalina truncate. Chem. Lett. 1984, 13, 779–780.
9. Braekman, J.C.; Daloze, D.; Devijver, C.; Dubut, D.; Soest, R.W.M. A new C-20 polyacetylene from the sponge Callyspongia pseudoreticulata. J. Nat. Prod. 2003, 66, 871–872.
10. Shirouzu, T.; Watari, K.; Ono, M.; Koizumi, K.; Saiki, I.; Tanaka, C.; Soest, R.W.M.; Miyamoto, T. Structure, synthesis, and biological activity of a C-20 bisacetylenic alcohol from a marine sponge Callyspongia sp. J. Nat. Prod. 2013, 76, 1337–1342.
11. Sobahi, T.R.A.; Ayyad, S.E.N.; Abdel-Lateff, A.; Algandaby, M.M.; Alorfi, H.S.; Abdel-Naim, A.B. Cytotoxic metabolites from Callyspongia siphonella display antiproliferative activity by inducing apoptosis in HCT-116 cells. Pharmacogn. Mag. 2017, 13, 37–40.
12. Ayyad, S.E.N.; Angawy, R.; Alarif, W.M.; Saqer, E.A.; Badria, F.A. Cytotoxic polyacetylenes from the red sea sponge Siphonochalina siphonella. Z. Nat. C 2014, 69, 117–123.
13. Balansa, W.; Trianto, A.; Voogd, N.J.; Tanaka, J. A new cytotoxic polyacetylenic alcohol from a sponge Callyspongia sp. Nat. Prod. Commun. 2017, 12, 1909–1911.
14. Youssef, D.T.A.; Soest, R.W.M.; Fusetani, N. Callyspongenols A-C, new cytotoxic C22-polyacetylenic alcohols from a red sea sponge, Callyspongia species. J. Nat. Prod. 2003, 66, 679–681.
15. Chiu, C.W.; Su, H.J.; Lu, M.C.; Wang, W.H.; Sheu, J.H.; Su, J.H. Cytotoxic polyacetylenes from a formosan marine sponge Callyspongia sp. Bull. Chem. Soc. Jpn. 2014, 87, 1231–1234.
16. Ki, D.W.; El-Desoky, A.H.; Wong, C.P.; Abdel-Ghani, M.; El-Beih, A.A.; Mizuguchi, M.; Morita, H. New cytotoxic polyacetylene alcohols from the egyptian marine sponge Siphonochalina siphonella. J. Nat. Med. 2020, 74, 409–414.
17. Fujita, M.; Nakao, Y.; Matsunaga, S.; Soest, R.W.M.; Itoh, Y.; Seiki, M.; Fusetani, N. Callysponginol sulfate A, an MT1-MMP inhibitor isolated from the marine sponge Callyspongia truncata. J. Nat. Prod. 2003, 66, 569–571.

18. Uno, M.; Ohta, S.; Ohta, E.; Ikegami, S. Callyspongins A and B: Novel polyacetylene sulfates from the marine sponge Callyspongia truncata that inhibit fertilization of starfish gametes. J. Nat. Prod. 1996, 59, 1146–1148.

19. Rooney, F.; Capon, R.J. Callyspongynes A and B: New polyacetylenic lipids from a southern Australian marine sponge, Callyspongia sp. Lipids 1998, 33, 639–642.

20. Urban, S.; Capon, R.J. A New lipid from an australian marine sponge, Callyspongia sp. Lipids 1997, 32, 675–677.

21. El-Hawary, S.S.; Sayed, A.M.; Mohammed, R.; Hassan, H.M.; Rateb, M.E.; Amin, E.; Mohammed, T.A.; El-Mesery, M.; Muhsinah, A.B.; Alsayari, A.; et al. Bioactive brominated oxindole alkaloids from the red sea sponge Callyspongia siphonella. Mar. Drugs 2019, 17, 465.

22. Nakao, Y.; Uehara, T.; Matunaga, S.; Fusetani, N.; Soest, R.W.M. Callyspongynic acid, a polyacetylenic acid which inhibits α-glucosidase, from the marine sponge Callyspongia truncata. J. Nat. Prod. 2002, 65, 922–924.

23. Xiao-Jian, L.; Shi-Hai, X.; Qi-Chang, H.; Dong-Hong, H. Studies on chemical constituents from Callyspongia fibrosa. Chin. J. Spectrosc. Lab. 2005, 22, 281–283.

24. Rao, T.S.P.; Sarma, N.S.; Murthy, Y.L.N.; Kantamreddi, V.S.S.N.; Wright, C.W.; Parameswaran, P.S. New polyhydroxy sterols from the marine sponge Callyspongia fibrosa (Ridley & Dendly). Tetrahedron Lett. 2010, 51, 3583–3586.

25. Youssef, D.T.A.; Soest, R.W.M.; Fusetani, N. Callyspongamide A, a new cytotoxic polyacetylenic amide from the red sea sponge Callyspongia fistularis. J. Nat. Prod. 2003, 66, 861–862.

26. Rotem, M.; Kashman, Y. New polyacetylenes from the sponge Siphonochalina sp. Tetrahedron Lett. 1979, 20, 3193–3196.

27. Delbeke, E.I.P.; Everaert, J.; Uitterhaegen, E.; Verweire, S.; Verlee, A.; Talou, T.; Soetaert, W.; Bogaert, I.N.A.; Stevens, C.V. Petroselinic acid purification and its use for the fermentation of new sophorolipids. AMB Express 2016, 6, 28.

28. Garg, H.S.; Agraval, S. Callyspinol, a new diterpene from sponge Callyspongia spinossima. Tetrahedron Lett. 1995, 36, 9035–9038.

29. Kurnianda, V.; Faradilla, S.; Karina, S.; Agustina, S.; Ulfah, M.; Octavina, C.; Syahliza, F.; Ramadhan, M.R.; Purnawan, S.; Musman, M. Polyoxygenated diterpene produced by the indonesian marine sponge Callyspongia sp. as an inhibitor of the human pancreatic cancer cells. Microbiol. Indones. 2019, 13, 70–74.

30. Fukami, A.; Ikeda, Y.; Kondo, S.; Naganawa, H.; Takeuchi, T.; Furuya, S.; Hirabayashi, Y.; Shimoike, K.; Hosaka, S.; Watanabe, Y.; et al. Akaterpin, a novel bioactive triterpene from the marine sponge Callyspongia sp. Tetrahedron Lett. 1997, 38, 1201–1202.

31. Gray, C.A.; Lira, S.P.; Silva, M.; Pimenta, E.F.; Thiemann, O.H.; Oliva, G.; Hajdu, E.; Andersen, R.J.; Berlinck, R.G.S. Sulfated meroterpenoids from the brazilian sponge Callyspongia sp. are inhibitors of the antileishmaniasis target adenosine phosphoribosyl transferase. J. Org. Chem. 2006, 71, 8685–8690.

32. Jain, S.; Abraham, I.; Carvalho, P.; Kuang, Y.H.; Shaala, L.A.; Youssef, D.T.A.; Avery, M.A.; Chen, Z.S.; Sayed, K.A. Sipholane triterpenoids: Chemistry, reversal of ABCB1/P-glycoprotein-mediated multidrug resistance, and pharmacophore modeling. J. Nat. Prod. 2009, 72, 1291–1298.

33. Kashman, Y.; Yosief, T.; Carmeli, S. New triterpenoids from the red sea sponge Siphonochalina siphonella. J. Nat. Prod. 2001, 64, 175–180.

34. Carmely, S.; Kashman, Y. Neviotine-A, a new triterpene from the red sea sponge Siphonochalina siphonella. J. Org. Chem. 1986, 51, 784–788.

35. Ayyad, S.E.N.; Angawi, R.F.; Saqer, E.; Abdel-Lateff, A.; Badria, F.A. Cytotoxic neviotane triterpene-type from the red sea sponge Siphonochalina siphonella. Pharmacogn. Mag. 2014, 10, 334–341.

36. Al-Massarani, S.M.; El-Gamal, A.A.; Al-Said, M.S.; Al-Lihaibi, S.S.; Basoudan, O.A. In vitro cytotoxic, antibacterial and antiviral activities of triterpenes from the red sea sponge, Siphonochalina siphonella. Trop. J. Pharm. Res. 2015, 14, 33–40.

37. El-Beih, A.A.; El-Desoky, A.H.; Al-hammady, M.A.; Elshamy, A.I.; Hegazy, M.E.F.; Kato, H.; Tsukamoto, S. New inhibitors of RANKL-induced osteoclastogenesis from the marine sponge Siphonochalina siphonella. Fitoterapia 2018, 128, 43–49.

38. Shmueli, U.; Carmely, S.; Groweiss, A.; Kashman, Y. Sipholenol and Sipholenone, two new triterpenes from the marine sponge Siphonochalina Siphonella. Tetrahedron Lett. 1981, 22, 709–712.

39. Jain, S.; Shirode, A.; Yacoub, S.; Barbo, A.; Sylvester, P.W.; Huntimer, E.; Halaweish, F.; Sayed, K.A. Biocatalysis of the anticancer sipholane triterpenoids. Planta Med. 2007, 73, 591–596.

40. Jain, S.; Laphookhieo, S.; Shi, Z.; Fu, L.W.; Akiyama, S.I.; Chen, Z.S.; Youssef, D.T.A.; Soest, R.W.M.; Sayed, K.A. Reversal of P-glycoprotein-mediated multidrug resistance by sipholane triterpenoids. J. Nat. Prod. 2007, 70, 928–931.

41. Al-Lhaibi, S.S.; Abdel-Lateff, A.; Alarif, W.M.; Nogata, Y.; Ayyad, S.E.N.; Okino, T. Potent antifouling metabolites from re d sea organisms. Asian J. Chem. 2015, 27, 2252–2256.

42. Carmely, S.; Kashman, Y. The sipholanes: A novel group of triterpenes from the marine sponge Siphonochalina siphon ella. J. Org. Chem. 1983, 48, 3517–3525.

43. Carmely, S.; Loya, Y.; Kashman, Y. Siphonellinol, a new triterpene from the marine sponge Siphonochalina siphonella. Tetrahedron Lett. 1983, 24, 3673–3676.

44. Alam, P.; Alqahtani, A.S.; Husain, F.M.; Rehman, M.T.; Alajmi, M.F.; Noman, O.M.; Gamal, A.A.; Al-Massarani, S.M.; Kh an, M.S. Siphonocholin isolated from red sea sponge Siphonochalina siphonella attenuates quorum sensing controlled virulence and biofilm formation. Saudi Pharm. J. 2020, 28, 1383–1391.

45. Carmely, S.; Kashman, Y. The study of sipholanes by two-dimensional NMR spectroscopy. Magn. Reson. Chem. 1986, 24, 332–336.

46. Youssef, D.T.A.; Ibrahim, A.K.; Khalifa, S.I.; Mesbah, M.K.; Mayer, A.M.S.; Soest, R.W.M. New Anti-inflammatory sterols from the red sea sponges Scalarispongia aqabaensis and Callyspongia siphonella. Nat. Prod. Commun. 2010, 5, 27–3 1.

47. Plisson, F.; Prasad, P.; Xiao, X.; Piggott, A.M.; Huang, X.C.; Khalil, Z.; Capon, R.J. Callyspongisines A-D: Bromopyrrole alkaloids from an australian marine sponge, Callyspongia sp. Org. Biomol. Chem. 2014, 12, 1579–1584.

48. Yang, B.; Tao, H.; Zhou, X.; Lin, X.P.; Liu, Y. Two new alkaloids from marine sponge Callyspongia sp. Nat. Prod. Res. 2 012, 27, 1–5.

49. Yang, B.; Huang, J.; Lin, X.; Zhang, Y.; Tao, H.; Liu, Y. A new diketopiperazine from the marine sponge Callyspongia sp ecies. Rec. Nat. Prod. 2016, 10, 117–121.

50. Yang, B.; Hu, J.; Lei, H.; Chen, X.Q.; Zhou, X.F.; Liu, Y.H. Chemical constituents of marine sponge Callyspongia sp. fro m the south China sea. Chem. Nat. Compd. 2012, 48, 350–351.

51. Wang, G.Y.S.; Kuramoto, M.; Uemura, D.; Yamada, A.; Yamaguchi, K.; Yazawa, K. Three Novel Anti-mierofouling nitroal kyl pyridine alkaloids from the Okinawan marine sponge Callyspongia sp. Tetrahedron Lett. 1996, 37, 1813–1816.

52. Buchanan, M.S.; Carroll, A.R.; Addepalli, R.; Avery, V.M.; Hooper, J.N.A.; Quinn, R.J. Niphatoxin C, a cytotoxic tripyridin e alkaloid from Callyspongia sp. J. Nat. Prod. 2007, 70, 2040–2041.

53. Huang, R.M.; Ma, W.; Dong, J.D.; Zhou, X.F.; Xu, T.; Lee, K.J.; Yang, X.; Xu, S.H.; Liu, Y. A new 1,4-diazepine from sou th China sea marine sponge Callyspongia species. Molecules 2010, 15, 871–877.

54. Kapojos, M.M.; Abdjul, D.B.; Yamazaki, H.; Ohshiro, T.; Rotinsulu, H.; Wewengkang, D.S.; Sumilat, D.A.; Tomoda, H.; N amikoshi, M.; Uchida, R. Callyspongiamides A and B, sterol O-acyltransferase inhibitors, from the Indonesian marine sp onge Callyspongia sp. Bioorg. Med. Chem. Lett. 2018, 28, 1911–1914.

55. Yang, B.; Dong, J.; Zhou, X.; Yang, X.; Lee, K.J.; Wang, L.; Zhang, S.; Liu, Y. Proline-containing dipeptides from a mari ne sponge of a Callyspongia Species. Helv. Chim. Acta. 2009, 92, 1112–1117.

56. Chen, Y.; Peng, Y.; Gao, C.; Huang, R. A new diketopiperazine from South China Sea marine sponge Callyspongia sp. Nat. Prod. Res. 2014, 28, 1010–1014.

57. Sperry, S.; Crews, P. A novel alkaloid from the indo-pacific sponge Clathria basilana. Tetrahedron Lett. 1996, 37, 2389–2390.

58. Gopichand, Y.; Schmitz, F.J. Two novel lactams from the marine sponge Halichondria melanodocia. J. Org. Chem. 197 9, 44, 4995–4997.

59. Jayatilake, G.S.; Thornton, M.P.; Leonard, A.C.; Grimwade, J.E.; Baker, B.J. Metabolites from an antarctic sponge-asso ciated bacterium, Pseudomonas aeruginosa. J. Nat. Prod. 1996, 59, 293–296.

60. Adamczeski, M.; Reed, A.R.; Crews, P. New and known diketopiperazines from the Caribbean sponge, Calyx cf. Podat ypa. J. Nat. Prod. 1995, 58, 201–208.

61. Gautschi, M.; Schmid, J.P.; Peppard, T.L.; Ryan, T.P.; Tuorto, R.M.; Yang, X. Chemical characterization of diketopiperaz ines in beer. J. Agric. Food Chem. 1997, 45, 3183–3189.

62. Stark, T.; Hofmann, T. Structures, sensory activity, and dose/response functions of 2,5-diketopiperazines in roasted coc oa nibs (Theobroma cacao). J. Agric. Food Chem. 2005, 53, 7222–7231.

63. Fdhila, F.; Vázquez, V.; Sánchez, J.L.; Riguera, R. DD-diketopiperazines: Antibiotics active against Vibrio anguillarum is olated from marine bacteria associated with cultures of Pecten maximus. J. Nat. Prod. 2003, 66, 1299–1301.

64. Kim, C.K.; Woo, J.K.; Lee, Y.J.; Lee, H.S.; Sim, C.J.; Oh, D.C.; Oh, K.B.; Shin, J. Callyazepin and (3R)-methylazacyclo decane, nitrogenous macrocycles from a Callyspongia sp. sponge. J. Nat. Prod. 2016, 79, 1179–1183.

65. López, S.; Fernández-Trillo, F.; Midón, P.; Castedo, L.; Saá, C. First stereoselective syntheses of (−)-siphonodiol and (−)-tetrahydrosiphonodiol, bioactive polyacetylenes from marine sponges. J. Org. Chem. 2005, 70, 6346–6352.
66. Díaz, Y.M.; Laverde, G.V.; Gamba, L.R.; Wandurraga, H.M.; Arévalo-Ferro, C.; Rodríguez, F.R.; Beltrán, C.D.; Hernández, L.C. Biofilm inhibition activity of compounds isolated from two Eunicea species collected at the Caribbean Sea. Rev. Bras. Farmacogn. 2015, 25, 605–611.
67. Shi, Z.; Jain, S.; Kim, I.W.; Peng, X.X.; Abraham, I.; Youssef, D.T.A.; Fu, L.W.; Sayed, K.; Ambudkar, S.V.; Chen, Z.S. Sipholenol A, a marine-derived sipholane triterpene, potently reverses P-glycoprotein (ABCB1)-mediated multidrug resistance in cancer cells. Cancer Sci. 2007, 98, 1373–1380.
68. Ma, J.; Fu, G.; Wu, J.; Han, S.; Zhang, L.; Yang, M.; Yu, Y.; Zhang, M.; Lin, Y.; Wang, Y. 4-cholesten-3-one suppresses lung adenocarcinoma metastasis by regulating translocation of HMGB1, HIF1α and Caveolin-1. Cell Death Dis. 2016, 7, e2372.
69. Villaseñor, I.M.; Angelada, J.; Canlas, A.P.; Echegoyen, D. Bioactivity studies on β-sitosterol and its glucoside. Phytother. Res. 2002, 16, 417–421.
70. Choi, S.; Kim, K.W.; Choi, J.S.; Han, S.T.; Park, Y.I.; Lee, S.K.; Kim, J.S.; Chung, M.H. Angiogenic activity of β-sitosterol in the ischaemia/reperfusion-damaged brain of mongolian gerbil. Planta Med. 2002, 68, 330–335.
71. Beltrame, F.L.; Pessini, G.L.; Doro, D.L.; Filho, B.P.D.; Bazotte, R.B.; Cortez, D.A.G. Evaluation of the antidiabetic and antibacterial activity of Cissus sicyoides. Braz. Arch. Biol. Technol. 2002, 45, 21–25.
72. Sen, A.; Dhavan, P.; Shukla, K.K.; Singh, S.; Tejovathi, G. Analysis of IR, NMR and antimicrobial activity of β-sitosterol isolated from Momordica charantia. Sci. Secur. J. Biotech. 2012, 1, 9–13.
73. Kiprono, P.C.; Kaberia, F.; Keriko, J.M.; Karanja, J.N. The in vitro Anti-fungal and anti-bacterial activities of β-sitosterol from Senecio lyratus (Asteraceae). Verl. Z. Nat. 2000, 55, 485–488.
74. Zeb, M.A.; Khan, S.U.; Rahman, T.U.; Sajid, M.; Seloni, S. Isolation and biological activity of β-sitosterol and stigmasterol from the roots of Indigofera heterantha. Pharm. Pharmacol. Int. J. 2017, 5, 204–207.
75. Nirmal, S.A.; Pal, S.C.; Mandal, S.C.; Patil, A.N. Analgesic and anti-inflammatory activity of β-sitosterol isolated from Nyctanthes arbortristis leaves. Inflammopharmacology 2012, 20, 219–224.
76. Gupta, M.B.; Nath, R.; Srivastava, N.; Shanker, K.; Kishor, K.; Bhargava, K.P. Anti-inflammatory and antipyretic activities of β-sitosterol. Planta Med. 1980, 39, 157–163.
77. Loizou, S.; Lekakis, I.; Chrousos, G.P.; Moutsatsou, P. β-Sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. Mol. Nutr. Food Res. 2010, 54, 551–558.
78. Chai, J.W.; Kuppusamy, U.R.; Kanthimathi, M.S. Beta-sitosterol induces apoptosis in MCF-7 Cells. Malays. J. Biochem. Mol. Biol. 2008, 16, 28–30.
79. Awad, A.B.; Holtz, R.L.; Cone, J.P.; Fink, C.S.; Chen, Y.C. Beta-sitosterol inhibits growth of HT-29 human colon cancer cells by activating the sphingomyelin cycle. Anticancer Res. 1998, 18, 471–473.
80. Park, C.; Moon, D.O.; Rhu, C.H.; Choi, B.T.; Lee, W.H.; Kim, G.Y.; Choi, Y.H. β-sitosterol induces anti-proliferation and apoptosis in human leukemic U937 cells through activation of caspase-3 and induction of Bax/Bcl-2 ratio. Biol. Pharm. Bull. 2007, 30, 1317–1323.
81. Vundru, S.S.; Kale, R.K.; Singh, R.P. β-sitosterol induces G1 arrest and causes depolarization of mitochondrial membrane potential in breast carcinoma MDA-MB-231 cells. BMC Complement. Altern. Med. 2013, 13, 280.
82. Zhao, Y.; Chang, S.K.C.; Qu, G.; Li, T.; Cui, H. β-sitosterol inhibits cell growth and induces apoptosis in SGC-7901 human stomach cancer cells. J. Agric. Food Chem. 2009, 57, 5211–5218.
83. Holtz, R.L.; Fink, C.S.; Awad, A.B. β-sitosterol activates the sphingomyelin cycle and induces apoptosis in LNCaP human prostate cancer cells. Nutr. Cancer 1998, 32, 8–12.
84. Sugano, M.; Morioka, H.; Ikeda, I. A Comparison of hypocholesterolemic activity of β-sitosterol and β-sitostanol in rats. J. Nutr. 1977, 107, 2011–2019.
85. Fraile, L.; Crisci, E.; Córdoba, L.; Navarro, M.A.; Osada, J.; Montoya, M. Immunomodulatory properties of beta-sitosterol in pig immune responses. Int. Immunopharmacol. 2012, 13, 316–321.