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MARINE BROMOPHENOLS FROM POLYSIPHONIA: STRUCTURAL FEATURES, BIOLOGICAL ACTIVITY CORRELATIONS, AND FUTURE THERAPEUTIC APPLICATIONS

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Abstract:
This review presents a timeline of bromophenols discovered globally in various Polysiphonia species, a type of red alga (Rhodomelaceae). Researchers have focused on the authentication, extraction, isolation, and structural elucidation of the compounds. The compounds exhibited antioxidant, antibacterial, anticancer, anticoagulant, antidiabetic, and antifungal properties. The review suggests that DPPH free radical scavenging activity and cytotoxic studies were done extensively. Compounds like Chaetopyranin (35 µg/ml), Isotetrahydroauroglaucin (26 µg/ml), (E)-4-(3-bromo-4,5-dihydroxyphenyl)-but-3-en-2-one (9.67 µg/ml), and 4,7-dibromo-9,10-dihydrophenanthrene-2,3,5,6-tetraol (6.1 µM) were found to be potent, and several scaffolds have been identified to be correlated with activity, like the chroman ring, phenanthrene, oxepines, and many more. Several side chains like prenylated (3-methylbut-2-enyl), but-3-en-2-one (vinyl ketone), propionic acid chain, a butyl ester (acetic acid ester), and an ethane bridge also had an effect on biological activity. The interesting bromination pattern establishes bioactivity with the number and position of bromine atoms in the rings. Researchers have several potential to study these structures and develop safe drugs for the pharmaceutical and cosmetic industry.

Key words: Polysiphonia, Bromophenols, DPPH, Cytotoxicity, Rhodomelaceae, IC_{50}.

INTRODUCTION

Dr. William Fenical, a pioneer in marine chemistry of natural products at the Scripps Institution of Oceanography, UC San Diego, has aptly remarked that "the ocean is clearly the world's greatest resource for the discovery of potential new drugs" [1]. Underneath the waves, from sun-drenched coral reefs to the cold, rocky shores of polar seas, resides Polysiphonia, an often-undervalued architect of marine ecosystems that harbors a multitude of red algal species. Approximately 200 species of this filamentous alga constitute a complex global network of red seaweeds [2]. With a unique polysiphonous structure of central filaments sheathed by concentric layers of cells, it boasts a complex, triphasic life cycle [3]. Polysiphonia species form an intricate ecological partnership with damselfish on Indo-Pacific coral reefs, where they are deliberately cultivated and protected to become a monoculture food crop [4]. Polysiphonia symbolizes adaptation and interdependence in the world's oceans.

Several research articles have been published about the chemical constituents identified in various Polysiphonia species around the world. The nature of chemical constituents present in each species varies from continent to continent due to tidal variations, seasonal changes, salinity of the seawater, pH, temperature, weather, etc. [5]. The different chemical classes reported are bromophenols and derivatives, sulfated polysaccharides, phytosterols, fatty acids & lipids, proteins & amino acids, flavonoids & phenolics, halogenated monoterpenes, and minerals & trace elements [5]. The range of biological activity observed in this genus is reported as having antioxidant, antidiabetic, anticoagulant (heparin-like), antiviral, anti-inflammatory, hypocholesterolemic, potential anticancer, and antimicrobial action [5, 6].

Assorted Bromophenols (BPs) are signature compounds present in Polysiphonia species. BPs are polyphenolic compounds that are brominated at different positions of the aromatic ring. BPs are marine secondary metabolites that are biosynthesized in the presence of some enzyme like bromoperoxidases, hydrogen peroxide (H_2O_2), bromide ion (Br^-), and acids to brominate the phenolic rings [7, 8]. Generally, the biochemical pathways initiate with tyrosine as a precursor and with the formation of a highly reactive quinone methide intermediate, which ultimately gives rise to the final products [9, 10].

The content of bromophenols present in different Polysiphonia species varies with season, as reported by Shoeib et.al. [22]. The bromophenol concentration in *Polysiphonia lanosa* exhibited significant variation between late spring and early autumn. The minimum content was observed in samples gathered in early May, followed by an increase that culminated in a peak in July [11].

This review emphasizes the bioactivities with structure activity relationship, and pharmaceutical applications of the bromophenols isolated from various species of Polysiphonia, as well as their chemical structure. We have disregarded the extraction and bioactivity studies of the extracts conducted by various researchers, which did not involve any effort to isolate the chemical substances.

Discovery of Bromophenols from Polysiphonia Species-The Timeline

Brominated polyphenolic compounds are present in large numbers in the red algae belonging to the family Rhodomelaceae. In the beginning the presence of bromophenolic compounds was identified by paper chromatography and sensitive-colored reactions with $FeCl_3$ [12].

Several research groups have isolated and created a large dataset of bromophenols present in different species of Polysiphonia at different times. Hence, a timeline with their discoveries is presented in a tabular format (Table 1).

Table: 1. *A timeline showing list of chemical compounds isolated from different species of Polysiphonia*

Year	Authors	Isolated Compounds	Polysiphonia species
1955	T. Saito and Y. Ando [13]	• $C_7H_{20}O_9S_2Br_2K_2$ • $C_7H_3O_9S_2BrK_2$	*Polysiphonia lanosa*
1956	Augier and Mastagli [14]	• 5-bromo-3,4-dihydroxybenzaldehyde	*Polysiphonia morrowii*
1965	Hodgkin et. al. [15]	• 2,3-dibromobenzyl alcohol 4,5-disulfate (dipotassium salt)	*Polysiphonia lanosa*
1974	M. Pedersen, P. Saenger and L. Fries [16]	• 2,6-dibromo-4-(hydroxymethyl)phenol • 3-bromo-4,5-dihydroxybenzaldehyde • 3-bromo-5-(hydroxymethyl)benzene-1,2-diol • 2,3-dibromo-4,5-dihydroxybenzaldehyde • 3,4-dibromo-5-(hydroxymethyl)benzene-1,2-diol • 3,4-dibromo-5-(methoxymethyl)benzene-1,2-diol	*Polysiphonia brodiaei* *Polysiphonia urceolata* *Polysiphonia nigrescens*
	Glombitza et al., [17]	• 3–bromo–4,5–dihydroxybenzaldehyde • 2,3–dibromo–4,5–dihydroxybenzaldehyde • 3–bromo–4,5–dihydroxybenzylalcohol • 2,3–dibromo–4,5–dihydroxybenzylalcohol (Lanosol) • 2,3,6–tribromo–4,5-dihydroxybenzylalcohol • 2,3–dibromo–4,5–dihydroxybenzyl-n -propylether, 3–bromo–4-hydroxybenzylalcohol • 3,5–dibromo–4–hydroxybenzylalcohol • lanosol–1',4–disulfate	*Polysiphonia lanosa,* *Polysiphonia fruticulosa,* *Polysiphonia elongate,* *Polysiphonia nigrescens,* *Polysiphonia nigra,* *Polysiphonia brodiaei,* *Polysiphonia violacea,* *Polysiphonia thuyoides,* *Polysiphonia urceolata*
1976	Kurata and colleagues [18]	• 3,3'-dibromo-4,4',5,5'-tetrahydroxydibenzyl • 3,5-dibromo-4-hydroxybenzyl methyl ether • 3-bromo-4,5-dihydroxybenzyl methyl ether • 3-bromo-4,5-dihydroxybenzaldehyde	*Polysiphonia urceolata*
1980	Kurata et.al. [19]	• 3,3'-dibromo-4,4',5,5'-tetrahydroxydibenzyl • 3-bromo-4,5-dihydroxybenzaldehyde • 3-bromo-4,5-dimethoxybenzoic acid • 2,6-dibromo-4-(ethoxymethyl)phenol • 3,5-dibromo-4-hydroxybenzaldehyde • 2,6-dibromo-4-(hydroxymethyl)phenol • 3-bromo-5-(hydroxymethyl)benzene-1,2-diol	*Polysiphonia urceolata*
1985	Glombitza et al., [20]	• **Rhodomelol** (3R)-3-(2,3-dibromo-4,5-dihydroxybenzyl)-3,3a,6-trihydroxytetrahydrofuro[3,2-b]furan-2(5H)-one • **Methylrhodomelol** [(3R)-3-(2,3-dibromo-4,5-dihydroxybenzyl)-3a,6-dihydroxy-3-methoxytetrahydrofuro[3,2-b]furan-2(5H)-one]	*Polysiphonia urceolata*
2000	Flodin and Whitfield [21]	• 2,4-dibromoanisole, • 2,4,6-tribromoanisole, • 3-bromocresol, 3,5-dibromocresol, • 3-bromo-4-hydroxybenzaldehyde, • 3,5-dibromo-4-hydroxybenzaldehyde, • 2-bromophenol, • 4-bromophenol, • 2,4-dibromophenol, • 2,6-dibromophenol, • 2,4,6-tribromophenol	*Polysiphonia sphaerocarpa*

2004	Shoeib et al. [22]	• Lanosol • Methyl, ethyl, and n-propyl ethers of lanosol • aldehyde of lanosol • Synthesized bromophenols e.g., 2,5-Dibromo-3,4-dihydroxybenzyl Alcohol • 2,5-Dibromo-3,4-dihydroxybenzyl Methyl Ether • 2,5-Dibromo-3,4-dihydroxybenzyl Ethyl Ether • 2,5-Dibromo-3,4-dihydroxybenzyl n-Propyl Ether	*Polysiphonia lanosa*
	Gabriele M. Konig [23]	• Myrocin A • Apiosporic acid • Monomethyl ester of 9-hydroxyhexylitaconic acid • (-)-enantiomer of (+)-hexylitaconic acid • (+)-Epiepoxydon • (+)-Epoxydon monoacetate • R-Mellein • R-8-Methoxymellein • 5-Hydroxymethylfuran-2-carboxylic acid • Anomalin A.	*Polysiphonia violacea*
2006	B.G. Wang et. al. [24]	• Chaetopyranin, • 2-(2′,3-epoxy-1′,3′-heptadienyl)-6-hydroxy-5-(3-methyl-2-butenyl)benzaldehyde • Isotetrahydroauroglaucin • Erythroglaucin • Parietin • Asperentin (also known as cladosporin) • 5′-hydroxy-asperentin-8-methylether • Asperentin-8-methyl ether • 4′-hydroxyasperentin	*Polysiphonia urceolata*
2007	B.G. Wang et. al. [25]	• 3-(3-bromo-4,5-dihydroxyphenyl)-2-(3,5-dibromo-4-hydroxyphenyl)propionic acid • (E)-4-(3-bromo-4,5-dihydroxyphenyl)-but-3-en-2-one • (3,5-dibromo-4-hydroxyphenyl)acetic acid butyl ester • 1,2-bis(3-bromo-4,5-dihydroxyphenyl)ethane	*Polysiphonia urceolata*
2008	Bin-Gui Wang et. al. [26]	• 7-bromo-9,10-dihydrophenanthrene-2,3,5,6-tetraol • 4,7-dibromo-9,10-dihydrophenanthrene-2,3,5,6-tetraol • 1,8-dibromo-5,7-dihydrodibenzo[c,e]oxepine-2,3,9,10-tetraol • Urceolatol • 3-bromo-4,5-dihydroxybenzaldehyde • 3,5-dibromo-4-hydroxybenzaldehyde	*Polysiphonia urceolata*
	Bin-Gui Wang et. al. [27]	• 6-Bromo-1-(3-bromo-4,5-dihydroxybenzyl)phenanthrol[4,5-bcd]furan-2,3,5-triol (Urceolatin)	*Polysiphonia urceolata*
2009	Quan-Wen Liu et.al. [28]	• (5S, 10S)-2,7-Dibromo-3,8-dihydroxy-5,10-dimethoxyl-5,10-dihydro-chromeno[5,4,3-cde]chromene	*Polysiphonia urceolata*
2011	So Young Kang et.al. [29]	• 3-Bromo-4,5-dihydroxybenzyl methyl ether • 3-Bromo-4,5-dihydroxybenzaldehyde	*Polysiphonia morrowii*
2013	Elisabeth K. Olsen et.al. [30]	• 2,3-Dibromo-4,5-dihydroxybenzylaldehyde • 2,2′,3-Tribromo-3′,4,4′,5-tetrahydroxy-6′-hydroxymethyl diphenylmethane • Bis(2,3-dibromo-4,5-dihydroxylbenzyl) ether, 5,5″-oxybis(methylene)bis(3-bromo-4-(2′,3′-dibromo-4′,5′-dihydroxylbenzyl)benzene-1,2-diol)	*Vertebrata lanosa ((Polysiphonia lanosa))*
2014	Li Sun et.al. [31]	• Phycobilisomes • R-Phycocyanin	*Polysiphonia urceolata*
	Li Sun et.al. [32]	• Allophycocyanin (AP) • R-Phycocyanin	*Polysiphonia urceolata*
2017	Eun-Sook Yoo et.al. [33]	• 3-Bromo-4,5-dihydroxybenzaldehyde	*Polysiphonia morrowii*
2018	Youn Kyung Choi et.al. [34]	• Bis (3-bromo-4,5-dihydroxybenzyl) ether	*Polysiphonia morrowii*
	Seung-Hong Lee et.al. [35]	• 5-Bromo-3,4-dihydroxybenzaldehyde	*Polysiphonia morrowii*
2019	Sylvia Urban et.al. [36]	• Polysiphonol, • α-O-methyllanosol,	*Polysiphonia decipiens*

		• Lanosol, • 5-(2-bromo-3,4-dihydroxy-6 -(hydroxymethyl)benzyl)-3,4-dibromobenzene-1,2-diol, • 5-(2-bromo-3,4-dihydroxy-6-(methoxymethyl)benzyl)-3,4-dibromobenzene-1,2-diol, • Rhodomelol	
	Su-Hyeon Cho et.al. [37]	• 3-Bromo-4,5-Dihydroxybenzaldehyde	*Polysiphonia morrowii*
2020	Eui Jeong Han et.al. [38]	• 5-Bromo-3,4-dihydroxybenzaldehyde	*Polysiphonia morrowii*
2022	Verena Spiegler et.al. [39]	• lanosol-4,7-disulfate • 3,5-dibromotyrosine, • 3-bromo-5-sulfodihydroxyphenylalanine, • 3-bromo-6-lanosyl dihydroxyphenylalanine, • 3-(6′-lanosyl lanosyl) tyrosine • 5-sulfovertebratol • 4-sulfo-7-dimethylsulfonium lanosol • ((5Z,8Z,11Z,14Z,17Z)-eicosapentaenoic acid • 3′-[(6′-O-galactopyranosyl-D-galactopyranosyl)]-1-glycerol ester, • Porphyra-334, • Aplysiapalythine A • Palythine	*Vertebrata lanosa (Polysiphonia lanosa)*
	Eun-A Kim [40]	• 3,4-dihydroxybenzaldehyde	*Polysiphonia morrowii*

Using this primitive technology, T. Saito and Y. Ando first isolated two brominated sulfonated hydroxybenzoic acids from *Polysiphonia lanosa* in **1955**, which have the empirical formulas $C_7H_{20}O_9S_2Br_2K_2$ and $C_7H_3O_9S_2BrK_2$. No biological activity study was carried out [13].

Another bromophenolic compound from *Polysiphonia morrowii* was reported in **1956** when Augier and Mastagli proved the presence of 5-bromo-3,4-dihydroxybenzaldehyde in the red algae (Fig.1). No biological activity study was carried out [14].

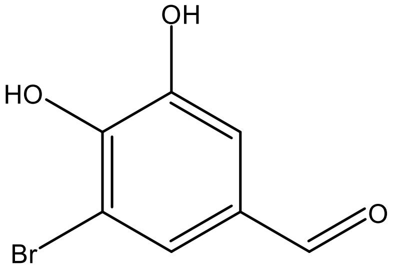


Fig.1. Structure of 5-bromo-3,4-dihydroxybenzaldehyde

In 1965, Hodgkin et al. successfully isolated a natural aromatic bromophenolic compound in 5% yield from the boiling aqueous ethanolic extract (80%) of *Polysiphonia lanosa*, 2,3-dibromobenzyl alcohol 4,5-disulfate (dipotassium salt) (Fig. 2). The structure was confirmed by IR and HNMR and later synthesized in the laboratory from 5,6-dibromovanillin. No biological activity study was carried out. This landmark study established that brominated phenolic compounds were characteristic constituents of this genus [15].

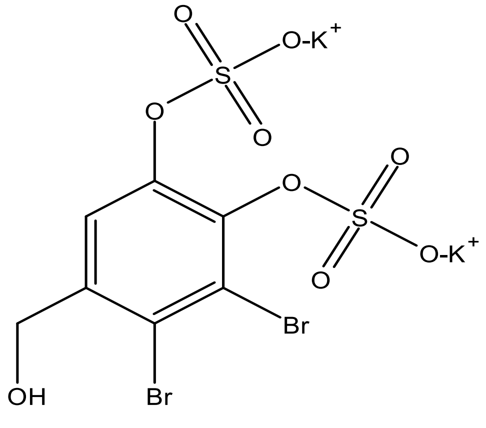


Fig.2. Structure of 2,3-dibromobenzyl alcohol 4,5-disulfate (dipotassium salt)

In 1974, M. Pedersen, P. Saenger, and L. Fries examined twenty-three species of red algae, encompassing nine orders, for the presence of simple bromophenols. Five bromophenols were identified. Furthermore, lanosol was identified in sea water from the *Polysiphonia Brodiaei*, *Polysiphonia urceolata*, and *Polysiphonia nigrescens*. The compounds isolated were, 2,6-dibromo-4-(hydroxymethyl) phenol, 3-bromo-4,5-dihydroxybenzaldehyde, 3-bromo-5-(hydroxymethyl) benzene-1,2-diol, 2,3-dibromo-4,5-dihydroxybenzaldehyde, 3,4-dibromo-5-(hydroxymethyl) benzene-1,2-diol, and 3,4-dibromo-5-(methoxymethyl) benzene-1,2-diol (Fig.3). No biological activity study was carried out [16].

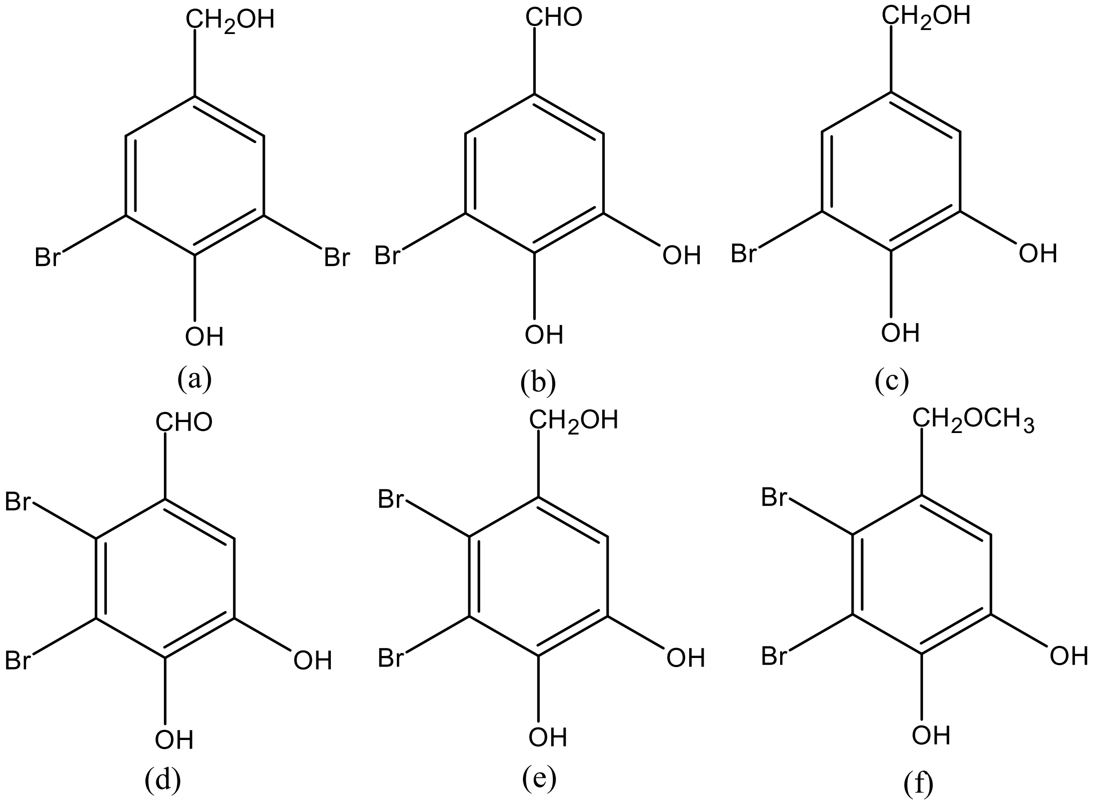
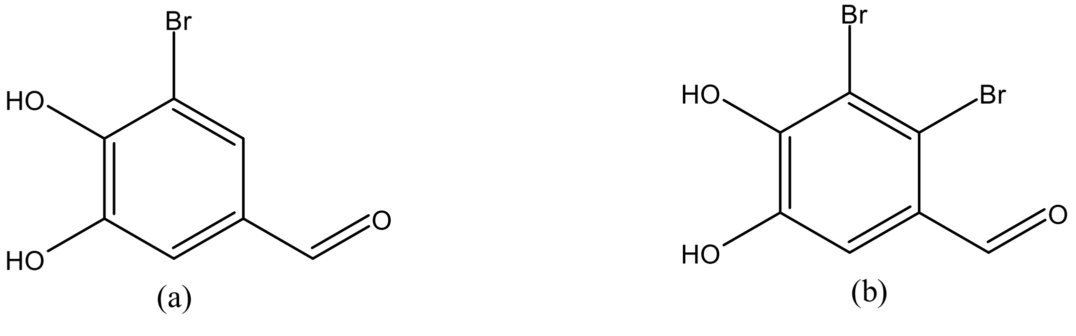
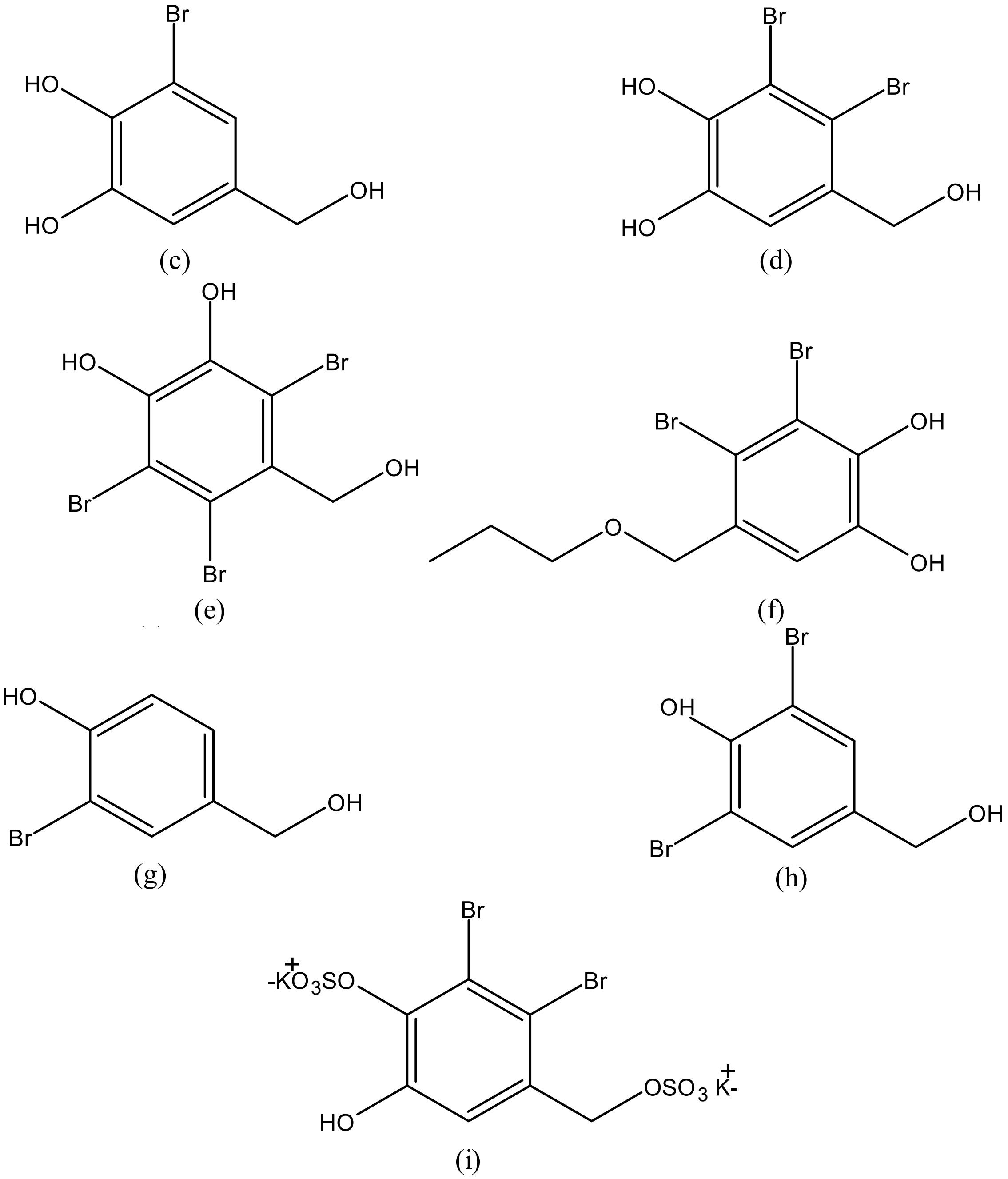


Fig.3. Structure of (a) 2,6-dibromo-4-(hydroxymethyl) phenol (b) 3-bromo-4,5-dihydroxybenzaldehyde (c) 3-bromo-5-(hydroxymethyl) benzene-1,2-diol (d) 2,3-dibromo-4,5-dihydroxybenzaldehyde (e) 3,4-dibromo-5-(hydroxymethyl) benzene-1,2-diol (f) 3,4-dibromo-5-(methoxymethyl) benzene-1,2-diol

The same year, Glombitza et al. conducted a study on thirteen distinct Rhodomelaceae species. The species were systematically examined to identify simple bromophenols. The following polysiphonia species were selected for the study: *Polysiphonia lanosa, Polysiphonia fruticulosa, Polysiphonia elongate, Polysiphonia nigrescens, Polysiphonia nigra, Polysiphonia brodiaei, Polysiphonia violacea, Polysiphonia thuyoides,* and *Polysiphonia urceolata*.

The compounds that were identified are 3–bromo–4,5–dihydroxybenzaldehyde, 2,3–dibromo–4,5–dihydroxybenzaldehyde, 3–bromo–4,5–dihydroxybenzyl alcohol, 2,3–dibromo–4,5–dihydroxybenzyl alcohol (lanosol), 2,3,6–tribromo–4,5–dihydroxybenzyl alcohol, 2,3–dibromo–4,5–dihydroxybenzyl n-propylether, 3–bromo–4–hydroxybenzyl alcohol, 3,5–dibromo–4–hydroxybenzyl alcohol, and lanosol–1',4–disulfate (Fig. 4). The compounds demonstrated antibacterial and algicidal properties against both gram-positive and gram-negative organisms. *Bacillus subtilis*, *Escherichia coli*, *Sarcina pelagia*, and *Serratia marinorubra* were selected to investigate their antibacterial and algicidal properties. The MICs are typically between 100 and 200 μg/ml (Table. 2). All substances, with the exception of lanosol-1',4-disulfate, have demonstrated antibiotic properties, suggesting their potential efficacy in the prevention of bacterial infections in various environments [17].





(c) (d) (e) (f) (g) (h) (i)

Fig.4. Structures of the compounds: **(a)** 3-bromo-4,5-dihydroxybenzaldehyde, **(b)** 2,3-dibromo-4,5-dihydroxybenzaldehyde, **(c)** 3-bromo-4,5-dihydroxybenzyl alcohol, **(d)** 2,3-dibromo-4,5-dihydroxybenzylalcohol (Lanosol), **(e)** 2,3,6-tribromo-4,5-dihydroxybenzyl alcohol, **(f)** 2,3-dibromo-4,5-dihydroxybenzyl-n-propylether, **(g)** 3-bromo-4-hydroxybenzylalcohol, **(h)** 3,5-dibromo-4-hydroxybenzylalcohol, **(i)** lanosol-1,4-disulfate.

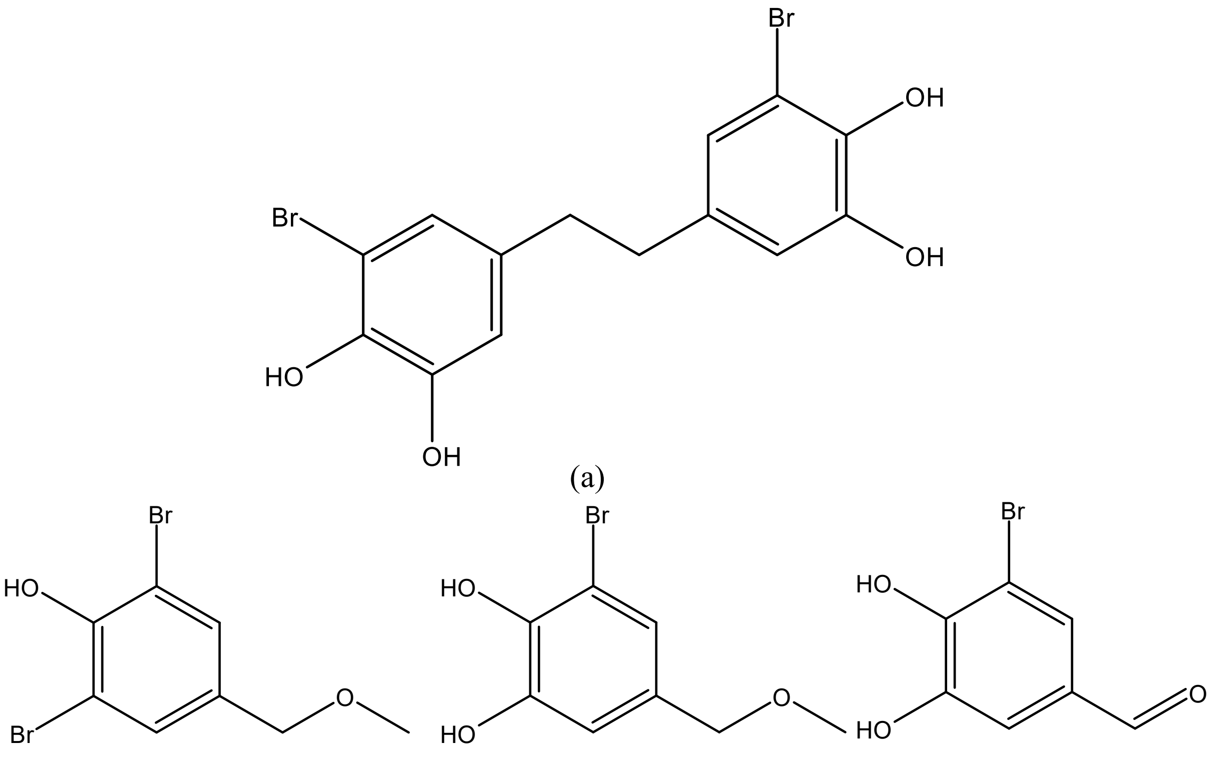
Table: 2. IC$_{50}$ of the antimicrobial activity and names of compounds.

Sl. No.	**IC$_{50}$** (In µg/ml)	**Compound name**
1.	50(a) 150(b) 100(c) 80(d) 100(e)	3–bromo–4,5–dihydroxybenzylalcohol
2.	280(a) 200(b) 300(c) 380(d) 180(e)	3–bromo–4,5–dihydroxybenzaldehyde
3.	120(a)	2,3–dibromo–4,5–dihydroxybenzylalcohol (Lanosol)

	160(b) 190(c) 350(d) 200(e)	
4.	100(a) 150(b) 100(c) >530(d) 130(e)	2,3–dibromo–4,5–dihydroxybenzaldehyde
5.	100(a) 420(b) 400(c) >600(d) 430(e)	Lanosol–1′,4–disulfate

Notes: a- *Bacillius subtillis*; b- *Escherichia coli*; c- *Sarcina pelagia*; d- *Serratia marinorubra*; e- *Vibrio phytoplankton*

In 1976, Kurata and colleagues investigated the red alga *Polysiphonia urceolata* from Japanese waters near Hokkaido, and reported a novel bromophenol, 3,3′-dibromo-4,4′,5,5′-tetrahydroxybibenzyl. Besides these, three other bromophenols were reported by them, viz., 3,5-dibromo-4-hydroxybenzyl methyl ether, 3-bromo-4,5-dihydroxybenzyl methyl ether, and 3-bromo-4,5-dihydroxybenzaldehyde **(Fig. 5)**. Extraction was carried out in methanol that was soaked for ten days with the algae. The compounds were characterized by IR, HNMR, and MS, and later the structures were proven by laboratory synthesis. No biological activity study was carried out [18].



(a)

(b) (c) (d)

Fig. 5. Structures of (a) 3,3′-dibromo-4,4′,5,5′-tetrahydroxybibenzyl (b) 3,5-dibromo-4-hydroxybenzyl methyl ether (c) 3-bromo-4,5-dihydroxybenzyl methyl ether (d) 3-bromo-4,5-dihydroxybenzaldehyde

Two years later in 1980, Kurata and colleagues investigated the red alga *Polysiphonia urceolata* and reported that they found the previous novel compound 3,3′-dibromo-4,4′,5,5′-tetrahydroxybibenzyl along with some novel compounds like 3-bromo-4,5-dihydroxybenzaldehyde, 3-bromo-4,5-dimethoxybenzoic acid, 2,6-dibromo-4-(ethoxymethyl)phenol, 3,5-dibromo-4-hydroxybenzaldehyde, 2,6-dibromo-4-(hydroxymethyl)phenol, and 3-bromo-5-(hydroxymethyl)benzene-1,2-diol **(Fig. 6)**. No biological activity study was carried out [19].

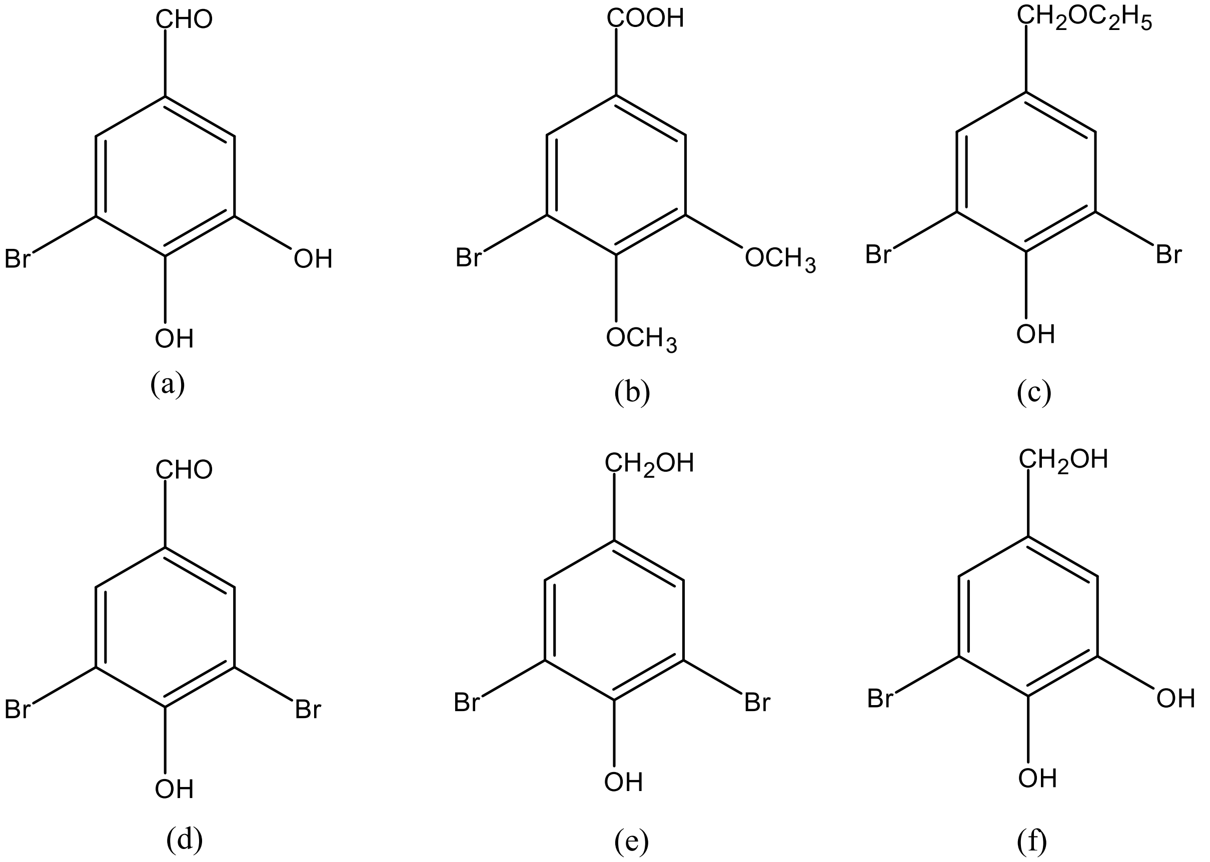
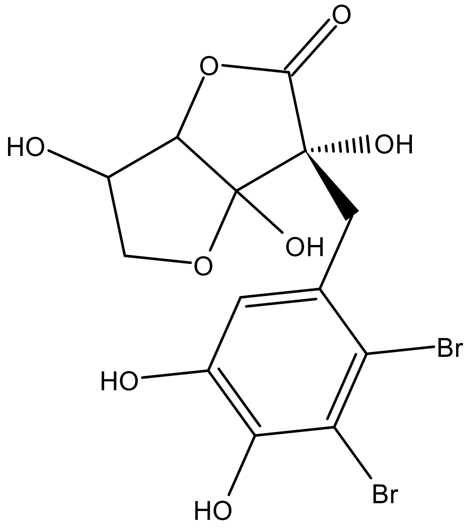
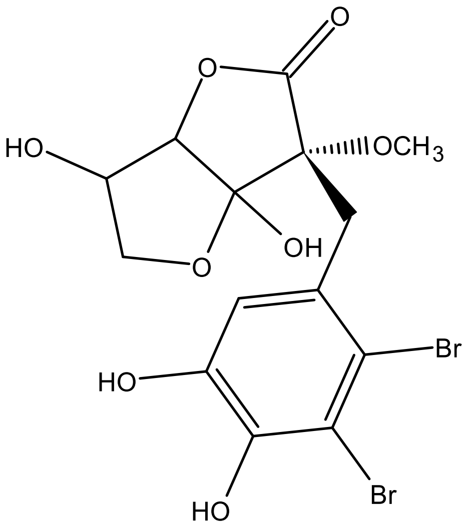


Fig. 6. Structures of (a) 3-bromo-4,5-dihydroxybenzaldehyde (b) 3-bromo-4,5-dimethoxybenzoic acid (c) 2,6-dibromo-4-(ethoxymethyl)phenol (d) 3,5-dibromo-4-hydroxybenzaldehyde (e) 2,6-dibromo-4-(hydroxymethyl)phenol (f) 3-bromo-5-(hydroxymethyl)benzene-1,2-diol

A significant advance came in 1985 when Glombitza and colleagues isolated rhodomelol (3R)-3-(2,3-dibromo-4,5-dihydroxybenzyl)-3,3a,6-trihydroxytetrahydrofuro[3,2-b]furan-2(5H)-one and methylrhodomelol [(3R)-3-(2,3-dibromo-4,5-dihydroxybenzyl)-3a,6-dihydroxy-3-methoxytetrahydrofuro[3,2-b]furan-2(5H)-one] from *Polysiphonia lanosa* **(Fig.7)**. These represented more complex, dimeric bromophenolic structures. They acetylated and methylated the compounds for structural elucidation using spectroscopic methods, complemented by an X-ray crystallographic study of a derivative. However, the absolute configuration of natural rhodomelol remained unassigned for over three decades. No biological activity study was carried out [20].



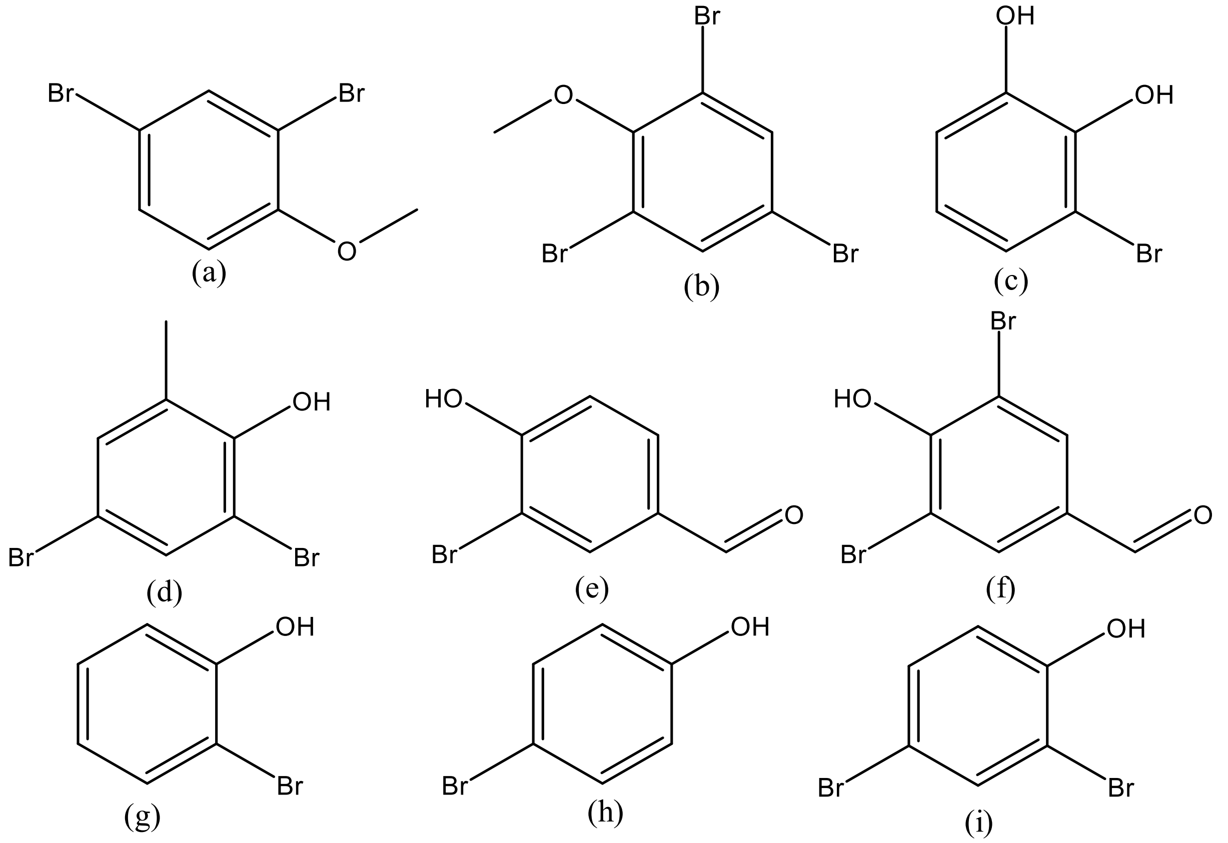
(a)



(b)

Fig. 7. Structure of (a) (3R)-3-(2,3-dibromo-4,5-dihydroxybenzyl)-3,3a,6-trihydroxytetrahydrofuro[3,2-b]furan-2(5H)-one Rhodomelol, (b) (3R)-3-(2,3-dibromo-4,5-dihydroxybenzyl)-3a,6-dihydroxy-3-methoxytetrahydrofuro[3,2-b]furan-2(5H)-one Methylrhodomelol

With the beginning of **Y2K**, a methodologically innovative study was carried out by Flodin and Whitfield that employed simultaneous steam distillation-solvent extraction coupled with GC-MS to analyze *Polysiphonia sphaerocarpa* from Australia. They identified eleven brominated compounds, including the brominated anisoles like 2,4-dibromoanisole, 2,4,6-tribromoanisole, brominated cresols like 3-bromocresol, 3,5-dibromocresol, and other compounds like 3-bromo-4-hydroxybenzaldehyde, 3,5-dibromo-4-hydroxybenzaldehyde, 2-bromophenol, 4-bromophenol, 2,4-dibromophenol, 2,6-dibromophenol, and 2,4,6-tribromophenol (Fig. 8). Significantly, this was the first time brominated anisoles and cresols had been detected in any marine alga, expanding the known chemical diversity of the genus. No biological activity study was carried out [21].



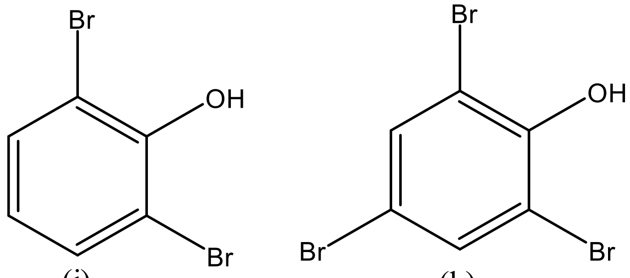
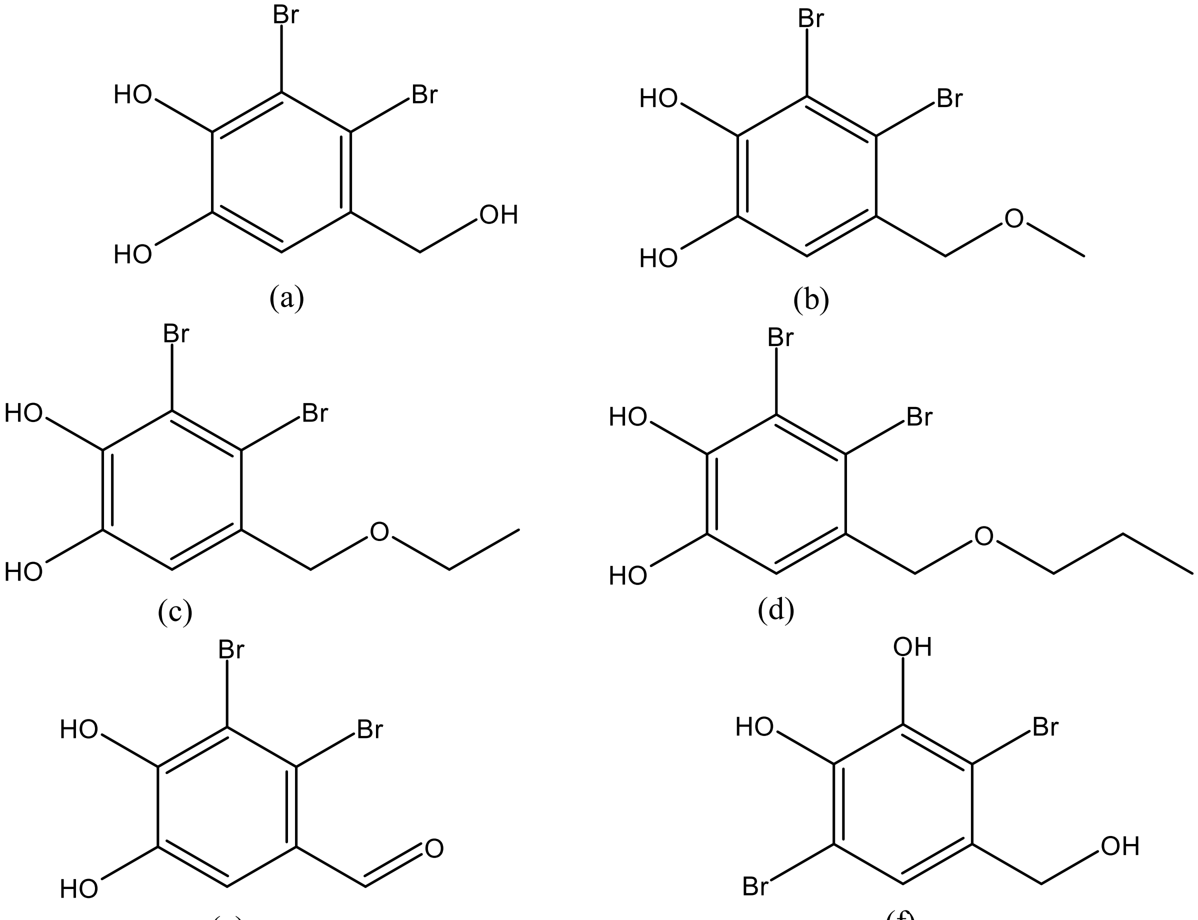

(j) (k)

Figure. 8. (a) 2,4-Dibromoanisole, (b) 2,4,6-tribromoanisole, (c) 3-bromocresol (d) 3,5-dibromocresol (e) 3-bromo-4-hydroxybenzaldehyde (f) 3,5-dibromo-4-hydroxybenzaldehyde (g) 2-bromophenol (h) 4-bromophenol (i) 2,4-dibromophenol (j) 2,6-dibromophenol (k) 2,4,6-tribromophenol

In July 2004, Shoeib et al. collected a cytotoxic chloroform extract from the *Polysiphonia lanosa* inhabiting the south coast of England. They subsequently employed bioassay-guided fractionation to identify the active components. They employed GLC-MS analysis to identify a series of bromophenol compounds in the most active fraction, including lanosol, methyl, ethyl, and n-propyl ethers of lanosol, and the aldehyde of lanosol. In addition to four novel isomers, such as 2,5-Dibromo-3,4-dihydroxybenzyl Alcohol, 2,5-Dibromo-3,4-dihydroxybenzyl Methyl Ether, 2,5-Dibromo-3,4-dihydroxybenzyl Ethyl Ether, and 2,5-Dibromo-3,4-dihydroxybenzyl n-Propyl Ether (Fig. 9), these compounds and other known bromophenols were synthesized. In laboratory tests conducted on human colorectal cancer cells (DLD-1 and HCT-116), the synthetic compound 2,5-Dibromo-3,4-dihydroxybenzyl-n-propyl ether was the most effective, exhibiting strong cytotoxicity at extremely low concentrations (IC_{50} values of 1.72 and 0.80 µmol, respectively) [Table: 3].

The impact of the active compound on the cell cycle was examined through flow cytometry analysis, utilizing various concentrations (0.65-10 µmol) and exposure durations (24, 48, 72, and 96 hours). The percentage of cells in G0/G1 elevated relative to the control cells at a 10 µmol dosage across all four exposure durations ($P < 0.01$), signifying that the cell cycle has been arrested at the G0/G1 phase. Cells in the S phase exhibited a substantial accumulation at a concentration of 5 µmol ($P < 0.001$), indicating a S phase arrest. Treatment with 2.5 µmol resulted in a significant elevation of the G2/M phase population ($P < 0.05$), indicating a blockade at the G2/M checkpoint. The cell cycle exhibited no substantial alterations at doses below 2.5 µmol [22].


(a) (b) (c) (d) (e) (f)

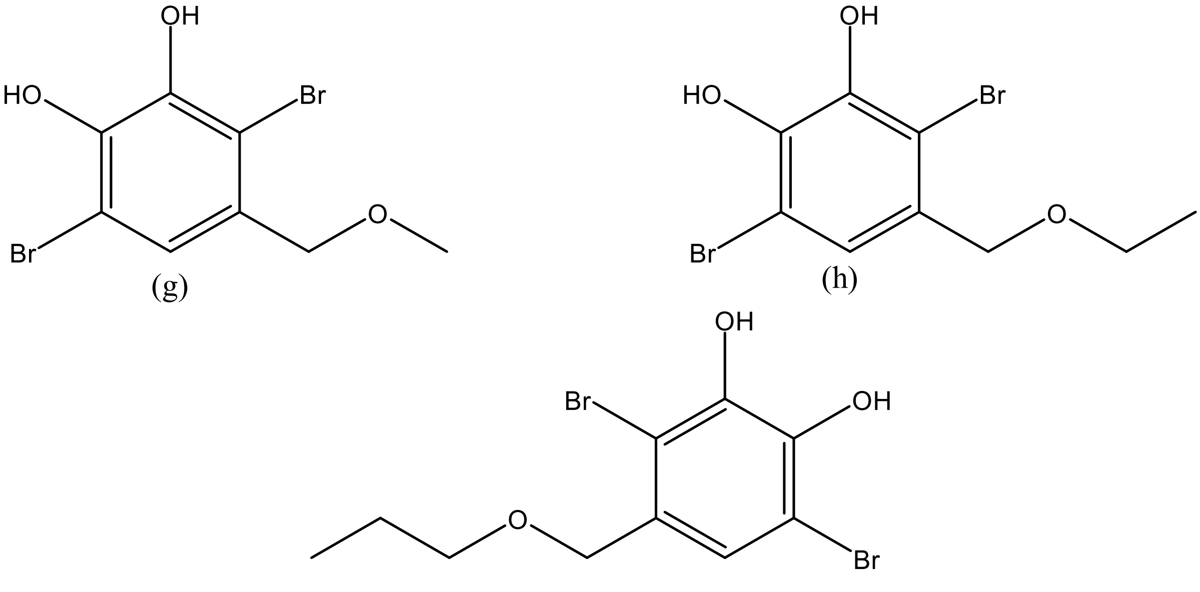

(g) (h) (i)

Fig. 9. Structure of (a) 3,4-Dibromo-5-(hydroxymethyl)benzene-1,2-diol (b) 2,3-dibromo-4,5-dihydroxybenzyl methyl ether (c) 3,4-dibromo-5-(ethoxymethyl)benzene-1,2-diol (d) 3,4-dibromo-5-(propoxymethyl)benzene-1,2-diol (e) 2,3-dibromo-4,5-dihydroxybenzaldehyde (f) 2,5-Dibromo-3,4-dihydroxybenzyl Alcohol (g) 2,5-Dibromo-3,4-dihydroxybenzyl Methyl Ether (h) 2,5-Dibromo-3,4-dihydroxybenzyl Ethyl Ether (i) 2,5-Dibromo-3,4-dihydroxybenzyl n-Propyl Ether

Table: 3. IC_{50} of the cytotoxic activity on human colorectal cancer cell line of the compounds

Sl. No.	**DLD-1 cells µmol**	**HCT-116 cells µmol**	**Compound name**
1.	18.3 ±0.94	20.4 ± 2.9	Lanosol
2.	14.6 ±3.1	14.1 ± 2.5	Methyl ethers of lanosol
3.	13.5±2.3	2.51± 0.95	Ethyl ethers of lanosol
4.	12.4 ±1.1	1.32±0.3	n-Propyl ethers of lanosol
5.	30.9 ±2.7	-	Aldehyde of lanosol
6.	15.0 ±2.0	21.4± 1.2	2,5-Dibromo-3,4-dihydroxybenzyl Alcohol
7.	7.27 ±1.5	3.48 ± 0.63	2,5-Dibromo-3,4-dihydroxybenzyl Methyl Ether
8.	7.15±1.4	1.54 ± 0.57	2,5-Dibromo-3,4-dihydroxybenzyl Ethyl Ether
9.	1.72 ±0.29	0.80±0.63	2,5-Dibromo-3,4-dihydroxybenzyl n-Propyl Ether
10.	7.38±0.92	4.93 ±0.81	5-Flurouracil (standard)

Each value is presented as the means ± SD (n = 3)

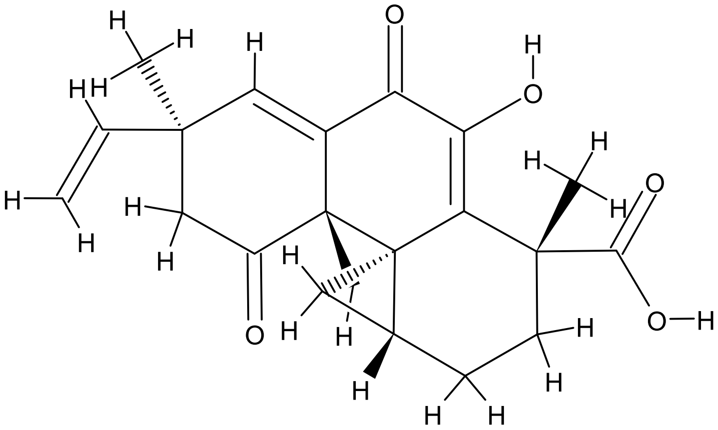
In **2004**, Gabriele M. Konig and his research team conducted a noteworthy investigation into an endophytic fungus, *Apiospora montagnei*, which resides within the inner tissues of *Polysiphonia violacea*. Their study revealed that the fungus synthesized a range of novel secondary metabolites, such as the diterpene myrocin A and the polyketide apiosporic acid. The research also uncovered the monomethyl ester of 9-hydroxyhexylitaconic acid, the (-)-enantiomer of the previously known (+)-hexylitaconic acid, alongside a suite of known compounds: (+)-epiepoxydon, (+)-epoxydon monoacetate, R-mellein, R-8-methoxymellein, 5-hydroxymethylfuran-2-carboxylic acid, and the xanthone derivative anomalin A **(Fig. 10)**.

Evaluating the crude extract and isolated compounds assessed their biological activity. They were tested for antibacterial, antifungal, and antialgal activity against Bacillus megaterium, *Escherichia coli*, *Microbotryum violaceum*, *Eurotium repens*, *Mycotypha microspora*, and *Chlorella fusca* using agar diffusion assays. None of the substances tested at 50 µg per disk showed inhibitory activity.

The brine shrimp lethality test assessed cytotoxicity of the crude extract and (+)-epiepoxydon (Table. 4). According to NCI protocols, myrocin A, (+)-epiepoxydon, and epoxydon monoacetate were tested against three human cancer cell lines: HM02 (gastric adenocarcinoma), HepG2, and MCF7. Results indicate (+)-epiepoxydon is the most effective, inhibiting growth at 0.7 µg/mL for HM02, 0.75 µg/mL for HepG2, and 0.8 µg/mL for MCF7 and achieving 50% GI50. It inhibited cell growth at 1.0, 4.6, and 1.5 µg/mL for the respective cell lines. The MCF7 breast adenocarcinoma line showed a 50% reduction in initial cell population (LC50) at 3.6 µg/mL, indicating significant cytotoxic effects [23].

Table: 4. The cytotoxic activity of (+)-Epiepoxydon on HM02 (gastric adenocarcinoma), HepG2 (hepatocellular carcinoma), and MCF7 (breast adenocarcinoma) cell line of the compounds

Sl. No.	Type of inhibition	HM02	HepG2	MCF7	Compound name
1.	50% growth inhibition (GI_{50})	0.7 µg/ml	0.75 µg/ml	0.8 µg/ml	(+)-Epiepoxydon
	Total growth inhibition (TGI)	1.0 µg/ml	4.6 µg/ml	1.5 µg/ml	
	50% reduction of the initial cell number (LC_{50})	>10 µg/ml	>10	>10 µg/ml	



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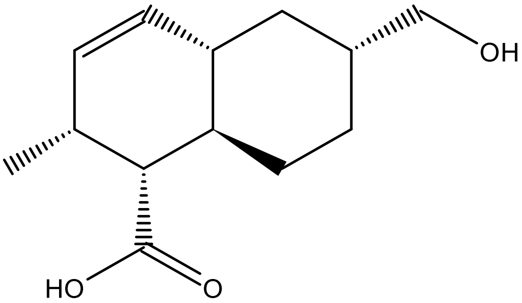

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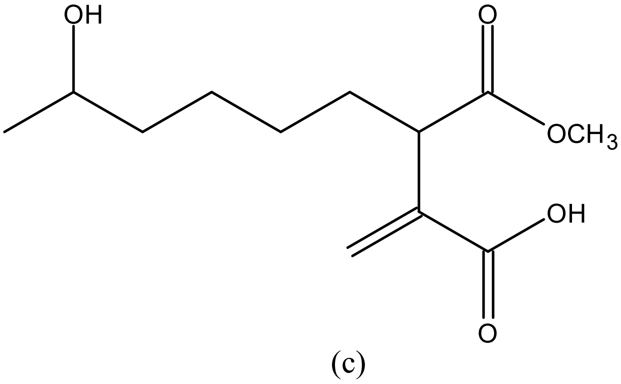

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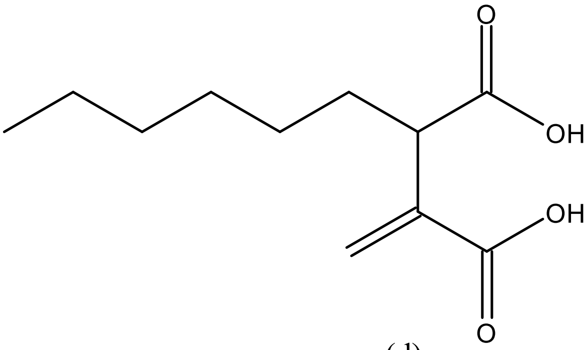

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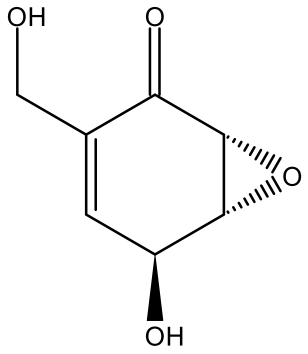


(e)

Fig. 10. Structure of (a) (3R,7S,9aS,9bR,10aR)-4-hydroxy-3,7,9a-trimethyl-5,9-dioxo-7-vinyl-1,2,3,5,7,8,9,9a,10,10a-decahydrocyclopropa[d]phenanthrene-3-carboxylic acid (Myrocin A) (b) (1R,2R,4aS,6R,8aR)-6-(hydroxymethyl)-2-methyl-1,2,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylic acid (Apisporic acid) (c) 8-hydroxy-3-(methoxycarbonyl)-2-methylenenonanoic acid (d) 2-Hexyl-3-methylenesuccinic acid (e) (+)-Epiepoxydon

A 2006 study by B.G. Wang et al. isolated Chaetomium globosum from the marine red alga *Polysiphonia urceolata* from the Qingdao coastline of the Yellow Sea, China, and cultured it. This isolated eleven compounds. The Chaetomium genus and marine algae-derived fungi have produced chaetopyranin, a novel benzaldehyde metabolite. Two benzaldehyde congeners, 2-(2′,3-epoxy-1′,3′-heptadienyl)-6-hydroxy-5-(3-methyl-2-butenyl) benzaldehyde and isotetrahy droauroglaucin, two anthraquinone derivatives, erythroglaucin and parietin, and five asperentin derivatives, including cladosporin, 5′-hydroxy-asperentin-8-methylether, Asperentin-8 methyl ether and 4′-hydroxyasperentin are known compounds (Fig. 11) [24].

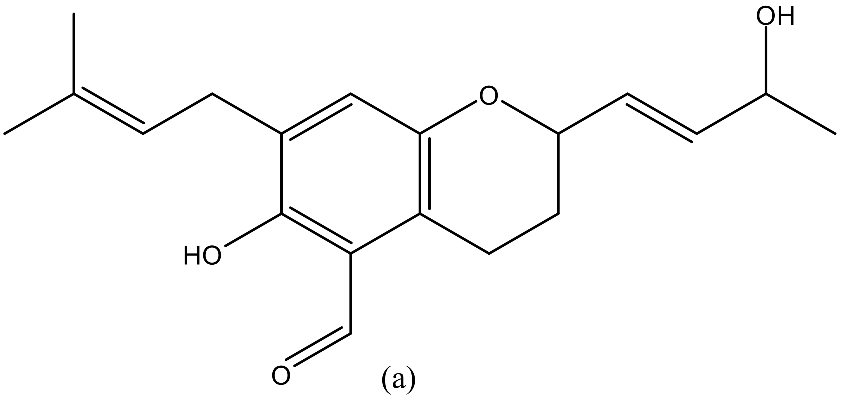
Chaetopyranin, 2-(2′,3-epoxy-1′,3′-heptadienyl)-6-hydroxy-5-(3-methyl-2-butenyl) benzaldehyde, isotetrahydroauroglaucin, and erythroglaucin had moderate antioxidant effects (Table. 5). Chaetopyranin, a novel compound, showed moderate or weak cytotoxic effects on three tumor cell lines, with IC_{50} values of 15.4 (HMEC), 28.5 (SMMC-7721), and 39.1 µg/mL (A549) (Table-6).

Table. 5. IC_{50} values of DPPH radical-scavenging property of the compounds

Sl. No.	IC_{50} in µg/ml	Compound name
1	35	Chaetopyranin
2	88	2-(2′,3-epoxy-1′,3′-heptadienyl)-6-hydroxy-5-(3-methyl-2-butenyl)benzaldehyde
3	26	Isotetrahydroauroglaucin
4	62	Erythroglaucin
5	18	Butylated hydroxytoluene (BHT)

Table: 6. The cytotoxic activity of Chaetopyranin on HM02 (gastric adenocarcinoma), HepG2 (hepatocellular carcinoma), and MCF7 (breast adenocarcinoma) cell line and name of compounds.

Sl. No.	**Human microvascular endothelial cells, HMEC** (IC_{50} in µg /mL)	**Hepatocellular carcinoma cells, SMMC-7721** (IC_{50} in µg /mL)	**Human lung epithelial cells A549** (IC_{50} in µg /mL)	Compound name
1.	15.4	28.5	39.1	Chaetopyranin



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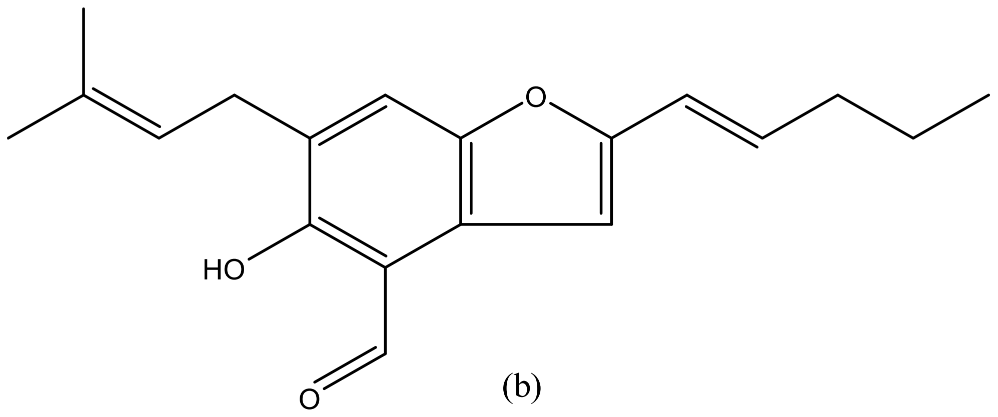

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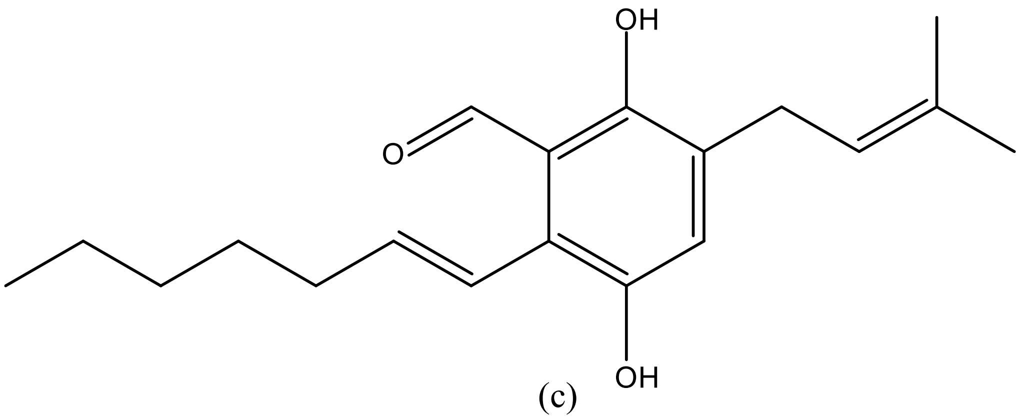

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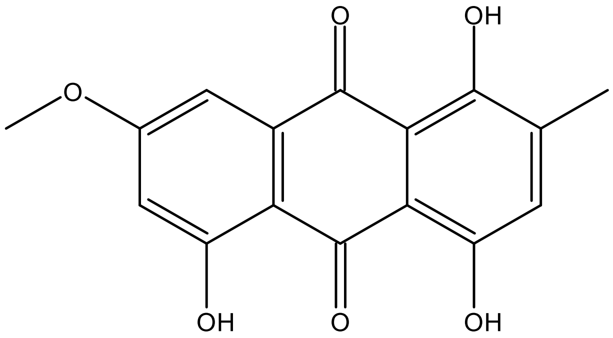

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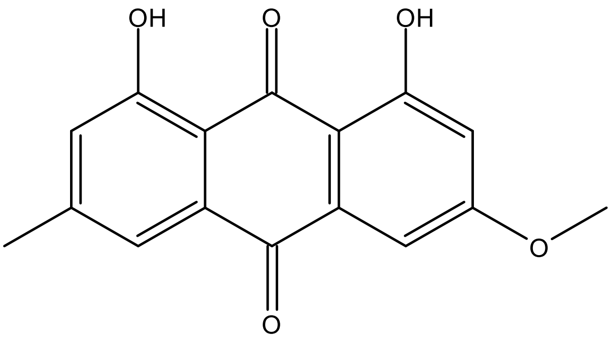

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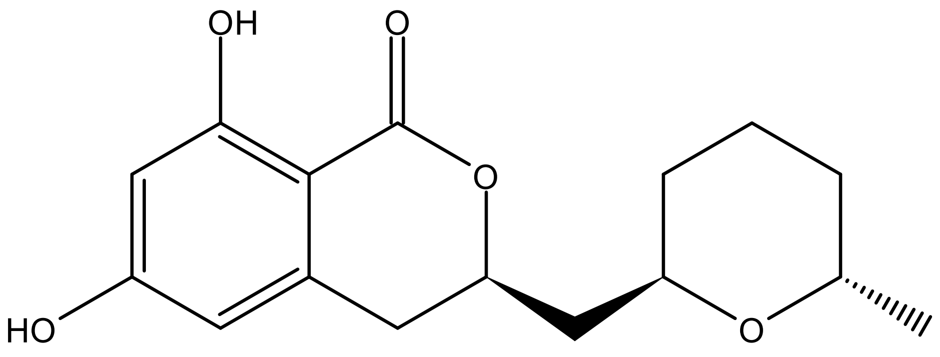

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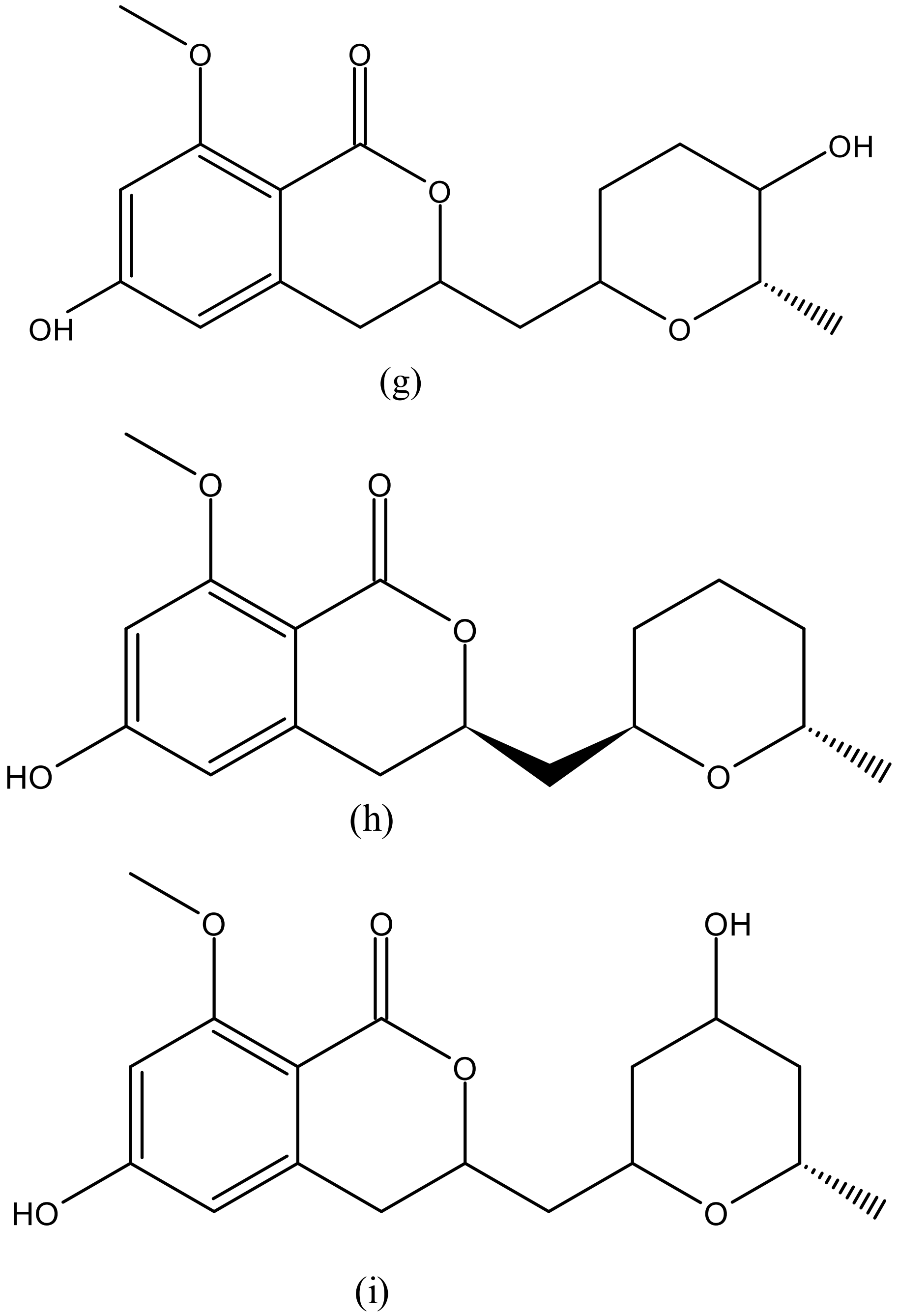


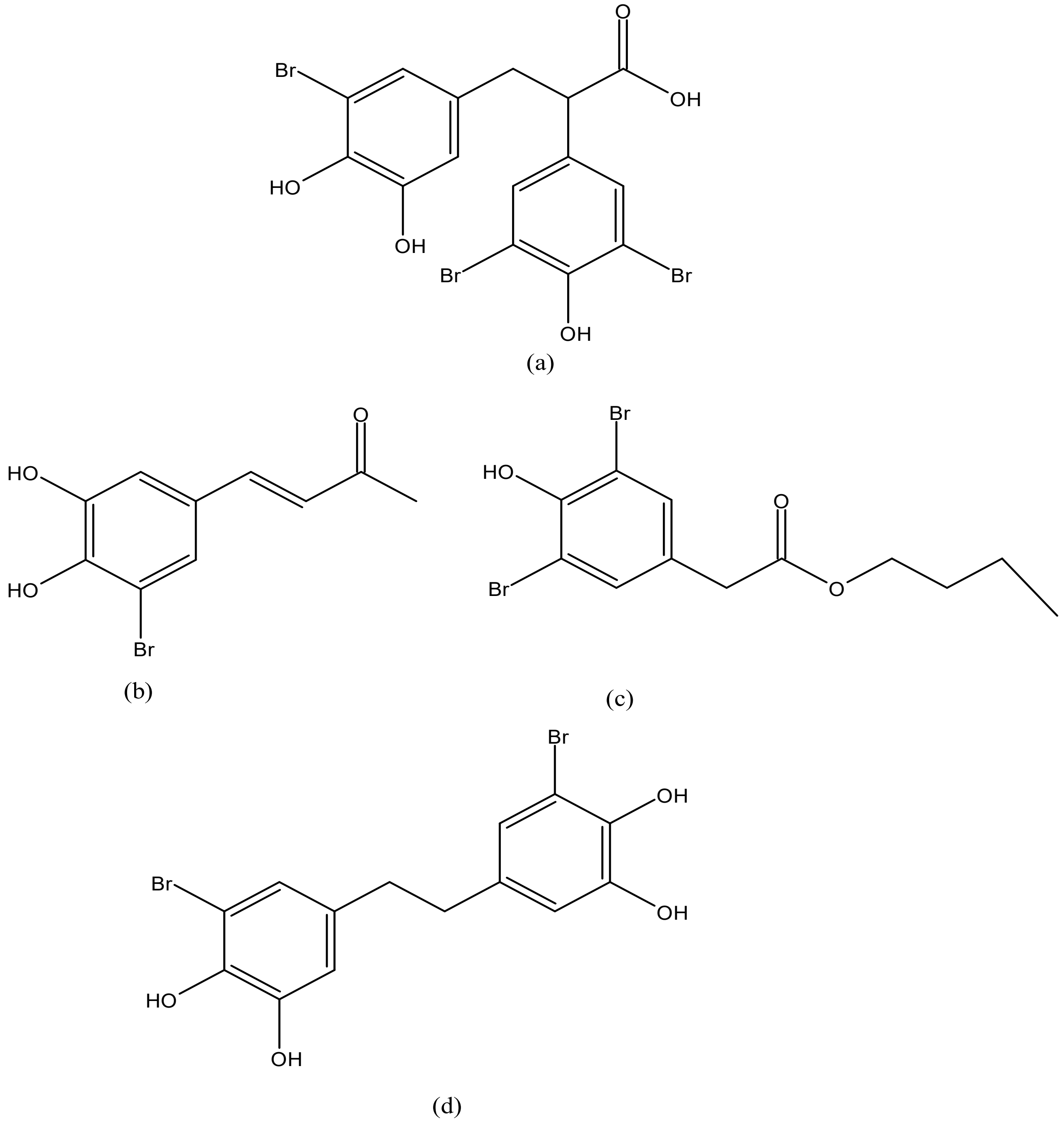
Fig. 11. Structure of (a) (E)-6-Hydroxy-2-(3-hydroxybut-1-enyl)-7-(3-methylbut-2-enyl)- chroman-5-carbaldehyde (Chaetopyranin) (b) (E)-5-hydroxy-6-(3-methylbut-2-en-1-yl)-2-(pent-1-en-1-yl)benzofuran-4-carbaldehyde (c) 2-[(E)-hept-1-enyl]-3,6-dihydroxy-5-(3-methylbut-2-enyl)benzaldehydeisotetrahydroauroglaucin (d) 1,4,5-trihydroxy-7-methoxy-2-methylanthracene-9,10-dione (erythroglaucin) (e) 1,8-dihydroxy-3-methoxy-6 -methylanthracene-9,10-dione (Parietin), (f) (R)-6,8-dihydroxy-3-(((2R,6S)-6-methyltetrahydro-2H-pyran-2-yl)methyl)isochroman-1-one Cladosporin or Asparentin (g) 5′-hydroxy-asperentin-8-methylether, (h) Asperentin-8-methyl ether (i) 4′-Hydroxyasperentin

The isolation of three new bromophenols was achieved in 2007 from *Polysiphonia urceolata* by the research group of B.G. Wang. The structures of these metabolites were identified as 3-(3-bromo-4,5-dihydroxyphenyl)-2-(3,5-dibromo-4-hydroxyphenyl)propionic acid, (E)-4-(3-bromo-4,5-dihydroxyphenyl)-but-3-en-2-one, (3,5-dibromo-4-hydroxyphenyl)acetic acid butyl ester, and 1,2-bis(3-bromo-4,5-dihydroxyphenyl)ethane (Fig. 12). The study's key finding was the potent antioxidant activity of these compounds. In DPPH radical scavenging assays, they exhibited IC_{50} values between 9.67 and 21.90 µM, demonstrating a remarkable efficacy that was several times higher than that of the established antioxidant butylated hydroxytoluene (BHT), which had an IC_{50} of 83.84 µM (Table. 7) [25].

Table. 7. IC_{50} values of DPPH radical-scavenging property of the compounds

Sl. No.	IC_{50} in µg/ml	Compound name
1	21.90 ± 0.1	3-(3-bromo-4,5-dihydroxyphenyl)-2-(3,5-dibromo-4-hydroxyphenyl)propionic acid
2	9.67 ± 0.04	(E)-4-(3-bromo-4,5-dihydroxyphenyl)-but-3-en-2-one
3	16.11 ± 0.06	(3,5-dibromo-4-hydroxyphenyl)acetic acid butyl ester
4	19.64 ± 0.09	1,2-bis(3-bromo-4,5-dihydroxyphenyl)ethane
5	83.84 ± 0.3	Butylated hydroxytoluene (BHT)

Each value is presented as the means ± SD (n = 3)



(a)

(b)

(c)

(d)

(Fig.12) Structure of (a) 3-(3-bromo-4,5-dihydroxyphenyl)-2-(3,5-dibromo-4-hydroxyphenyl) propionic acid (b) (E)-4-(3-bromo-4,5-dihydroxyphenyl)-but-3-en-2-one (c) (3,5-dibromo-4-hydroxyphenyl) acetic acid butyl ester (d) 1,2-bis(3-bromo-4,5-dihydroxyphenyl) ethane.

In 2008, Bin-Gui Wang and his team isolated and identified six bromophenols from *Polysiphonia urceolata*, three of which were novel. In (Fig. 13) the compounds are represented as 7-bromo-9,10-dihydrophenanthrene-2,3,5,6-tetraol, 4,7-dibromo-9,10-dihydrophenanthrene-2,3,5,6-tetraol, and 1,8-dibromo-5,7-dihydrodibenzo[c,e]oxepine-2,3,9,10 The compounds were urceolatol, 3-bromo-4,5-dihydroxybenzaldehyde, and 3,5-dibromo-4-hydroxybenzaldehyde were identified before. Each of the six compounds showed strong antioxidant activity against DPPH radicals. (Table. 8) shows that their IC_{50} values, ranging from 6.1 to 35.8 µM, significantly outperformed the positive control, butylated hydroxytoluene (BHT), with an IC50 of 83.8 µ [26].

Table. 8. IC_{50} values of DPPH radical-scavenging property of the compounds

Sl. No.	**IC_{50} in µM**	**Compound name**
1	6.8	7-bromo-9,10-dihydrophenanthrene-2,3,5,6-tetraol
2	6.1	4,7-dibromo-9,10-dihydrophenanthrene-2,3,5,6-tetraol
3	8.1	1,8-dibromo-5,7-dihydrodibenzo[c,e]oxepine-2,3,9,10-tetraol
4	15.1	Urceolatol
5	20.3	3-bromo-4,5-dihydroxybenzaldehyde
6	35.8	3,5-dibromo-4-hydroxybenzaldehyde
7	83.8	Butylated hydroxytoluene (BHT)

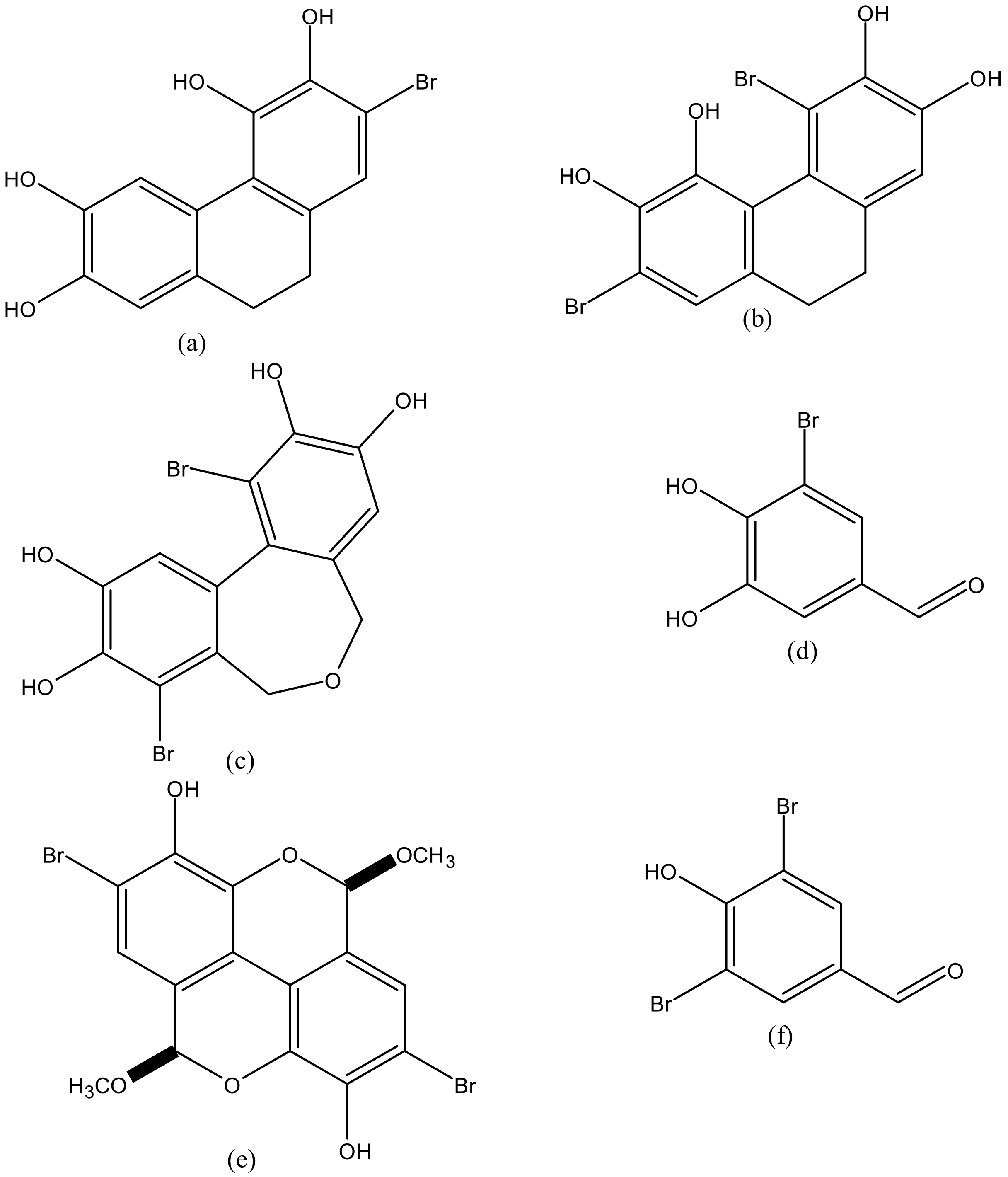


Fig. 13. Structure of (a) 7-bromo-9,10-dihydrophenanthrene-2,3,5,6-tetraol (b) 4,7-dibromo-9,10-dihydrophenanthrene-2,3,5,6-tetraol (c) 1,8-dibromo-5,7-dihydrodibenzo[c,e]oxepine-2,3,9,10-tetraol (d) 3-bromo-4,5-dihydroxybenzaldehyde (e) (5R,10R)-2,7-dibromo-5,10-dimethoxy-5,10-dihydrochromeno[5,4,3-cde]chromene-3,8-diol (Urceolatol) (f) 3,5-dibromo-4-hydroxybenzaldehyde

In the same year, Bin-Gui Wang and colleagues isolated a highly oxygenated bromophenol from *Polysiphonia urceolata*. Urceolatin, 6-bromo-1-(3-bromo-4,5-dihydroxybenzyl) phenanthro[4,5-bcd]furan-2,3,5-triol, has an unprecedented naturally occurring benzylphenanthro[4,5-bcd]furan skeleton (Fig. 14). Urceolatin showed significant DPPH radical-scavenging activity in bioactivity assays, with an IC_{50} value of 7.9 μM (Table. 9). This potency is ten times that of butylated hydroxytoluene, the positive control [27].

Table. 9. IC_{50} values of DPPH radical-scavenging property of the compounds

Sl. No.	**IC_{50} in μM**	**Compound name**
1	7.9	6-Bromo-1-(3-bromo-4,5-dihydroxybenzyl)phenanthro[4,5-bcd]furan-2,3,5-triol (urceolatin)
2	83.8	Butylated hydroxytoluene (BHT)

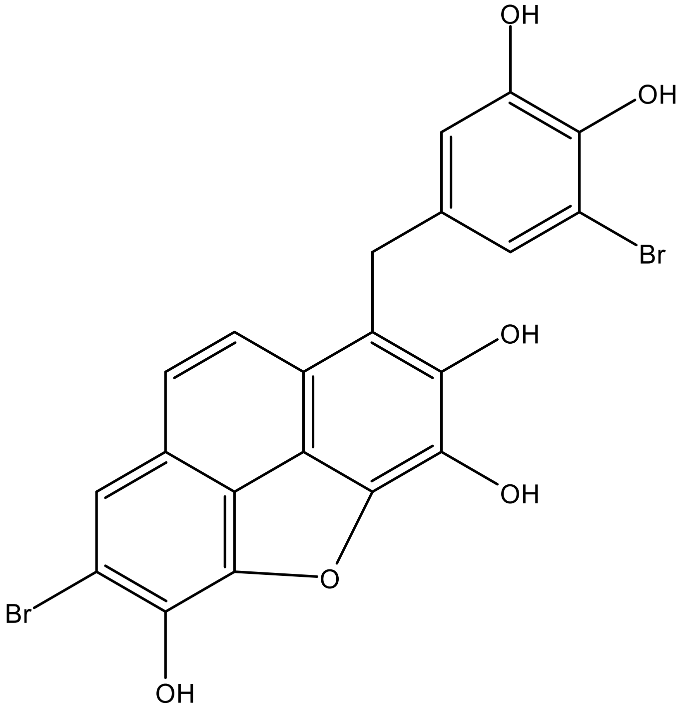


Fig. 14. Structure of 6-bromo-1-(3-bromo-4,5-dihydroxybenzyl)phenanthro[4,5-bcd]furan-2,3,5-triol (Urceolatin)

In 2009, for the first time, Quan-Wen Liu et al. identified a novel bromophenol metabolite derived from *Polysiphonia urceolata*. The configuration and definitive stereochemistry of this bromophenol were determined to be (5S,10S)-2,7-dibromo-3,8-dihydroxy-5,10-dimethoxyl-5,10-dihydro-chromeno[5,4,3-cde]chromene through spectroscopic methods and DFT theoretical analysis. (Fig. 15) No biological activity assessment has been conducted [28].

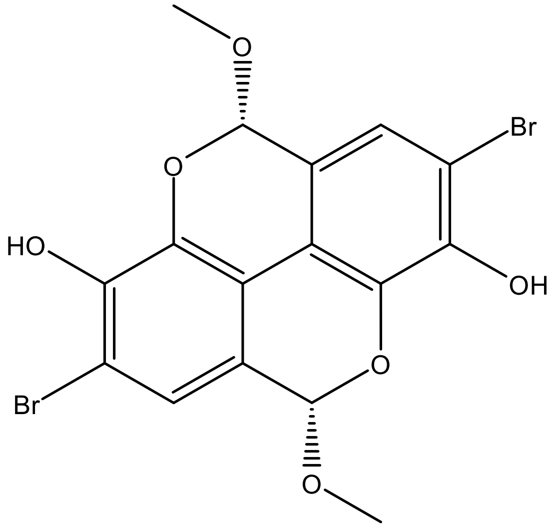


Fig. 15. Structure of (5S,10S)-2,7-dibromo-3,8-dihydroxy-5,10-dimethoxyl-5,10-dihydro-chromeno[5,4,3-cde]chromene

In 2011, So Young Kang et.al., previously extracted *Polysiphonia morrowii* with 90% methanol and the extract exhibited antiviral action on Infectious Hematopoietic Necrosis Virus (IHNV) and Infectious Pancreatic Necrosis Virus (IPNV).

The two isolated compounds are 3-bromo-4,5-dihydroxybenzyl methyl ether and 3-bromo-4,5-dihydroxybenzaldehyde (Fig. 16.). The antiviral activities of the compounds are enlisted in the table below which shows that 3-bromo-4,5-dihydroxybenzyl methyl ether is active against both the viral strains but 3-bromo-4,5-dihydroxybenzaldehyde is selectively active against IPNV (Table. 10) [29].

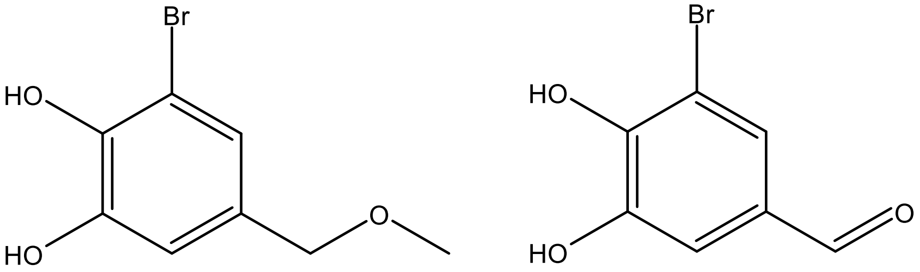
Table. 10. Antiviral activities of compounds against IHNV and IPNV in **µM**

Sl. No.	**IHNV**					**IPNV**				**Compound**
	Pretreatment test			Direct virucidal test		Pretreatment test		Direct virucidal test		
	CC^{a}_{50}	EC^{b}_{50}	SI^{c}	EC_{50}	SI	EC_{50}	SI	EC_{50}	SI	
1	926±153	27.0±6.3	34.3	19.0±4.1	48.7	22.0±0.6	42.1	26.0±8.5	35.6	3-bromo-4,5-dihydroxybenzyl methyl ether
2	1175±154	45.0±9.1	26.1	75.0±14.2	15.7	57.0±10.6	20.6	$>100^{d}$	-	3-bromo-4,5-dihydroxybenzaldehyde
3	475±11	2.0±0.4	237.5	2.6±1.5	182.7	4.0±0.4	118.8	$>50^{d}$	-	Ribavirin

a Concentration required to reduce cell growth by 50% (µg/ml)

b Concentration required to inhibit virus-induced CPE by 50% (µg/ml)

c Selective index=CC_{50}/EC_{50}



(a) (b)

Fig. 16. Structure of (a) 3-bromo-4,5-dihydroxybenzyl methyl ether (b) 3-bromo-4,5-dihydroxybenzaldehyde

In 2013, Elisabeth K. Olsen and coworkers for the first time demonstrated the cellular antioxidant activity of the three known bromophenols, 2,3-dibromo-4,5-dihydroxybenzylaldehyde, 2,2′,3-tribromo-3′,4,4′,5-tetrahydroxy-6′-hydroxymethyl diphenylmethane, and bis(2,3-dibromo-4,5-dihydroxylbenzyl) ether, along with one novel compound, 5,5″-oxybis(methylene)bis(3-bromo-4-(2′,3′-dibromo-4′,5′-dihydroxylbenzyl)benzene-1,2-diol), which were isolated from an extract of *Vertebrata lanosa* (Fig. 17).

The cellular study examined bioavailability of compounds, specifically capacity to traverse cellular membrane. One biochemical and two cellular assays—ORAC, CAA, and CLPAA—assessed the antioxidant efficacy of these four bromophenols. In the CAA and CLPAA assays, 2,2′,3-tribromo-3′,4,4′,5-tetrahydroxy-6′-hydroxymethyldiphenylmethane outperformed luteolin and quercetin as an antioxidant. A normal human lung fibroblast cell line (MRC-5) viability assay assessed cytotoxicity. We tested compounds at concentrations of 5, 25, and 50 µg/mL. Only the compound bis(2,3-dibromo-4,5-dihydroxylbenzyl) ether showed toxicity at 25 µg/mL. (Table.11) [30].

Table. 11. IC_{50} values and % inhibition of Antioxidant and cytotoxic Assay of the compounds

Sl. No.	**IC_{50} in µM**				**Compound name**
	ORAC Trolox equivalent At 1µg/ml	CAA At 10 µg/ml	CLPAA At 5 µg/ml	(MRC-5)	
1	0.8	No activity	18%	No activity	2,3-dibromo-4,5-dihydroxybenzylaldehyde
2	1	68%	45%	No activity	2,2′,3-tribromo-3′,4,4′,5-tetrahydroxy-6′-hydroxymethyl diphenylmethane
3	0.9	5%	2%	25 µg/ml	bis(2,3-dibromo-4,5-dihydroxylbenzyl) ether
4	0.3	8%	9%	No activity	5,5″-oxybis(methylene)bis(3-bromo-4-(2′,3′-dibromo-4′,5′-dihydroxylbenzyl)benzene-1,2-diol)
6	7	92%	35%	Low	Quercetin
7	10	58%	34%	Low	Luteolin

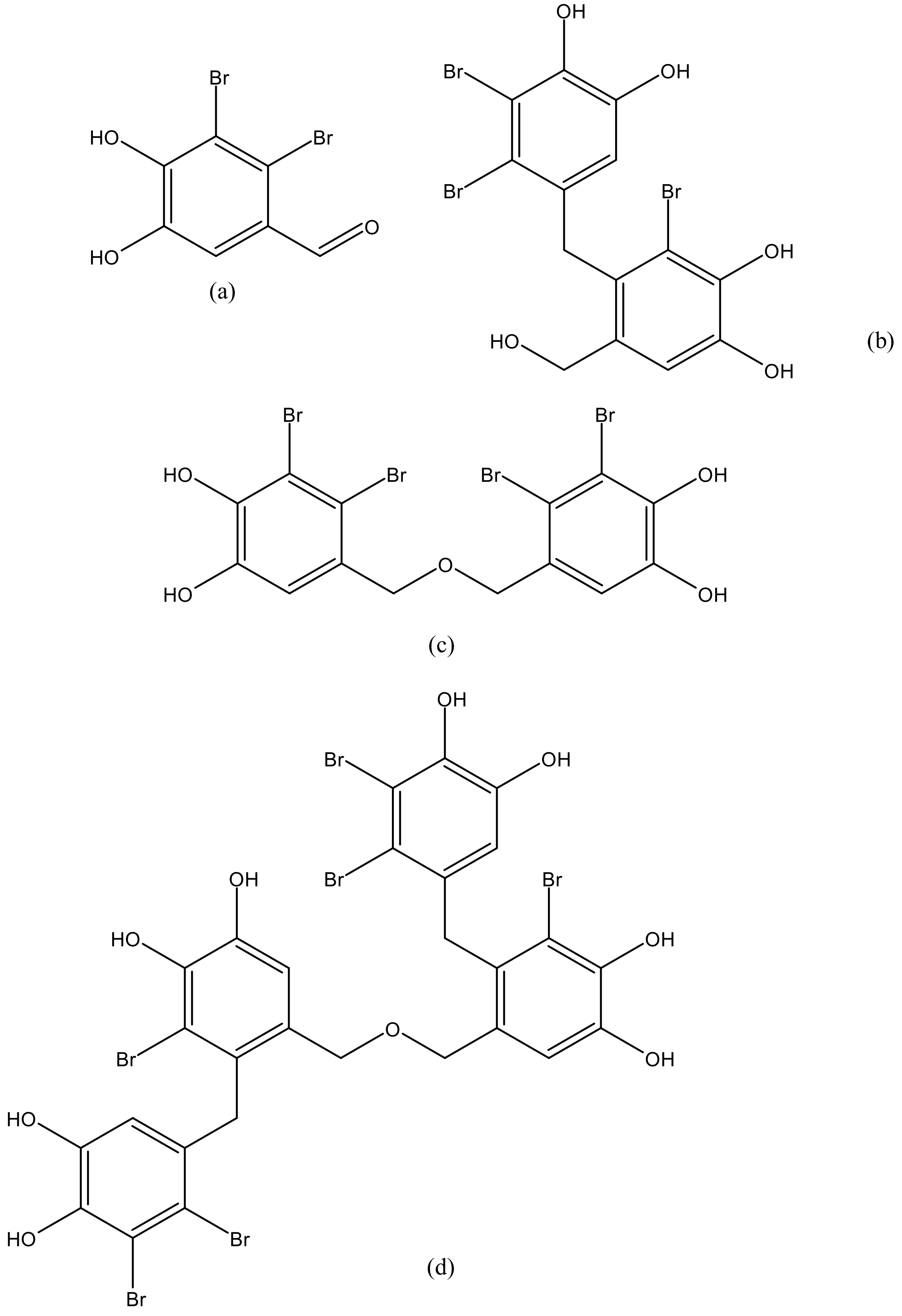


Fig. 17. Structure of (a) 2,3-dibromo-4,5-dihydroxybenzylaldehyde, (b) 2,2′,3-tribromo-3′,4,4′,5-tetrahydroxy-6′-hydroxymethyl diphenylmethane, (c) bis(2,3-dibromo-4,5-dihydroxylbenzyl) ether, (d) 5,5″-oxybis(methylene)bis(3-bromo-4-(2′,3′-dibromo-4′,5′-dihydroxylbenzyl)benzene-1,2-diol)

Li Sun et al. isolated phycobilisomes from *Polysiphonia urceolata* in 2014 using sucrose step-gradient ultracentrifugation. These phycobilisomes were gel-filtered on Sephadex G-150 and purified using DEAE-Sepharose Fast Flow ion exchange chromatography and native PAGE to obtain R-phycocyanin. The purified protein appeared as a homogeneous 136 kDa trimer on gel filtration, a single band on native PAGE, and a single band at pH 5.7 on native isoelectric focusing. The SDS-PAGE analysis showed a single α subunit (17.5 kDa) and two β subunits (21.3 and 22.6 kDa). Both denaturing IEF and two-dimensional PAGE yielded isoelectric point (pI) values of 6.4, 5.3, and 5.4 for α17.5, β21.3, and β22.6. MALDI-TOF MS analysis revealed peptide mass fingerprint differences between the two β subunits. The purified R-phycocyanin has one α and two β subunits, likely forming trimeric forms $(\alpha17.5)_3(\beta21.3)_2(\beta22.6)_1$ and $(\alpha17.5)_3(\beta21.3)_1(\beta22.6)_2$. The close pI values of these forms may explain their co-migration as a single band in native IEF [31].

Li Sun et al. reported three discontinuous native polyacrylamide gel electrophoresis (PAGE) systems for pH 7.0 and 6.5 in the same year, viz., imidazole–acetic acid, HEPES–imidazole/bis-tris, and bis-tris–HEPES–MES. These systems analyzed and prepared Allophycocyanin (AP) and R-phycocyanin (R-PC) from *Polysiphonia urceolata*, phycobiliproteins that are unstable in alkaline conditions. The HEPES–Imidazole/Bis-tris and Bis-tris–HEPES–MES systems resolved and separated AP and R-PC. Absorption and fluorescence spectra showed that these proteins retained their native spectroscopic properties. Alternatives to alkaline-buffered PAGE can separate proteins that are alkali-labile or poorly resolved [32].

Eun-Sook Yoo et al. investigated 3-Bromo-4,5-dihydroxybenzaldehyde (BDB), a bromophenol derived from red algae *Polysiphonia morrowii*, for its anti-inflammatory effects in a 2,4-dinitrochlorobenzene (DNCB)-induced mouse model of atopic dermatitis (AD) and lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages in 2017. (Fig. 18) In vivo, BDB treatment (100 mg/kg) reduced AD symptoms by lowering serum immunoglobulin E, lymph node size, ear edema, and ear tissue inflammatory cell infiltration. BDB (12.5-100 μM) dose-dependently reduced interleukin-6 production in LPS-stimulated macrophages in vitro. BDB prevented NF-κB and STAT1 (Tyr701) phosphorylation, which are crucial signaling mediators in inflammatory pathways. These findings suggest that BDB may treat atopic dermatitis by reducing allergic inflammation [33].

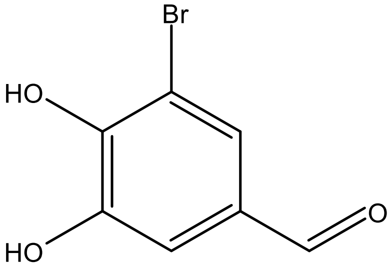


Fig. 18. Structure of 3-Bromo-4,5-dihydroxybenzaldehyde

Youn Kyung Choi et al. isolated Bis (3-bromo-4,5-dihydroxybenzyl) ether from *Polysiphonia morrowii* in 2018. (Fig. 19) Research shows that BBDE reduces inflammation in LPS-induced macrophage cells by reducing mediators like nitric oxide, prostaglandin E2, iNOS, COX2, and pro-inflammatory cytokines (TNF-α, interleukin-1β, and interleukin-6). The mechanism shows that BBDE selectively inhibited mitogen-activated protein kinase ERK phosphorylation. In addition, BBDE inhibited LPS-induced ROS in RAW 264.7 macrophages. BBDE inhibited LPS-induced ROS generation, inactivating ERK and causing inflammatory reaction. BBDE can treat inflammatory diseases [34].

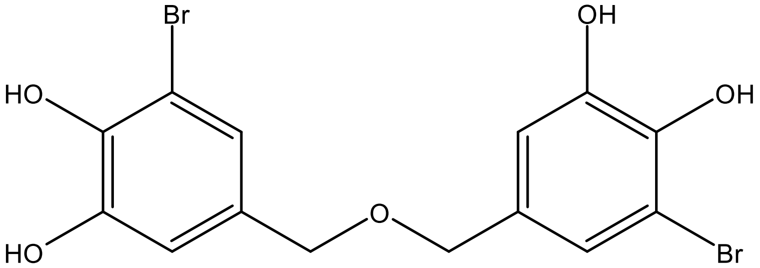


Fig. 19. Structure of Bis (3-bromo-4,5-dihydroxybenzyl) ether

In 2018, Seung-Hong Lee et al. studied the effect of *Polysiphonia morrowii*'s 5-bromo-3,4-dihydroxybenzaldehyde (BD) on 3T3-L1 preadipocytes adipogenesis and differentiation into mature adipocytes, as well as its potential mechanism of action (Fig.20). In BD-treated cells, lipid accumulation and triglyceride were significantly lower. BD treatment also decreased the expression of peroxisome proliferator-activated receptor-γ, CCAAT/enhancer-binding proteins α, and sterol regulatory element-binding protein 1 compared to the control group. It also decreased adiponectin, leptin, fatty acid synthase, and FABP4 expression. One BD effect was AMP-activated protein kinase activation. Research shows that BD inhibits adipogenesis by activating the AMP-activated protein kinase signal pathway [35].

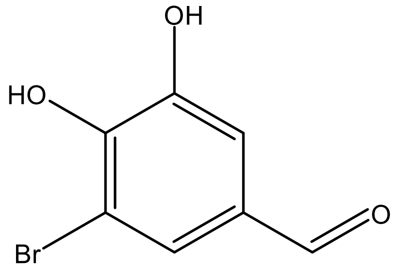
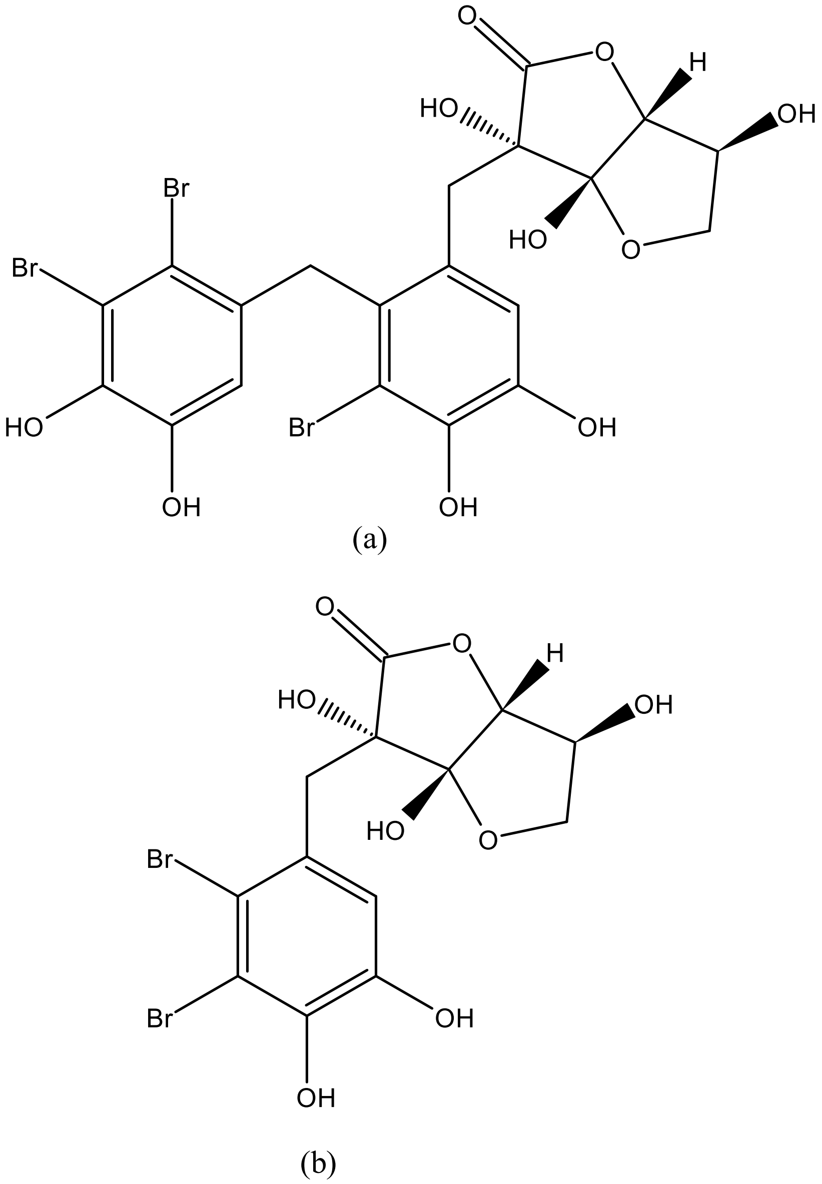


Fig. 20. Structure of 5-bromo-3,4-dihydroxybenzaldehyde

In 2019, Urban and colleagues isolated polysiphonol, a new bromophenol, from *Polysiphonia decipiens*, along with five known compounds: α-O-methyllanosol, lanosol, 5-(2-bromo-3,4-dihydroxy-6-(hydroxymethyl)benzyl)-3,4-dibromobenzene-1,2-diol, and 5-(2-bromo-3,4-dihydroxy-6-(methoxymethyl)benzyl. (Fig. 21) Spectroscopic analysis yielded the first unambiguous confirmation of rhodomelol's absolute configuration. Polysiphonol's biosynthetic configuration was tentatively absolute. Antibacterial activity against Gram-positive and Gram-negative bacteria was insignificant for all six compounds [36].



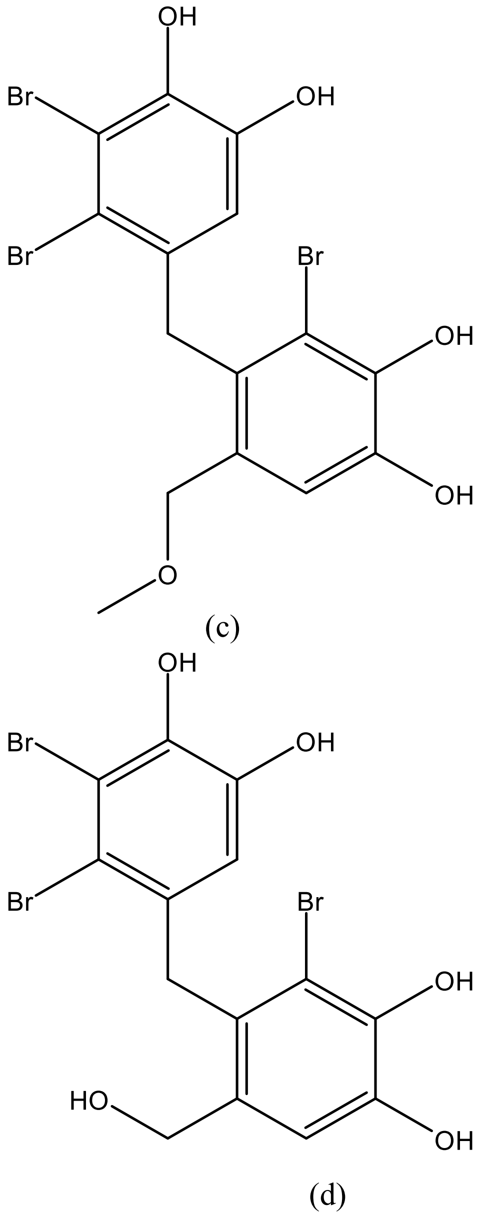
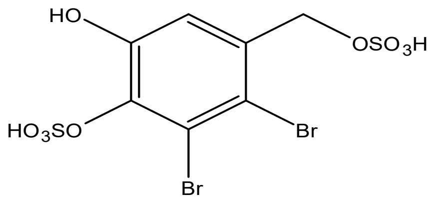


Fig. 21. Structure of (a) (3S,3aS,6S,6aR)-3-(3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl)-3,3a,6-trihydroxytetrahydrofuro[3,2-b]furan-2(5H)-one (Polysiphonol) (b) (3S,3aS,6S,6aR)-3-(2,3-dibromo-4,5-dihydroxybenzyl)-3,3a,6-trihydroxytetrahydrofuro[3,2-b]furan-2(5H)-one (Rhodomelol) (c) 5-(2-bromo-3,4-dihydroxy-6-(methoxymethyl)benzyl)-3,4-dibromobenzene-1,2-diol (d) 5-(2-bromo-3,4-dihydroxy-6-(hydroxymethyl)benzyl)-3,4-dibromobenzene-1,2-diol

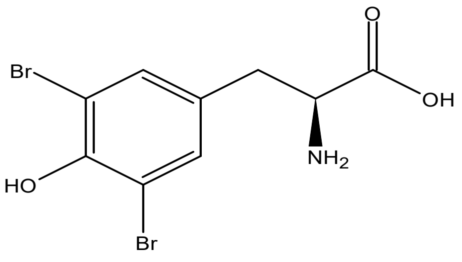
In 2019 Su-Hyeon Cho et al. investigated the protective effects of *Polysiphonia morrowii* Harvey's 3-bromo-4,5-dihydroxybenzaldehyde (BDB) (Fig.18) against H_2O_2-induced apoptosis in Vero cells. BDB scavenged DPPH, hydroxyl, and alkyl radicals. By inhibiting ROS production, BDB prevented H_2O_2-induced lipid peroxidation, cell death, and apoptosis in Vero cells. Western blot assay was used to evaluate the molecular mechanisms of apoptosis inhibition by assessing the expression of Bax/Bcl-xL and NF-κB. BDB significantly reduced PARP and caspase-9 cleavage and Bax levels in H_2O_2-induced Vero cells. Additionally, BDB reduced NF-κB phosphorylation and p65 translocation in H_2O_2-induced cells. These results showed that BDB inhibited ROS generation and cell death in zebrafish embryos, proving its antioxidant activity in vitro and in vivo [37].

In 2020, Soo-Jin Heo et al. examined the anti-allergic properties of the marine algal bromophenol, 3-bromo-4,5-dihydroxybenzaldehyde (BDB), (Fig.18) derived from *Polysiphonia morrowii* Harvey, in immunoglobulin (Ig)E/BSA-stimulated mouse bone marrow-derived cultured mast cells (BMCMCs) and a passive cutaneous anaphylaxis (PCA) mouse ear model. BDB significantly suppressed β-hexosaminidase release (IC50 = 80.12 μM) in IgE/BSA-stimulated BMCMCs without inducing cytotoxicity. BDB diminished the expression and secretion of cytokines, including IL-1β, IL-4, IL-5, IL-6, IL-10, IL-13, IFN-γ, TNF-α, and TARC. BDB also inhibited NFκB and the signaling pathways, such as SYK-LAT-GRB2-Gab2, that are activated by IgE/BSA stimulation. Furthermore, oral BDB administration to IgEsensitized mice diminished the IgE-mediated PCA reaction. The anti-allergic properties of BDB indicate that it warrants comprehensive testing [38].

In 2022, Verena Spiegler et al. investigated the bromophenol composition of *Vertebrata lanosa* from the shores of Europe and North America, with a particular emphasis on the polar components. A total of 23 compounds were isolated, including lanosol-4,7-disulfate and the new compounds 3,5-dibromotyrosine, 3-bromo-5-sulfodihydroxyphenylalanine, 3-bromo-6-lanosyl dihydroxyphenylalanine, 3-(6′-lanosyl lanosyl)tyrosine, and 5-sulfovertebratol. Additionally, 4-sulfo-7-dimethylsulfonium lanosol was also identified along with the principal glycerogalactolipids, another novel (5Z,8Z,11Z,14Z,17Z)-eicosapentaenoic acid 3′-[(6′-O-galactopyranosyl-D-galactopyranosyl)]. -1-glycerol ester, mycosporine-like amino acids, porphyra-334, aplysiapalythine A, and palythine (Fig. 21) [39]. No biological activity was studied.



(a)



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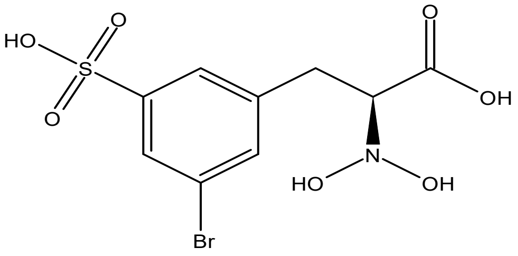

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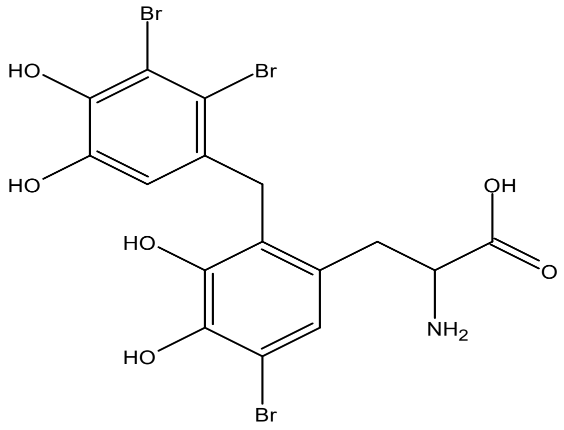

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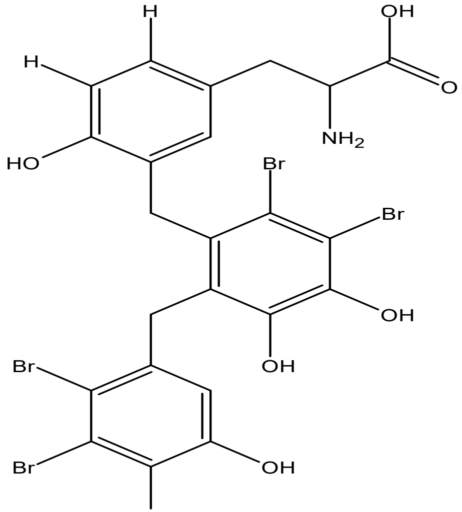

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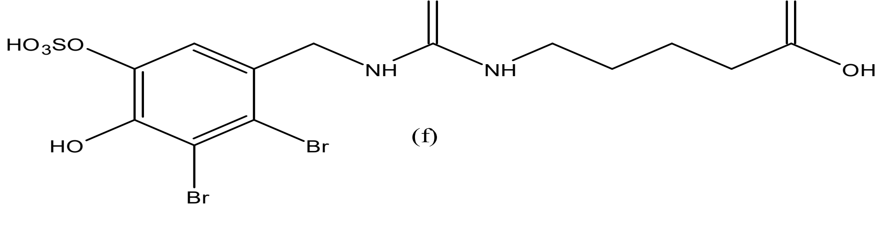
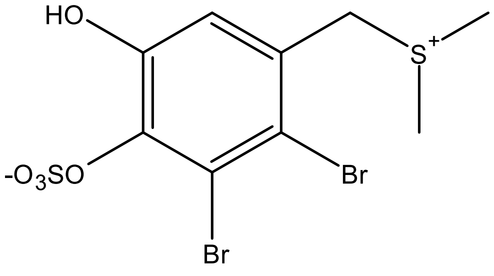


(f)



(g)

Fig. 21. Structures of (a) lanosol-4,7-disulfate, (b) 3,5-dibromotyrosine, (c) 3-bromo-5-sulfodihydroxyphenylalanine, (d) 3-bromo-6-lanosyl dihydroxyphenylalanine, (e) 3-(6′-lanosyl lanosyl) tyrosine, and (f) 5-sulfovertebratol, (g) 4-sulfo-7-dimethylsulfonium lanosol,

In 2022, Eun-A Kim et al. examined the anti-allergic effects of 3,4-dihydroxybenzaldehyde (DHB) (Fig. 22) extracted from *Polysiphonia morrowii* on mouse bone-marrow-derived cultured mast cells (BMCMCs) and passive cutaneous anaphylaxis (PCA) in anti-dinitrophenyl (DNP) immunoglobulin E (IgE)-sensitized mice. DHB reduced hexosaminidase release to inhibit IgE/BSA-induced BMCMC degranulation without cytotoxicity. DHB also dose-dependently decreased BMCMC surface IgE binding, high-affinity IgE receptor (Fc"RI) expression, and Fc"RI-IgE binding. DHB inhibited the release of allergic cytokines (IL-4, IL-5, IL-6, IL-13, TNF-α, and TARC) by regulating IB phosphorylation and NF-B translocation into the nucleus. DHB also reduced Evans blue exudation in mouse ears stimulated by IgE/BSA in passive cutaneous anaphylactic (PCA) reactions. These findings suggest that DHB may prevent and treat type I allergic disorders [40].

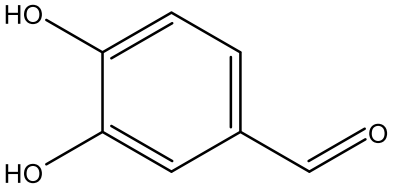


Fig. 22. Structure of 3,4-dihydroxybenzaldehyde

Widely Studied Bioactivities of the Bromophenols

a. DPPH radical scavenging assay **[41]**

Assessing antioxidant property of the extracts or isolated compounds is an important assay in the quest of bioactivities of natural or synthetic compounds. There are several invitro and in vivo models accepted globally. One such popular method is DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay which is a non-costly, rapid spectrophotometric method. The mechanism involves reduction of DPPH radicals by the active compounds while DPPH changes its color from purple to yellow and the absorbance is noted at 517 nm. The results are expressed as IC_{50} values. Lower the IC_{50} value higher is the antioxidant property. There are some complimentary assays such as Hydrogen Atom Transfer (HAT) Assays which includes Oxygen Radical Absorbance Capacity (ORAC) or Total Radical-Trapping Antioxidant Parameter (TRAP) [42, 43]. Electron Transfer (ET) Assays measures the capacity to reduce oxidants/metals, Ferric Reducing Antioxidant Power (FRAP) which includes Cupric Ion Reducing Antioxidant Capacity (CUPRAC) and DPPH & ABTS Assays [44, 45, 46].

b. Cytotoxic activity studies [47, 48]

The purpose of cytotoxicity studies is to assess a compound's safety and effectiveness as well as its therapeutic activity as an anticancer agent. A compound's ability to kill cells was first assessed, but thanks to advancements in cutting-edge technologies, it is now possible to confidently assess the compounds' potency and mechanism of action. Numerous in vitro assay methods are currently popular, such as MTT, MTS, and Alamar Blue methods for membrane integrity and metabolic activity investigations. Cell proliferation and structure are determined using Trypan Blue and LDH release methods; mechanistic approaches such as caspase activity, comet assay, and micronucleus assay are determined using Crystal Violet and clonogenic assay. For the targeted studies, a number of cell line panels are chosen, such as the NCI-60 Human Tumor Cell Line Screen and Commercial and Institutional Panels. The activities are reported as EC_{50} (Half-maximal Effective Concentration) and IC_{50} (Half-maximal Inhibitory Concentration) values.

Discussion

A critical analysis of several bromophenol derivatives across the tested biological activities of different Polysiphonia species gives a notion about their structure-activity relationship. Widely studied bioactivity is free radical scavenging action and cytotoxicity, along with a few antibacterial and antiviral studies.

a. Free radical scavenging/antioxidant action

The most common DPPH free radical scavenging method was to study bromophenolic compounds using BHT as the standard drug. **Table 5** illustrates that the most effective compounds, Chaetopyranin (35 µg/ml) and Isotetrahydroauroglaucin (26 µg/ml), are lipophilic prenylated (3-methylbut-2-enyl) aromatic aldehydes of chroman and trisubstituted benzaldehyde groups. The dihydropyran ring (chroman ring) in chaetopyranin was less active than isotetrahydroauroglaucin [24]. All four bromophenols in **table 7** are biologically active, with (E)-4-(3-bromo-4,5-dihydroxyphenyl)-but-3-en-2-one being the most active (9.67 µg/ml). In their scaffold, all compounds have at least one bromine atom, one hydroxyl group on the benzene ring, and a highly oxygenated benzene ring. But-3-en-2-one (vinyl ketone) side chains in one ring make the most active compound. Other compounds have linkers like a propionic acid chain ($–CH_2–CH(–COOH)–$), a butyl ester (acetic acid ester), and an ethane bridge ($–CH_2–CH_2–$) connecting two identical rings. Common bromination patterns have a bromine atom at position 3, but 3,5-dibromo derivatives are active [25]. According to **table 8**, all six compounds are highly active compared to the standard and share similarities like phenolic hydroxyl groups impregnated with bromine atoms and at least one fully aromatic ring. The four polycyclic compounds (1–4) are dimeric or cyclized variants with two monomeric units linked by C–C bonds (phenanthrenes), C–O–C bonds (oxepine), or fused heterocyclic systems. All six compounds have one or both of these two simple bromophenol motifs: 3-bromo-4,5-dihydroxyphenyl (Br at C3, OH at C4 and C5) and 3,5-dibromo-4-hydroxyphenyl [26]. **Table 9** features urceolatin, a highly active compound with an IC$_{50}$ of 7.9 µM. Table 11 shows antioxidant activity using ORAC, CAA, and CLPAA. The 2,3 dibromo 4,5 dihydroxyphenyl group underpins all four compounds. All compounds had biological activity. Simply put, Compound 1 is benzaldehyde derivative. The common motif and a brominated/hydroxylated ring linked by a methylene bridge make up Compound 2, a mixed dimer. Compound 3 is a symmetric dimer with an ether bridge connecting two identical units. Compound 4 is a higher oligomer with two benzyl side chains and a central 3-bromo-1,2 dihydroxybenzene core dimerized by a central $–CH_2–O–CH_2–$ linker. Thus, the 2,3 dibromo 4,5 dihydroxyphenyl motif is the structural signature of all four compounds [30].

b. Cytotoxic activity

Table 3 shows all active compounds have a catechol ring (1,2-dihydroxybenzene). Cytotoxicity requires this ortho-dihydroxy phenyl group as the "pharmacophore". Positioning the bromine atoms on the catechol ring is crucial. These compounds have two substitution patterns. The second, third, and fourth compounds (methyl, ethyl, and n-propyl ethers) have 2,3-Dibromo substitutions, and the sixth, seventh, eighth, and ninth compounds (alcohol, methyl, ethyl, and n-propyl ethers) have 2,5-Dibromo substitutions. The structure-activity relationship is strongest in the alkyl side chain. Anticancer activity was highest with a three-carbon n-propyl chain. The n-propyl ether derivative's increased potency proves this. The most effective compound, 3c (2,5-dibromo-3,4-dihydroxybenzyl n-propyl ether), combines a catechol core with an alternative 2,5-dibromo substitution pattern and an ideal n-propyl side chain. This compound had highly effective IC$_{50}$ values of 1.72 µM against DLD-1 and 0.80 µM against HCT-116 cells [22]. Structure activity relationship for antibacterial results is unfavorable.

Conclusion

The present review is a summary of the studies performed on Polysiphonia species from different continents, according to their availability. The main bioactivities explored for the compounds are antioxidant, antimicrobial, anti-inflammatory and anticancer properties. In this review, the main results from the past to the present are summarized, especially the discovery of new bromophenols and the isolation of major bromophenols from Polysiphonia. Besides the classical bioactivity studies, antiallergic activity was also applied to find other compounds with similar potency. The structure-activity relationship study of the bromophenols from Polysiphonia suggests a future scope for pharmacophore-based drug discovery which may lead to the identification of potent anticancer hit or lead compounds. These compounds with their free radical scavenging potential could be used to treat neurodegenerative diseases, cardiovascular diseases and inflammatory conditions, both as adjuncts to cancer therapy and in preventive and anti-aging therapies. However, there are still great opportunities in this field to explore the vast potential of marine organisms.

Acknowledgments

The authors express their gratitude to the institution and university departments for the support, motivation and encouragement for writing this review article.

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