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SYNTHESIS AND ANTIFUNGAL ACTIVITY OF POLYPHENOL ETHER DERIVATIVES AGAINST PLANT PATHOGENIC FUNGI *IN VITRO* AND *IN VIVO*

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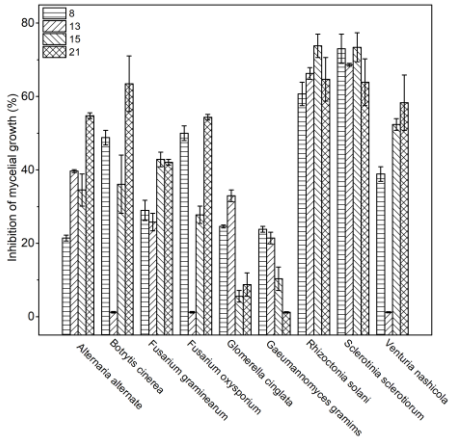
Phytopathogenic fungi have been considered as an enormous threat in the agricultural system. In our search of new antifungal natural products, twenty-seven polyphenol ether derivatives were synthesized. Their structures were elucidated based on extensive spectroscopic analysis. The results suggest that compound **8**, **13**, **15** and **21** exhibited strong antifungal activities against *R. solani and S. sclerotiorum in vitro*, and compound **13** displayed the best antifungal efficacy toward *E.* graminis *in vivo*. This work provides an effective strategy for searching antifungal candidate agents.



INTRODUCTION

Polyphenols are secondary metabolites of plats and widely exist in nature. They are a class of compounds with a wide range of pharmacological properties. At present, studies on polyphenols mainly focus on their bioavailability, antioxidant and anti-cancer activity, and bacteriostasis.[1] For example, considerable recent interest has concentrated on bioactive phenolic compounds in grape, as they possess many biological activities, such as antioxidant, cardioprotective, anti-cancer, anti-inflammation, anti-ageing and antimicrobial properties.[2] It is reported that berry phenolics have extensive beneficial effects because of their antioxidant and anti-inflammatory properties.[3] Y. Zhang found that *Chroogomphus rutilus* polyphenol extracts revealed a higher antioxidant, anti-inflammatory, and cytotoxic activities.[4] L. Delgado-Roche found that polyphenols have certain cytotoxicity to colorectal cancer cells, which can inhibit tumor growth and induce apoptosis.[5] In terms of bacteriostasis, research has shown that polyphenols from *Amygdalus*

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pcdunculata Pall seed coat had good antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Bacillus subtilis*.[6] Simultaneously, polyphenols were proved to play a beneficial role in the prevention and development of chronic diseases related to inflammation, such as diabetes, obesity, neurodegeneration, and cardiovascular diseases.[7]

Gallic acid (3,4,5 trihydroxybenzoic acid), a phenolic acid, is known to form various derivatives having a variety of the aforementioned biological functions.[8] D. Wang reported that gallic acid inhibited the activation of Epidermal Growth Factor Receptor (EGFR), repressed the proliferation and elevated apoptosis of Nonsmall-cell Lung Cancer (NSCLC) cells, and it has been confirmed in animal experiments that gallic acid exhibited an inhibitory effect on tumor growth *in vivo*.[9] Research has shown that gallic acid interacts with paclitaxel to increase its cytotoxic effect and cell induction.[10] According to research focused on gallic acid, it was found that the gallic acid can induce apoptosis of human gastric cancer cells.[11] Studies also suggested that gallic acid is anti-inflammatory via attenuating Lipopolysaccharide (LPS)-induced neuroinflammation, oxidative stress, and protein conjugation.[12]

Although a multitude of galloylated polyphenolic compounds distribute in nature, galloylated phenols also need to be produced synthetically to influence their biological properties.[13] In this paper, a series ofalkyl benzoate ether derivates were synthesized, and the antifungal activity of the prepared 27 compounds against plant pathogenic fungi *in vitro* and *in vivo* were investigated.

RESULTS AND DISCUSSION

In vitro activity against phytopathogenic fungi

The antifungal activity of the synthesized polyphenol ether derivatives against *Rhizoctonia solani* and *Sclerotinia sclerotiorum* were tested, and the results were listed in Table 1. It is clear that among this series of prepared compounds, compound **8**, **13**, **15** and **21** exhibited greater antifungal activity in comparison with other compounds, with the mycelial growth inhibition values of 60.7±3.3 (**8**), 66.2±1.7 (**13**), 73.7±3.3 (**15**), 64.9±5.6 (**21**) for *R. solani*, and 73.0±3.9 (**8**), 68.9±0.5 (**13**), 73.3±4.2 (**15**), 63.9±6.2 (**21**) for *S. sclerotiorum*, respectively. Particularly, compound **15** was the most potent that displayed the most efficient mycelial growth inhibition action toward *R. solani* and *S. sclerotiorum*.

To further investigate the antifungal effect of compound **8**, **13**, **15** and **21**, other seven pathogenic fungi including *Alternaria Alternate*, *Botrytis Cinerea*, *Fusarium Graminearum*, *Fusarium Oxysporium*, *Glomerella Cinglata*, *Gaeumannomyces Gramims*, *Rhizoctonia Solani*, *Sclerotinia Sclerotiorum*, and *Venturia Nashicola* were selected. The inhibition activity against these fungi as well as *R. solani* and *S. sclerotiorum* of compound **8**, **13**, **15** and **21** were given in Fig. 1. The results showed that these compounds were more susceptible to *R. solani and S. sclerotiorum* comparing with other fungi. Furthermore, it was noted that besides *R. solani and S. sclerotiorum*, compound **8** also exhibited notable antifungal activity against *B. Cinerea* and *F. Oxysporium* with the inhibition rates of mycelial growth of 48.8% and 50.0%, and compound **21** also displayed prominent antifungal action toward *A. Alternate*, *B. Cinerea* and *F. Oxysporium* with the inhibition rates of mycelial growth of 54.8%, 63.5% and 54.4%, respectively.

In vivo activity against Erysiphe graminis

The control effects on wheat powdery mildew of the series of polyphenol ether derivatives in greenhouse were also tested. Their fungicidal activities against *E. graminis* at concentration of 500 μg/ml were determined *in vivo*, and the results are listed in Table 2. The results showed that compound **13** had strong activity against *E. graminis in vivo* with the preventive and curative effects of 66.9 and 72.1%, respectively, although the effects are weaker than those of standard fungicide Triadifeon. However, compound **8**, **15** and **21** that possessed outstanding antifungal effect *in vitro* had almost no antifungal activity toward *E. graminis* in green house.

Table 1

Fungi toxicity of the prepared compounds on mycelial growth inhibition of *R. solani* and *S. sclerotiorum*

No. of compound	Inhibition of mycelial growth (%) (±SD)[a]	
	R. solani	S. sclerotiorum
1	(14.6±3.9) k	(8.4±2.1) K
2	(26.7±6.9) fg	(7.3±1.9) K
3	(16.0±3.5) ji	(19.8±1.4) JI
4	(45.3±3.4) d	(35.7±9.2) FGH
5	(44.3±8.7) d	(25.8±1.6) HI
6	(31.5±7.5) ef	(40.4±2.4) FG
7	(14.3±3.8) k	0 L
8	(60.7±3.3) bc	(73.0±3.9) A
9	(22.3±6.0) hgi	(34.0±4.6) FG
10	(23.6±3.6) gh	(41.1±2.2) FE
11	(37.1±2.4) e	(47.4±1.8) DE
12	(28.3±3.0) fg	(49.3±4.2) DC
13	(66.2±1.7) b	(68.9±0.5) AB
14	(10.8±3.4) k	(17.6±3.7) J
15	(73.7±3.3) a	(73.3±4.2) A
16	01	(35.5±4.0) FGH
17	(17.9±0.5) hji	(22.1±1.7) JI
18	(56.9±2.8) c	(55.3±7.6) C
19	(17.1±1.6) hji	(38.7±2.7) FG
20	(32.6±3.1) ef	(38.0±5.1) FG
21	(64.9±5.6) b	(63.9±6.2) B
22	01	0 L
23	01	(3.6±1.3) KL
24	(18.6±0.7) hji	(7.1±2.2) K
25	(17.6±1.0) ji	(9.2±0.8) K
26	(27.4±4.3) fg	(29.1±4.1) HG
27	(37.0±4.6) e	(33.5±4.5) FGH

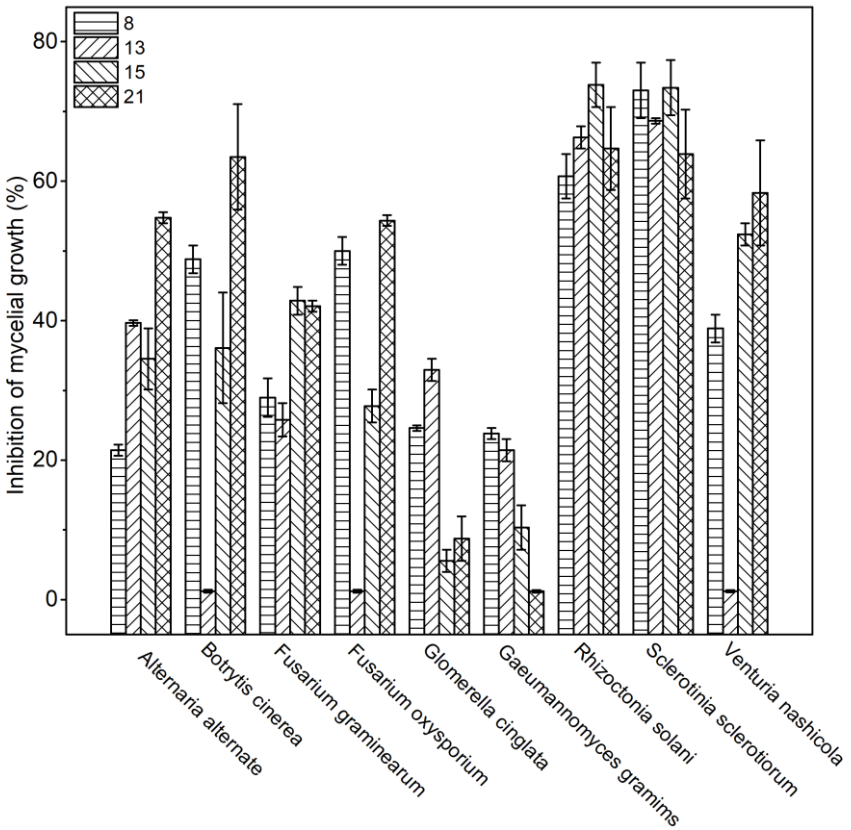


Fig. 1 – Antifungal activity spectra of compound **8**, **13**, **15** and **21** against nine pathogenic fungi.
Error bars represent the standard error of the mean of three replicates.

Table 2

In vivo control of Erysiphe graminis with protective and curative spray applications of polyphenol ether derivatives

No. of compound	Dose (mg/L)	Protective effects (%) (±SD) a	Curative effects (%) (±SD) b
		8 days	8 days
1	500	(15.0±3.3) jk	(12.7±2.8) KJ
2	500	0 l	(18.5±4.7) KJI
3	500	0 l	(38.4±6.0) DE
4	500	(20.9±1.3) hjki	(39.0±4.8) DE
5	500	(37.8±6.3) cde	(28.8±3.1) HFG
6	500	(13.5±5.8) k	(33.3±3.0) EDF
7	500	(17.0±5.3) jki	(11.0±3.0) K
8	500	(37.3±8.2) cde	(40.0±1.0) D
9	500	(25.4±6.2) hfg	(27.6±5.1) HFG
10	500	(32.5±6.7) cdef	0 L
11	500	0 l	(13.8±2.8) KJ
12	500	(35.5±1.2) cde	0 L
13	500	(66.9±4.3) b	(72.1±6.8) B
14	500	(40.0±2.4) c	(34.3±6.6) EDF
15	500	(31.4±7.6) cdef	(47.5 ±2.2) C
16	500	(29.8±1.9) efg	(14.1±6.1) KJ
17	500	0 l	(36.1±2.1) EDF
18	500	(30.9±6.6) def	(32.5±6.4) EDF
19	500	(14.7±1.6) jk	(49.2±7.7) C
20	500	(39.2±3.4) cd	(21.1±6.7) HJI
21	500	(22.3±4.8) hgji	(31.0±0.5) EGF
22	500	(15.1±3.0) jk	(30.5±5.2) EGF
23	500	(13.8±3.7) jk	(37.7±5.9) EDF
24	500	(3.9±4.2) l	(16.0±4.5) KJI
25	500	(24.7±8.0) hfgi	(14.7±3.6) KJI
26	500	(13.6±4.2) k	(19.1±5.8) KJI
27	500	(32.8±5.5) cdef	(23.0±3.8) HGI
Triadifeon	250	(82.58±1.95) a	(88.3±1.7) A

EXPERIMENTAL SECTION

Materials and Measurements

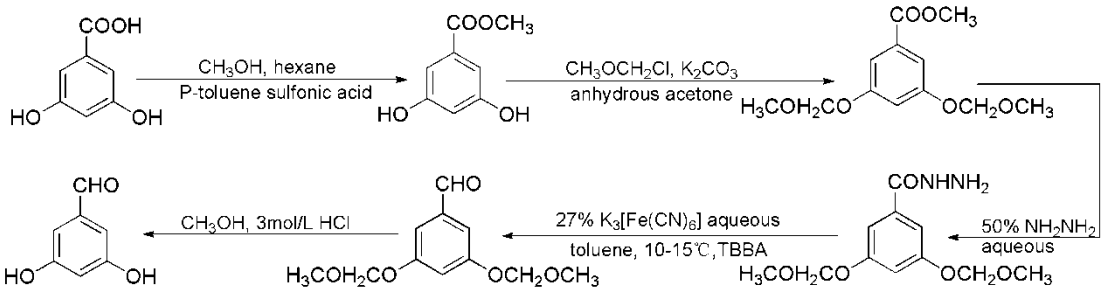
Alkanols were purchased from Shanghai Chemical Factory (China). Gallic acid, Dicyclohexylcarbodiimide (DCC), trans,trans-farnesyl bromide, 4-bromo-2-methyl-2-butene, 3,5-dihydroxybenzoic acid and chloromethyl methyl ether were purchased from Aldrich Chemical Co. (Milwaukee, WI). The organic solvents were purified and dried using appropriate procedures. EI-MS spectra were measured with a VG Autospec3000 mass spectrometer (VG, England). ^{1}H, ^{13}C-NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometer (Karlsruhe, Germany). The chemical shifts were reported in ppm (δ) relative to the internal standard tetramethylsilane (TMS). Air- and/or moisture-sensitive reactions were carried out under an argon or nitrogen atmosphere.

Synthesis

Synthesis of alkyl gallates ester

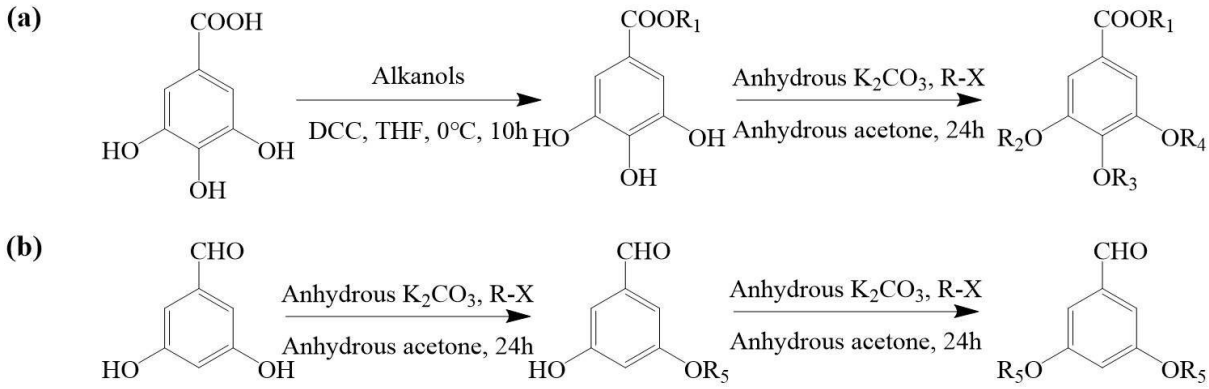
To a solution of gallic acid (2.00 mM) and the corresponding alcohol (CH_3CH_2OH, $(CH_3)_2CHCH_2CH_2OH$, $CH_3(CH_2)_{16}CH_2OH$, CH_3OH, $(CH_3)_2CHOH$, $(CH_3)_2CHCH_2CH_2OH$, $CH_3CH_2CH_2(CH_3)CHOH$, 2.00 mM) in Tetrahydrofuran (THF, 10 mL) cooled at 0°C was added a solution of DCC (4.2 mM) in THF (10 mL). After stirring for 10 h, the solvent of the resulted mixture was removed under reduced pressure. The residue was extracted with ethyl acetate five times and filtered. The filter was washed successively with 4 M HCl solution, saturated $NaHCO_3$ solution, and water, and then dried over Na_2SO_4 and evaporated. The crude products were purified by column chromatography on silica gel with petroleum ether/ethyl acetate (4:1, v/v) as eluent. The synthetic route of the esters was shown in Scheme 2.

Synthesis of 3,5-dihyrdroxybenzaldehyde



Scheme 1 – Synthesis of 3,5-dihydroxybenzaldehyde.

Synthesis of polyphenol ether derivatives



Scheme 2 – Synthesis of polyphenol ether derivatives.

In order to synthesize polyphenol ether derivatives, anhydrous K_2CO_3 (2 mM), anhydrous acetone (10 mL) and R-X (trans, trans-farnesyl bromide, 4-bromo-2-methyl-2-butene, chloromethyl methyl ether) were added to the solution of corresponding alkyl gallates ester (1 mM) or 3,5-dihydroxybenzaldehyde (1 mM). Then the reaction mixture was heated under reflux for 24h. The solvent was removed under reduced pressure. The residue was extracted with ethyl acetate several times and filtered. The filter was washed successively with 4M HCl solution, saturated $NaHCO_3$ solution and water, and then dried over Na_2SO_4 and evaporated. The crude products were purified by column chromatography on silica gel with petroleum ether/ethyl acetate (7:1, v/v) as eluent. Structures of the synthesized esters were illustrated in Table 3.

Characterization of the prepared compounds

Ethyl 4-((2E,6E),2,6,11-trimethyldodeca-2,6,10-trienyloxy)-3,5-dihydroxybenzoate **($C_{24}H_{34}O_5$, 1):** obtained in 34% yield as yellow oil. EI-MS: *m/z* 402M^+ (4), 265 (3), 249 (12), 219 (13), 205 (26), 198 (55), 183 (19), 170 (30), 153 (64), 149 (34), 137 (51), 121 (43), 107 (33), 95 (48), 81(100), 69 (84), 55 (18); ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.3 (s, CO), 149.1 (s), 145.3 (s), 137.3 (s), 135.7 (s), 131.4 (s), 126.6 (s), 124.3 (d), 123.4 (d), 118.6 (d), 109.5 (d), 69.9 (t, OCH2), 61.1 (t, OCH_2), 39.6 (t), 32.0 (t), 26.7 (t), 26.1 (t), 25.7 (q, CH_3), 17.7 (q, CH_3), 16.4 (q, CH_3), 16.0 (q, CH_3), 14.7 (q, CH_3), 14.2 (q, CH_3); ^{1}H NMR ($CDCl_3$, 400 M) δ: 7.23 (s, 2H, ArH), 4.63 (d, J=6.0 Hz, 2H, OCH_2), 4.34 (dd, J=5.7 Hz, J=11.4 Hz, 2H, OCH_2), 5.73 (s, 2H, OH), 5.54 (t, 1H, CH), 5.08 (t, 2H, CH), 2.06 (m, 2H, CH_2), 1.97 (m, 2H, CH_2), 1.26 (t, CH_3), 1.60 (s, 6H, CH_3), 1.68 (s, 3H, CH_3), 1.71 (s, 3H, CH_3).

Ethyl 4-(3-methylbut-2-enyloxy)-3,5-dihydroxybenzoate **($C_{14}H_{18}O_5$, 2):** obtained in 45% yield as a colorless solid. Positive FAB-MS M+H^+ 267; ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.3 (s, CO), 149.1 (s), 149.1 (s), 141.7 (s), 137.8 (s), 126.5 (s), 119.1 (d), 109.5 (d), 109.5 (d), 70.0 (t), 61.1 (t), 25.8 (q), 18.0 (q), 14.2 (q); ^{1}H NMR ($CDCl_3$, 400 M) δ: 7.25 (s, 2H, ArH), 5.85 (s, 2H, OH), 5.23 (t, 1H, CH), 4.62 (d, J=6.0 Hz, 2H, OCH_2), 4.34 (dd, J=5.7 Hz, J=11.4 Hz, 2H, OCH_2), 1.77 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 1.37 (s, 3H, CH_3).

Ethyl 3-(3-methylbut-2-enyloxy)-4,5-dihydroxybenzoate **($C_{14}H_{18}O_5$, 3):** obtained in 56% yield as yellow colorless oil. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.2 (s, CO), 145.3 (s), 143.0 (s), 139.1 (s), 136.8 (s), 121.6 (s), 118.5 (d), 110.4 (d), 65.8 (t, OCH_2), 60.8 (t, OCH_2), 25.5 (q), 17.9 (q), 14.0 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.39 (s, 1H, ArH), 7.24 (s, 1H, ArH), 4.59 (d, J=6.8 Hz, H, OCH_2), 4.31 (dd, J=7.1 Hz, J=15.1 Hz, 2H, OCH_2), 5.45 (t, 1H, CH), 1.40 (t, 3H, CH_3), 1.77 (s, 3H, CH_3), 1.93 (s, 3H, CH_3).

Isopentyl 4-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-3,5-dihydroxybenzoate **($C_{27}H_{40}O_5$,4):** obtained in 45% yield as colorless oil. EI-MS: *m/z* 444 M^+ (5), 375 (4), 362 (4), 331 (3), 291 (18), 240 (4), 205 (15), 191 (25), 183 (40), 170 (86), 153 (57), 137 (41), 121 (37), 93 (52), 81 (83), 69 (100), 55 (22); HR-ESI M+Na^+ 467.2772, calc. 467.2773; ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.9 (s, CO), 149.3 (s), 149.3 (s), 144.6 (s), 137.5 (s), 135.5 (s), 131.2 (s), 125.8 (s), 124.2 (d), 123.4 (d), 118.8 (d), 109.4 (d), 109.4 (d), 25.6 (d), 69.6 (t, OCH_2), 63.9 (t, OCH_2), 39.6 (t), 39.5 (t), 37.2 (t), 26.6

(t), 26.1 (t), 25.0 (q), 22.4 (q), 17.6 (q), 16.4 (q), 16.3 (q), 15.9 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.29 (s, 2H, ArH), 4.32 (t, 2H, OCH_2), 4.69 (d, J=7.3 Hz, 2H, OCH_2), 5.07 (br 2H, CH), 5.52 (t, 1H, CH), 6.54 (br, 2H, OH), 097 (d, J=12.9 Hz, 6H, CH_3), 1.59 (br s, 6H, CH_3), 1.67 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 1.95 (m, 1H, CH), 2.07 (m, 8H, CH_2).

Isobutyl 4-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-3,5-dihydroxybenzoate) **($C_{26}H_{38}O_5$, 5):** obtained in 32% yield as yellow oil. HR-ESI M+Na^+ 453.2617, calc. 453.2616; ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.7 (s, CO), 149.3 (s), 144.6 (s), 137.7 (s), 135.6 (s), 131.2 (s), 126.2 (s), 124.4 (d), 123.5 (d), 123.5 (d), 119.0 (d), 109.6 (d), 109.6 (d), 26.7 (d), 71.3 (t, OCH_2), 69.8 (t, OCH_2), 39.6 (t), 39.6 (t), 26.7 (t), 26.2 (t), 25.5 (q, CH_3), 19.1 (q, CH_3), 19.1 (q, CH_3), 17.6 (q, CH_3), 16.4 (q, CH_3), 15.9 (q, CH_3); ^{1}H NMR ($CDCl_3$, 400 M) δ: 6.22 (s, 2H, ArH), 4.68 (d, J=7.4 Hz, 2H, OCH_2), 4.08 (d, J=6.5 Hz, 2H, OCH_2), 5.53 (t, 1H, CH), 5.08 (t, 2H, CH), 2.01 (m, 1H, CH), 0.97 (d, J=18.2 Hz, 6H, CH_3), 1.67 (s, 6H, CH_3), 1.60 (s, 6H, CH_3), 2.08 (m, 8H, CH_2).

Isobutyl 3-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-4,5-dihydroxybenzoate **($C_{26}H_{38}O_5$, 6):** obtained in 22% yield as yellow oil. Positive FAB-MS M+H^+ 431; HRESI-MS M+Na^+ 453.2629, cacl. 453.2616; ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.8 (s, CO), 150.0 (s), 143.6 (s), 143.0 (s), 137.3 (s), 135.9 (s), 131.7 (s), 122.4 (s), 124.6 (d), 123.8 (d), 118.9 (d), 111.0 (d), 106.4 (d), 28.2 (d), 71.3 (t, OCH_2), 66.5 (t, OCH_2), 40.0 (t), 39.8 (t), 27.0 (t), 26.5 (t), 26.0 (q, CH_3), 19.5 (q, CH_3), 19.5 (q, CH_3), 18.0 (q, CH_3), 17.0 (q, CH_3), 16.3 (q, CH_3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.34 (s, 1H, ArH), 7.22 (s, 1H, ArH), 4.65 (d, J=5.3 Hz, 2H, OCH_2), 4.07 (d, J=5.3 Hz, 2H, OCH_2), 5.07 (br s, 2H, OH), 5.89 (br t, 1H, CH), 5.44 (br t, 2H, CH), 2.04 (br t, 8H, CH_2), 0.98 (d, J=6.7 Hz, 6H, CH_3), 1.58 (br s, 6H, CH_3), 1.67 (d, J=12.7 Hz, 3H, CH_3), 1.76 (d, J=21.8 Hz, 3H, CH_3).

Octadecyl 4-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-3,5-dihydroxybenzoate **($C_{40}H_{66}O_5$, 7):** obtained in 65% as colorless oil. HR-ESI M+Na^+ 649.4816, calc. 649.4807; ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.8 (s, CO), 149.3 (s), 149.3 (s),144.8 (s), 137.4 (s), 135.6 (s), 131.3 (s), 126.1 (s), 124.2 (d), 123.4 (d), 118.8 (d), 109.5 (d), 109.5 (d), 69.7 (t, OCH_2), 65.5 (t, OCH_2), 39.6 (t), 39.6 (t), 39.6 (t), 31.1-29.3 (br t,11CH_2), 29.3 (t), 28.6 (t), 26.7 (t), 26.2 (t), 26.0 (t), 25.7 (q), 22.7 (q), 16.4 (q), 16.0 (q), 14.1 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.27 (s, 2H, ArH), 6.29 (br s, 2H, OH), 4.28 (t, 2H, OCH_2), 4.68 (d, J=7.5 Hz, 2H, OCH_2), 5.26 (t, 1H, CH), 5.08 (t, 2H, CH), 0.88 (t, 3H, CH_3), (br s, 6H, CH_3), 1.77 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 2.07 (m, 8H, CH_2), 1.26 (br m, 32H, CH_2).

Methyl 3,4-bis(3-methylbut-2-enyloxy)-5-hydroxybenzoate **($C_{18}H_{24}O_5$, 8):** obtained in 65% as colorless solid. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.8 (s, CO), 151.3 (s), 149.6 (s), 140.4 (s), 138.5 (s), 138.3 (s), 125.4 (s), 119.4 (d), 119.2 (d), 109.4 (d), 106.8 (d), 69.4 (t, OCH_2), 66.2 (t,OCH_2), 65.7 (t,OCH_2), 52.1 (q, OCH_3), 25.8 (q, CH_3), 25.8 (q, CH_3), 18.2 (q), 17.9 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.28 (s, 1H, ArH), 7.20 (s, 1H, ArH), 3.88 (s, OCH_3), 4.67 (d, J=7.5 Hz, 2H, OCH_2), 4.60 (d, J=5.4 Hz, 2H, OCH_2), 5.86 (s, 1H, OH), 5.50 (br t, 2H, CH), 1.66 (s, 3H, CH_3), 1.75 (br s, 6H, CH_3), 1.80 (s, 3H, CH_3).

Methyl 3,4,5-tris(3-methylbut-2-enyloxy)benzoate **($C_{23}H_{32}O_5$, 9):** obtained in 56% yield as colorless oil. HR-ESI M+1$^+$ 389.2327, calc. 389.2344; ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 167.2 (s, CO), 149.3 (s), 145.8 (s), 143.6 (s), 141.2 (s), 139.3 (s), 137.5 (s), 121.4 (s), 119.2 (d), 118.8 (s), 110.9 (d), 118.8 (d), 110.9 (d), 109.5 (d), 106.2 (d), 69.7 (t, OCH_2), 60.5 (t, OCH_2), 66.1 (t, OCH_2), 52.1 (q, OCH_3), 25.8 (q), 25.7 (q), 25.5 (q), 21.0 (q), 18.1 (q), 17.9 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.34 (s, 1H, ArH), 7.23 (s, 1H, ArH), 4.65 (d, J=7.5 Hz, 2H, OCH_2), 4.59 (d, J=6.6 Hz, 4H, OCH_2), 4.13 (br s, 3H, 3H), 3.88 (s, 3H, OCH_3), 1.64 (s, 6H,CH_3), 1.75 (br s, 6H, CH_3), 2.05 (s,6H, CH_3).

Methyl 3,4-bis((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-5-hydroxybenzoate **($C_{38}H_{56}O_5$, 10):** obtained in 65% yield as colorless oil. HR-ESI M+Na^+ 615.4025, cacl. 615.4025; ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.7 (s, CO), 151.3 (s), 149.6 (s), 143.7 (s), 141.4 (s), 138.3 (s), 135.3 (s), 135.3 (s), 131.1 (s), 131.1 (s), 125.3 (s), 124.2 (d), 124.2 (d), 123.5 (d), 123.5 (d), 119.1 (d), 119.0 (d), 109.4 (d), 106.5 (d), 69.1 (t, OCH_2), 65.6 (t, OCH_2), 39.6 (t), 39.6 (t), 39.5 (t), 39.5 (t), 26.6 (t), 26.6 (t), 26.1 (t), 26.1 (t), 52.0 (q, OCH_3), 25.8 (q, CH_3), 25.6 (q, CH_3), 17.6 (q, CH_3), 17.6 (q, CH_3), 16.6 (q, CH_3), 16.2 (q, CH_3), 15.9 (q, CH_3), 15.9 (q, CH_3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.23 (s, 1H, ArH), 7.16 (s, 1H, ArH), 3.83 (s, 3H, OCH_3), 4.65 (d, J=7.4 Hz, OCH_2), 4.58 (d, J=6.5 Hz, OCH_2), 5.99 (s, 1H, OH), 1.54 (s, 6H, CH_3), 1.62 (s, 6H, CH_3), 1.66 (s, 6H, CH_3), 1.75 (s, 6H, CH_3); 5.48 (br t, 2H, CH), 5.05 (m, 4H, CH), 2.06 (br, m, 8H, CH_2), 1.94 (br t, 8H, CH_2).

Methyl 4-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-3,5-dihydroxybenzoate **($C_{23}H_{32}O_5$, 11):** obtained in 67% yield as colorless oil. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.8 (s, CO), 149.3 (s), 144.8 (s), 137.4 (s), 135.6 (s), 131.3 (s), 126.0 (s), 124.3 (d), 123.4 (d), 118.8 (s), 109.4 (s), 69.6 (t, OCH_2), 61.3 (t, OCH_2), 39.6 (t), 39.6 (t), 26.6 (t), 26.1 (t), 25.6 (q, CH_3), 17.6 (q, CH3), 16.4 (q，CH_3), 15.9 (q, CH_3), 14.2 (q, CH_3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.38 (s, 2H, ArH), 4.68 (d, J=7.5 Hz, 2H, OCH_2), 4.34 (dd, J=7.1 Hz, J=14.2 Hz, 2H, OCH_2), 5.52 (t, 1H, CH), 5.07 ((br t, 2H, CH), 1.37 (t, 3H, CH_3), 1.59 (br s, 6H, CH_3), 1.65 (s, CH_3), 1.71 (s, CH_3), 2.04 (br tm, 8H, CH_2).

3,5-bis(3-methylbut-2-enyloxy)benzaldehyde **($C_{17}H_{22}O_3$, 12):** obtained in yield 42% as colorless oil. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 191.9 (d, CHO), 160.3 (s, CO), 160.3 (s, CO), 138.7 (s), 138.2 (s), 138.2 (s), 118.9 (d), 118.9 (d), 108.3 (d), 107.7 (d), 107.7 (d), 65.1 (t, OCH_2), 65.1 (t, OCH_2), 25.7 (q, CH_3), 25.7 (q, CH_3), 18.1 (q, CH_3), 18.1 (q, CH_3); ^{1}H NMR ($CDCl_3$, 400 M) δ: 9.97 (s, CHO), 7.11 (s, 2H, ArH), 6.84 (s, 1H, ArH), 4.64 (d, J=6.7 Hz, 4H, OCH_2), 5.59 (br t, 2H, CH), 1.93 (br s, 6H, CH_3), 1.86 (br s, 6H, CH_3).

Isopropyl 3,4-bis(3-methylbut-2-enyloxy)-5-hydroxybenzoate ($C_{20}H_{28}O_5$, 13): obtained in 45% as colorless oil. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 165.8 (s, CO), 151.2 (s), 149.5 (s), 140.2 (s), 138.3 (s), 138.1 (s), 126.1 (s), 119.6 (d), 119.4 (d), 109.3 (d), 106.6 (d), 68.3 (d), 69.3 (t, OCH_2), 65.6 (t, OCH_2), 25.7 (q), 25.7 (q), 21.9 (q), 21.9 (q), 18.2 (q), 17.8 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.63 (s, 1H, ArH), 7.55 (s, 1H, ArH), 5.02 (d=7.5 Hz, 2H, OCH_2), 4.96 (d, J=6.7 Hz, 2H, OCH_2), 5.56 (m, 1H, CH), 5.83 (br t, 2H,CH), 6.32 (s, 1H, OH), 1.70 (d, J=6.5 Hz, 6H, CH_3), 2.00 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.15 (s, 3H, CH_3).

Isopropyl 3,4,5-tris(3-methylbut-2-enyloxy)benzoate **($C_{25}H_{36}O_5$, 14):** obtained in 46% yield as colorless oil. HR-ESI M+Na^+ 439.2457, calc. 439.2460; ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 165.9 (s, CO), 152.6 (s), 152.6 (s), 142.0 (s), 138.3 (s), 137.6 (s), 125.4 (s), 123.4 (s), 121.6 (d), 119.9 (d), 108.4 (d), 108.4 (d), 69.2 (t, OCH_2), 68.3 (d, OCH_2), 66.0 (t, OCH_2), 66.0 (t, OCH_2), 25.5 (q), 22.0 (q), 18.2 (q), 17.9 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.26 (s, 2H, ArH), 4.61 (d, J=6.6 Hz, 4H, OCH_2), 4.56 (d, J=7.4 Hz, 2H, OCH_2), 5.23 (m, 1H, CH), 5.54 (br t, 3H, CH), 1.36 (d, J=6.0 Hz, 6H,CH_3), 1.54 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 1.77 (br s, 6H, CH_3).

Isopropyl 4-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-3,5-dihydroxybenzoate **($C_{25}H_{36}O_5$, 15):** obtained in 36% as colorless solid.^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.2 (s, CO), 149.5 (s), 145.3 (s), 137.7 (s), , 136.0 (s), 131.6 (s), 127.2 (s), 124.6 (d), 123.8 (d), 119.2 (d), 109.8 (d), 109.8

(d), 68.9 (d, CH), 70.2 (t, OCH_2), 40.0 (t), 40.0 (t), 27.1 (t), 26.5 (t), 26.0 (q, CH_3), 22.2 (q, CH_3), 22.2 (q, CH_3), 18.0 (q, CH_3), 16.8 (q, CH_3), 16.3 (q, CH_3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.23 (s, 2H, ArH), 4.66 (d, J=7.5 Hz, 2H, OCH_2), 5.21 (m, 1H, CH), 5.54(t, 1H, CH), 5.21 (br t 2H, CH), 5.98 (s, 2H, OH), 1.34 (br s, 6H, CH_3), 1.68 (br s, 6H, CH_3), 1.76 (br s, 6H, CH_3), 1.98 (br t, 4H, CH_2), 2.00 (br m, 4H, CH_2).

3,5-bis((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)benzaldehyde **($C_{37}H_{54}O_3$,16):** obtained in 43% yield as yellow oil. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 192.0 (s, CHO), 160.5 (s), 141.8 (s), 141.8 (s), 138.3 (s), 135.5 (s), 135.5 (s), 135.5 (s), 131.3 (s), 131.3 (s), 124.3 (d), 124.3 (d), 123.6 (d), 123.6 (d), 118.9 (d), 118.9 (d), 108.4 (d), 107.9 (d), 107.9 (d), 65.8 (OCH_2), 65.3 (OCH_2), 65.3 (OCH_2), 39.7 (t), 39.7 (t), 39.6 (t), 39.6 (t), 26.7 (t), 26.7 (t), 26.2 (t), 26.2 (t), 25.7 (q), 25.7 (q), 17.7 (q), 17.7 (q), 16.7 (q), 16.7(q), 16.0 (q), 16.0 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.89 (s, 1H, CHO), 7.02 (s, 2H, ArH), 6.74 (s, 1H, ArH), 4.56 (d, J=6.5 Hz, 4H, OCH_2), 5.49 (t, 2H, CH), 5.10 (t, 4H, CH), 1.59 (br s, 12H, CH_3), 1.75 (br s, 6H, CH_3), 1.96 (br s, 6H, CH_3), 2.15-2.04 (br s, 16H, CH_2).

3-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-5-hydroxybenzaldehyde **($C_{22}H_{30}O_3$, 17):** obtained in 34% yield as colorless solid. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 191.7 (s, CHO), 160.3 (s), 157.4 (s), 141.9 (s), 138.7 (s), 138.7 (s), 135. 9 (s), 131.3 (s), 124.4 (d), 123.7 (d), 119.0 (d), 108.9 (d), 108.3 (d), 65.5 (t, OCH_2), 39.7 (t), 39.6 (t), 26.8 (t), 26.3 (t), 26.8 (t), 25.6 (q, CH_3), 17.6 (q, CH_3), 16.7 (q, CH_3), 16.0 (q, CH_3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.87 (s, CHO), 7.00 (s, 1H, ArH), 6.67 (s, 1H, ArH), 5.47 (t, 1H, CH), 5.10 (br t, 3H, 2H), 4.57 (d, J=6.5 Hz, 2H, OCH_2), 1.59 (br s, 6H, CH_3), 1.74 (s, 3H, CH_3), 1.96 (s, 3H, CH_3), 2.13-2.03 (br t, 8H, CH_2).

Isopentyl 4-(3-methylbut-2-enyloxy)-3,5-dihydroxybenzoate **($C_{17}H_{24}O_5$, 18):** obtained in 45% yield as white solid. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.8 (s, CO), 149.2 (s), 149.2 (s), 141.5 (s), 137.4 (s), 126.1 (s), 119.1 (d), 109.4 (d), 109.4 (d), 25.0 (d), 69.8 (d, OCH_2), 63.9 (t, OCH_2), 37.2 (t), 26.0 (q), 25.8 (q), 22.4 (q), 22.4 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.23 (s, 2H, ArH), 6.24 (br 2H, OH), 5.48 (t, 1H, CH), 4.28 (t, 2H, OCH_2), 0.92 (d, J=6.6 Hz, 6H, CH_3), 1.61 (s, 6H, CH_3), 1.77 (br, 3H, CH_2, CH).

Pentan-2-yl 3,4-bis(3-methylbut-2-enyloxy)-5-hydroxybenzoate **($C_{22}H_{32}O_5$, 19):** obtained in 45% yield as colorless oil. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.9 (s, CO), 151.2 (s), 149.4 (s), 140.3 (s), 138.2 (s), 138.1 (s), 126.1 (s), 119.5 (d), 119.4 (d), 109.2 (d), 106.5 (d), 71.7 (d), 69.3 (t, OCH_2), 65.6 (t, OCH_2), 35.7 (t), 27.5 (t), 25.7 (q), 22.5 (q), 20.0 (q), 18.2 (q), 17.8 (q), 14.0 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.24 (s, 1H, ArH), 7.16 (s, 1H, ArH), 4.62 (d, J=7.5 Hz, 2H, OCH_2), 4.57 (d, J=6.7 Hz, 2H, OCH_2), 5.07 (br t, 1H, CH), 5.44 (br t, 2H, CH), 5.96 (s, 1H, OH), 0.86 (t, 3H, CH_3), 1.82 (s, 6H, CH_3), 1.28 (d, J=6.2 Hz, 3H, CH_3), 1.75 (s, 6H, CH_3), 1.26 (m, 4H, CH_2).

Pentan-2-yl 4-(3-methylbut-2-enyloxy)-3,5-dihydroxybenzoate **($C_{17}H_{24}O_5$, 20):** obtained in 21% yield as a white solid. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.1 (s, CO), 149.1 (s), 141.6 (s), 137.3 (s), 126.8 (s), 123.3 (s), 119.1 (d), 109.4 (d), 109.4 (d), 72.1 (d), 69.9 (t, OCH_2), 35.7 (t), 25.8 (q), 22.7 (t), 20.0 (q), 18.0 (q), 14.2 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.24 (s, 2H, ArH), 4.60 (d, J=7.5 Hz, 2H, OCH_2), 5.49 (t, 1H, CH), 5.06 (m, 1H, CH), 0.87 (t, 3H, CH_3), 1.33 (br t, m, 7H, $2CH_2$, $1CH_3$), 1.66 (s, 3H, CH_3), 1.72 (s, 3H, CH_3), 6.00 (br, 2H, OH).

Pentan-2-yl 3-(3-methylbut-2-enyloxy)-4,5-dihydroxybenzoate **($C_{17}H_{24}O_5$, 21):** obtained in 22% yield as colorless solid. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 166.2 (s, CO), 145.6 (s), 143.3 (s), 139.3 (s), 137.0 (s), 122.3 (s), 118.8 (d), 110.7 (d), 106.0 (d), 71.7 (t), 66.0 (t, OCH_2), 35.7 (t), 27.6 (t), 25.6 (q), 22.5 (q), 20.0 (q), 18.2 (q), 14.0 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.29 (s, 1H, ArH), 7.24 (s, 1H, ArH), 4.58 (d, J=6.8 Hz, 2H, OCH_2), 5.08 (m, 1H, OCH), 5.43 (t, 1H, CH), 0.86 (t, 3H, CH_3), 1.71 (s, 3H, CH_3), 1.76 (s, 3H, CH_3), 1.29, 1.27 (br m, 7H, CH_3, $2CH_2$).

Pentan-2-yl-4-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-Trienyloxy)-3-hydroxy-5-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyl) benzoate **($C_{42}H_{64}O_5$, 22):** obtained in 32% yield as colorless oil. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 165.9 (s, CO), 151.3 (s), 149.5 (s), 143.9 (s), 141.5 (s), 138.3 (s), 135.5 (s), 135.4 (s), 131.3 (s), 126.2 (s), 114.4 (s), 124.3 (d), 123.6 (d), 119.2 (d), 109.7 (d), 106.6 (d), 71.7 (d), 69.3 (t, OCH_2), 65.7 (t, OCH_2), 39.7 (t), 39.6 (t), 39.6 (t), 39.6 (t), 35.7 (t), 27.6 (t), 26.7 (t), 26.2 (t), 25.9 (t), 22.6 (t), 25.7 (q), 25.7 (q), 20.0 (q), 20.0 (q), 17.7 (q), 17.7 (q), 16.7 (q), 16.7 (q), 16.4 (q), 16.4 (q), 16.0 (q), 14.0 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.28 (s, 1H, ArH), 7.20 (s, 1H, ArH), 4.69 (d, J=7.4 Hz, 2H, OCH_2), 4.64 (d=6.5 Hz, 2H, OCH_2), (s, 1H, OH), 5.50 (m, 1H, CH), 5.10 (br t, 6H, CH), 0.90 (t, 3H, CH_3), 1.33 (d, J=9.2 Hz, 3H, CH_3), 1.67 (m, 12H, CH_3), 1.71 (m, 12 H, CH_3).

Methyl 3,4,5-tris(methoxymethoxy)benzoate **($C_{14}H_{20}O_8$, 23):** obtained in 85% yield as colorless oil. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 165.7 (s, CO), 150.2 (s), 150.2 (s), 140.1 (s), 125.3 (s), 110.8 (d), 110.8 (d), 97.9 (t), 94.6 (t), 94.6 (t), 56.5 (q), 55.8 (q), 55.8 (q), 51.6 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.54 (s, 2H, ArH), 5.20 (d, J=5.0 Hz, 6H, OCH_2), 3.60 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.50 (s, 6H, OCH_3).

1-(3-methylbut-2-enyloxy)-4-methylbenzene **($C_{12}H_{16}O$, 24):** side product, obtained in 87% yield as colorless oil. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 156.6 (s, CO), 137.8 (s), 129.8 (d), 129.6 (d), 119.8 (d), 114.3 (d), 114.3 (d), 64.6 (t, OCH_2), 25.7 (q, CH_3), 20.4 (q, CH_3), 18.1 (q, CH_3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.12 (d, 2H, J=8.1 Hz, 2H, ArH), 6.87 (d, J=7.0 Hz, 2H, ArH), 5.57 (t, 1H, CH), 2.35 (s, 3H, CH_3), 1.85 (s, 3H, CH_3), 1.80 (s, 3H, CH_3). The compound is a by-product of the reaction.

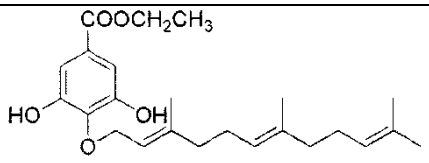
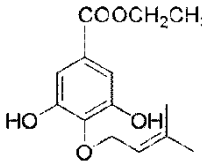
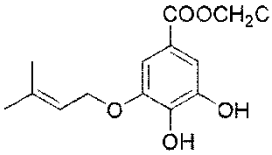
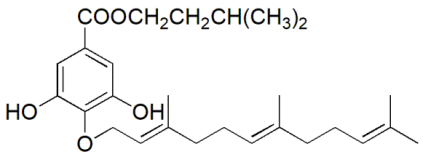
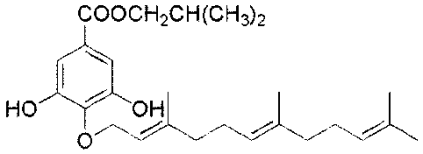
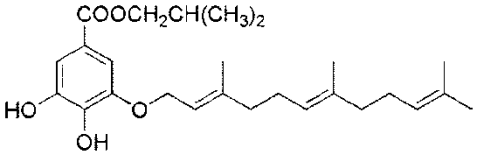
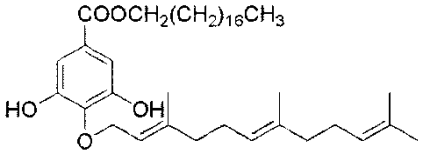
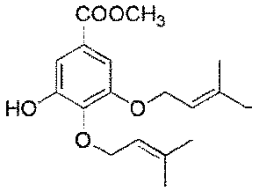
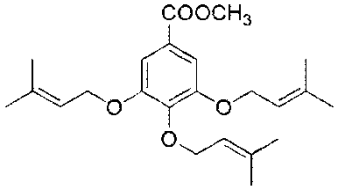
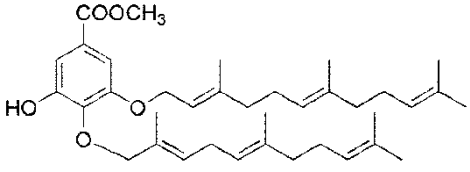
4-(3-Methylbut-2-enyloxy)-3-methoxybenzaldehyde **($C_{13}H_{16}O_3$, 25):** side product, obtained in yield 72% as yellow oil. HRESI-MS *m/s* $M+Na^+$ 243.1000, calc. 243.0997; ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 190.7 (s, CHO), 153.7 (s, CO), 149.6 (s, CO), 138.6 (s), 129.7 (s), 126.6 (d), 118.7 (d), 111.4 (d), 108.7 (d), 66.7 (t, OCH_2), 55.8 (q, OCH_3), 25.7 (q, CH_3), 18.1 (q, CH_3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.76 (s, CHO), 7.35 (d, J=8.2 Hz, 1H, ArH), 7.33 (d, J=6.8 Hz, 1H, ArH), 6.90 (d, J=8.1 Hz, 1H, ArH), 5.44 (t, 1H, CH), 4.59 (d, J=6.6 Hz, 2H, CH_2), 3.84 (s, 3H, CH_3), 1.70 (s, 3H, CH_3), 1.68 (s, 3H, CH_3). The compound is the by-product of the reaction.

4-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)-3-methoxybenzaldehyde **($C_{23}H_{32}O_3$, 26):** side product, obtained in 62% yield as yellow colorless oil. ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 191.0 (d, CHO), 153.8 (s), 149.8 (s), 141.7 (s), 135.5 (s), 131.3 (s), 129.9 (s), 126.8 (d), 124.3 (d), 123.5 (d), 118.7 (d), 111.6 (d), 109.7 (d), 66.0 (t, OCH_2), 56.0 (q, OCH_3), 39.7 (t), 39.5 (t), 26.7 (t), 26.1 (t), 24.8 (q), 17.7 (q), 16.8 (q), 16.0 (q); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9,81 (s, CHO), 7.39 (d, 2H, J= 10.5 Hz, ArH), 6.93 (d, 1H, J=8.1 Hz, ArH), 4.68 (d, 2H, J=8.1 Hz, OCH_2), 3.89 (3H, s, OCH_3), 5.47 (1H, t, CH), 5.05 (2H, t, CH). The compound is the by-product of the reaction.

3,5-dihydroxybenzaldehyde **($C_7H_6O_3$, 27):** obtained in yield 86% as yellow oil. ^{13}C NMR (MeOD, 400 MHz) δ: 194.2 (d, CHO), 160.4 (s), 160.4 (s), 140.0 (s), 109.8 (d), 108.7 (d), 108.7 (d); ^{1}H NMR (MeOD, 400 MHz) δ: 9.75 (s, CHO), 6.78 (br s, 2H, ArH), 6.54 (s, 1H, ArH).

Table 3

Structures of the prepared compounds

No. of the compound	Structure of the compound
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

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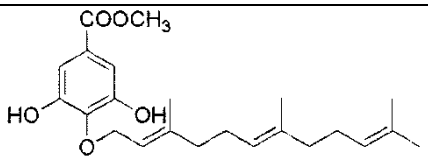
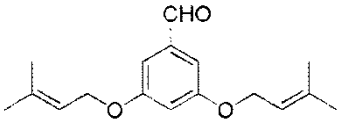
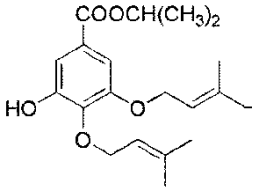
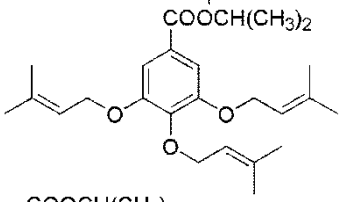
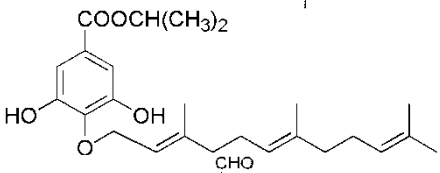
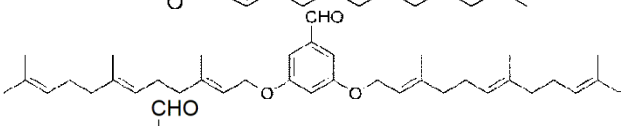
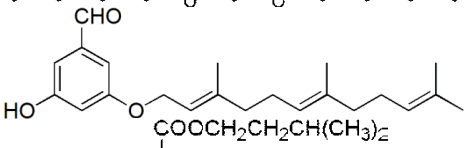
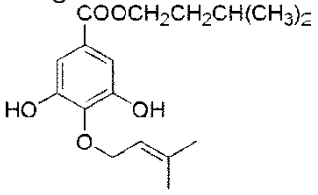
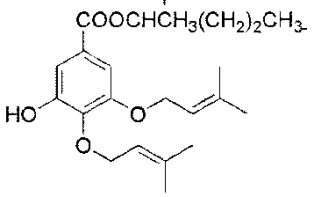
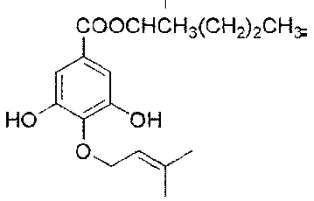
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	

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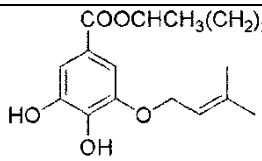
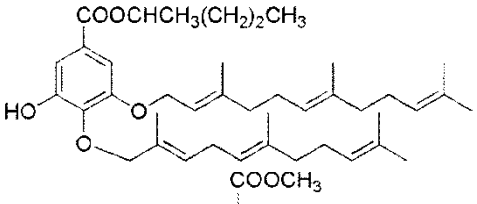
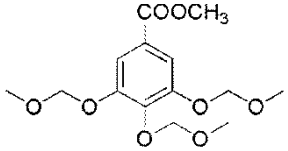
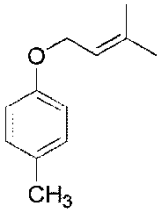
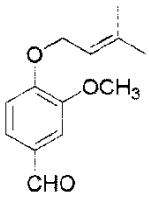
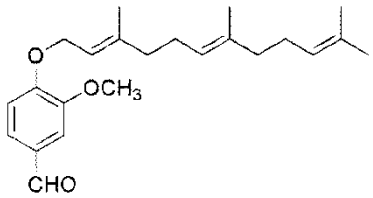
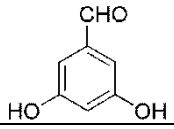
21	
22	
23	
24	
25	
26	
27	

Biological assay

Mycelial growth inhibition test in vitro

The prepared compounds were dissolved in acetone and tested for antifungal activities *in vitro* by a Poison Food Technique.[14] Potato dextrose agar (PDA) was used as the medium for all test fungi. The media incorporating test compounds at concentration of 50 μg/mL was inoculated at the center of the test fungi in agar discs (4 mm diameter). Three replicate plates for each fungi were incubated at 26±2°C. Control plates containing media mixed with acetone (1 ml) were included. After incubation for 2-6 days, the mycelial growth of fungi (mm) in both treated (T) and control (C) Petri dishes were measured diametrically in three different directions until the fungal growth in the control dishes was almost complete. The percentage of growth inhibition (*I*) was calculated using the formula:

$$I\,(\%) = (C - T)/C \times 100 \qquad (1)$$

The corrected inhibition (IC) was then calculated as follows:

$$IC = (I - \mathrm{CF})/(100 - CF) \times 100 \qquad (2)$$

where $CF = (90 - C_0)/C_0 \times 100$, 90 is the diameter (mm) of the Petri dish, and C_0 is the growth (mm) of the fungus in the control.

Analysis of variance was performed on the data with the PROCGLM procedure (SAS Institute, Cary, NC, USA). If the value of P > F was less than 0.01, means were separated with the least significant different (LSD) test at the p = 0.05 level.

In vivo assay

In order to further investigate the *in vivo* antifungal activities of the synthesized compounds, such as the duration of protection and curative activity, the plant disease of wheat powdery mildew (*Erysiphe graminis*) was used in the test. The effects of the test compounds on disease development and spread were determined using potted plants in a greenhouse.

The potted plants were arranged randomly in two groups in a greenhouse and watered twice daily with tap water. The potted plant seedlings were sprayed with the solutions of test compounds in water/acetone (95:5 v:v) that contained Tween 20 (250 μg/mL) as wetter, and allowed to stand for 24h.

For the test of preventive effects, the plants in first group were inoculated with the pathogen of the plant disease, one day after being sprayed with either the test compounds or a standard fungicide at dose 500, 250 g/mL. For the test of curative effects, the plants in second group were firstly inoculated with the plant pathogenic fungi, one day before the application of the test compounds and a standard fungicide at dose 500, 250 μg/mL. Control plants in each group were similarly treated with distilled water/acetone containing Tween 20.

For the development of wheat powdery mildew, the treated wheat seedlings at the first stage were inoculated with *E. graminis* by shaking the infected leaves over them. The inoculated wheat seedlings were incubated for 8 days at 20±1°C and 60% RH (relative humidity) of the day and 18±1°C and 60% RH of the night with 16 h of daylight per day in artificial climate chambers (RP-300, R. P. China), and then the disease severity was determined. The disease severity was recorded on a 0-5 scale, where 0 = no colonies visible to the unaided eye; 1 = few scattered, small discrete colonies; 2 = larger, but still discrete colonies; 3 = colonies merging to form larger mildew lesions; 4 = mildew covering half the total leaf surface and 5 = mildew covering the total leaf surface.[15]

The experiment was conducted three times and the mean value of the three estimates for each treatment was converted into percentage fungal control by the equation:

$$\text{control (\%)} = 100 \times (A\text{-}B)/A \quad (3)$$

where A = disease incidence (%) on leaves or steam sprayed with Tween 20 solution alone and B = disease incidence (%) on treated leaves or sheaths.

The percentage disease incidence was determined using the formula:

$$\text{disease incidence (\%)} = (\Sigma\text{scale} \times \text{number of plant leaves infected})/(\text{highest scale} \times \text{total number of leaves}) \times 100 \quad (4)$$

Analysis of variance was performed on the data with the PROC GLM procedure (SAS Institute, Cary, NC, USA). If the value of P > F less than 0.01, means were separated with the least significant different (LSD) test at the p = 0.05 level.

CONCLUSION

In conclusion, a series of polyphenol ether derivatives were prepared, some of which possess high fungicidal activity. In particular, compound **8**, **13**, **15** and **21** exhibited strong antifungal activities against *R. solani and S. sclerotiorum in vitro*, and compound **13** displayed the best antifungal efficacy toward *E.* graminis *in vivo*. The results suggest that the polyphenol ether derivatives have the potential to be developed as candidates of antifungal products.

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