

Supporting documents

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Table S1. Relative monosaccharide compositions (Mol%) of unfractionated polysaccharides of six red seaweed species quantified by GC-FID

Monosaccharide	<i>Prionitis</i>	<i>Gracilariopsis</i>	<i>Callophyllis</i>	<i>Mastocarpus</i>	<i>Palmaria</i>	<i>Mazzaella</i>
AnGal	12.7±0.2	27.3±0.9	18.1±0.2	34.1±0.6	0.2±0.0	17.7±4.3
Ara	0.5±0.1	1.1±0.1	2.3±0.1	0.9±0.0	2.0±0.4	0.3±0.0
Gal	71.6±3.0	54.1±2.0	54.1±1.8	59.0±0.1	11.6±0.8	72.7±4.8
Glc	10.9±3.7	14.9±3.4	16.3±1.5	4.4±0.5	6.6±0.4	7.3±0.3
Man	0.3±0.1	0.5±0.0	2.9±0.2	0.3±0.0	0.1±0.0	0.3±0.0
Xyl	3.9±0.6	2.1±0.4	6.2±0.2	1.3±0.0	79.5±1.5	1.7±0.2

Note: AnGal, 3,6-anhydrogalactose; Ara, arabinose; Gal, galactose; Glc, glucose; Man, mannose; Xyl, xylose. Two separate experiments were conducted to generate averages and standard deviations.

Table S2. Estimated polysaccharide compositions (Mol%) from linkage compositions of six red seaweed species quantified GC-FID

Polysaccharide	<i>Prionitis</i>	<i>Gracilariopsis</i>	<i>Callophyllis</i>	<i>Mastocarpus</i>	<i>Palmaria</i>	<i>Mazzaella</i>
XN	1.5±0.9	1.2±0.2	4.5±0.2	0.4±0.0	72.8±1.0	0.3±0.0
MN	0.1±0.0	0.4±0.0	1.3±0.0	0.2±0.0	0.0±0.0	0.1±0.0
GN	84.3±3.2	81.4±2.9	72.3±1.6	93.1±0.5	11.7±0.8	90.4±0.5
CE	8.5±3.1	10.2±1.8	12.7±0.9	1.8±0.2	4.0±0.2	3.3±1.1
FS	2.0±0.4	4.4±1.4	3.0±0.5	1.5±0.1	0.9±0.4	3.1±0.3
UA	3.6±0.5	2.4±0.2	6.2±0.0	3.0±0.1	10.7±0.4	2.7±0.3

Note: XN: xylans; MN: mannans; GN: galactans; CE: cellulose; FS: Floridean starch; UA: unassigned linkages. Two separate experiments were conducted to generate averages and standard deviations.

Table S3. Estimation of polysaccharide compositions based on linkage compositions

Polysaccharide	Calculation
XN	Sum of 3-Xylp and 4-Xylp
MN	4-Manp
GN	Sum of all Galp and AnGalp linkages
FS	Calculated based on 4,6-Glcp using an average degree of branching of 4.8
CE	Total 4-Glcp subtracted by 4-Glcp in FS
UA	All remaining linkages

Note: XN: xylan; MN: mannan; GN: galactan; CE: cellulose; FS: Floridean starch; UA: unassigned linkages.

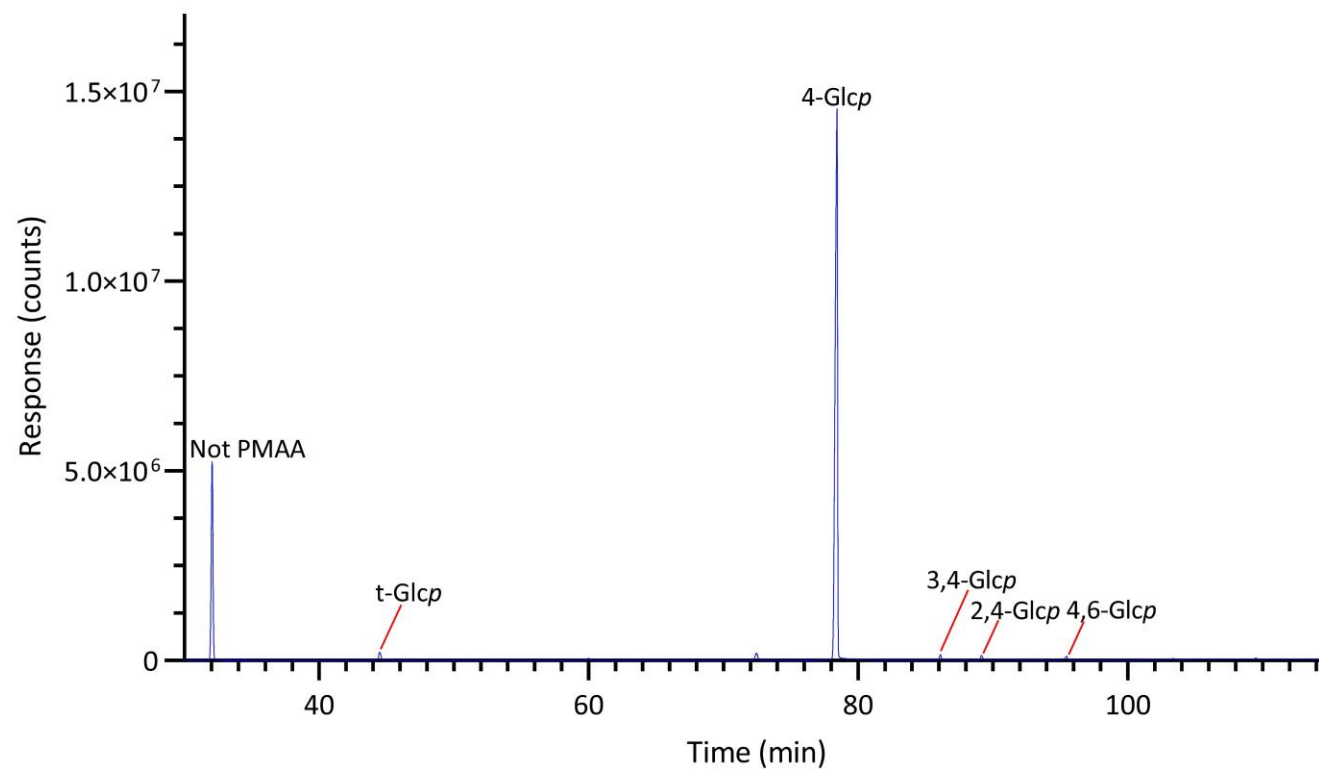


Figure S1. GC-TIC chromatogram of PMAA standards prepared from microcrystalline cellulose.

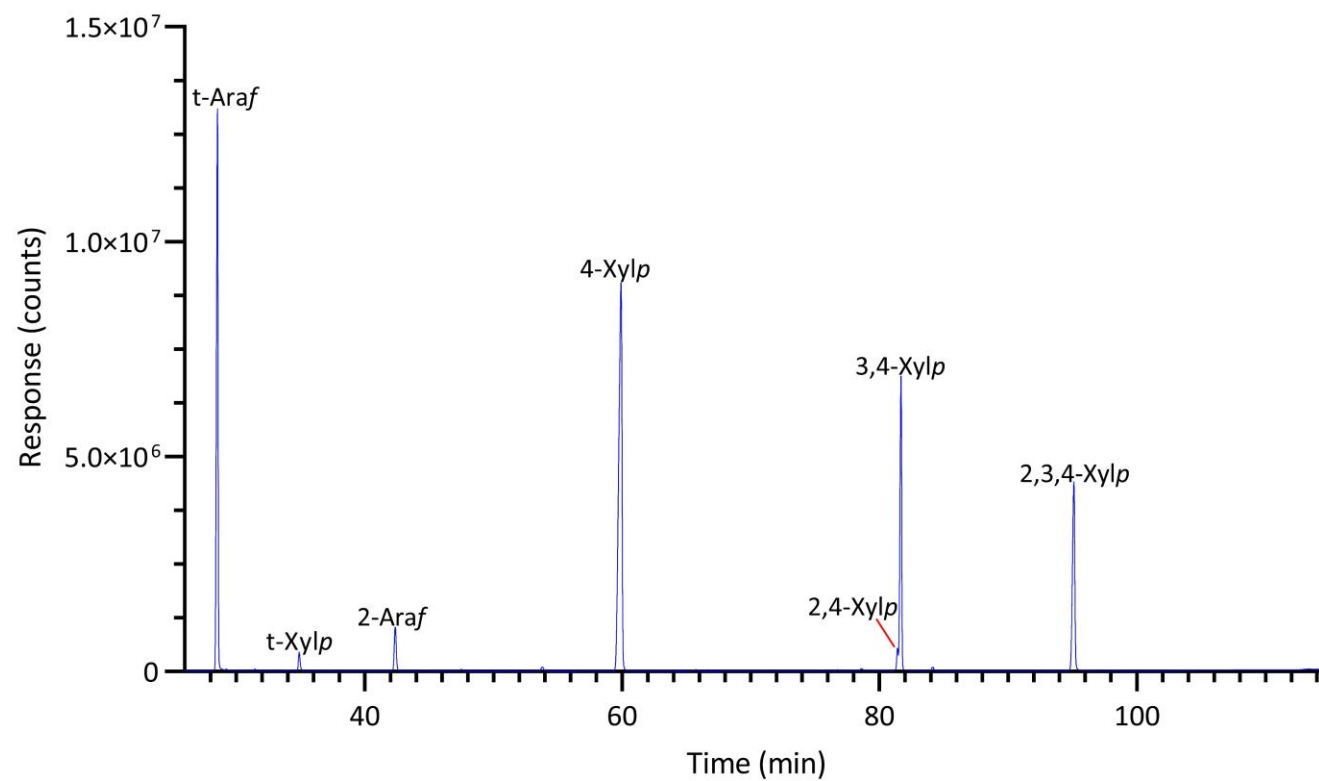


Figure S2. GC-TIC chromatogram of PMAA standards prepared from commercial arabinoxylan.

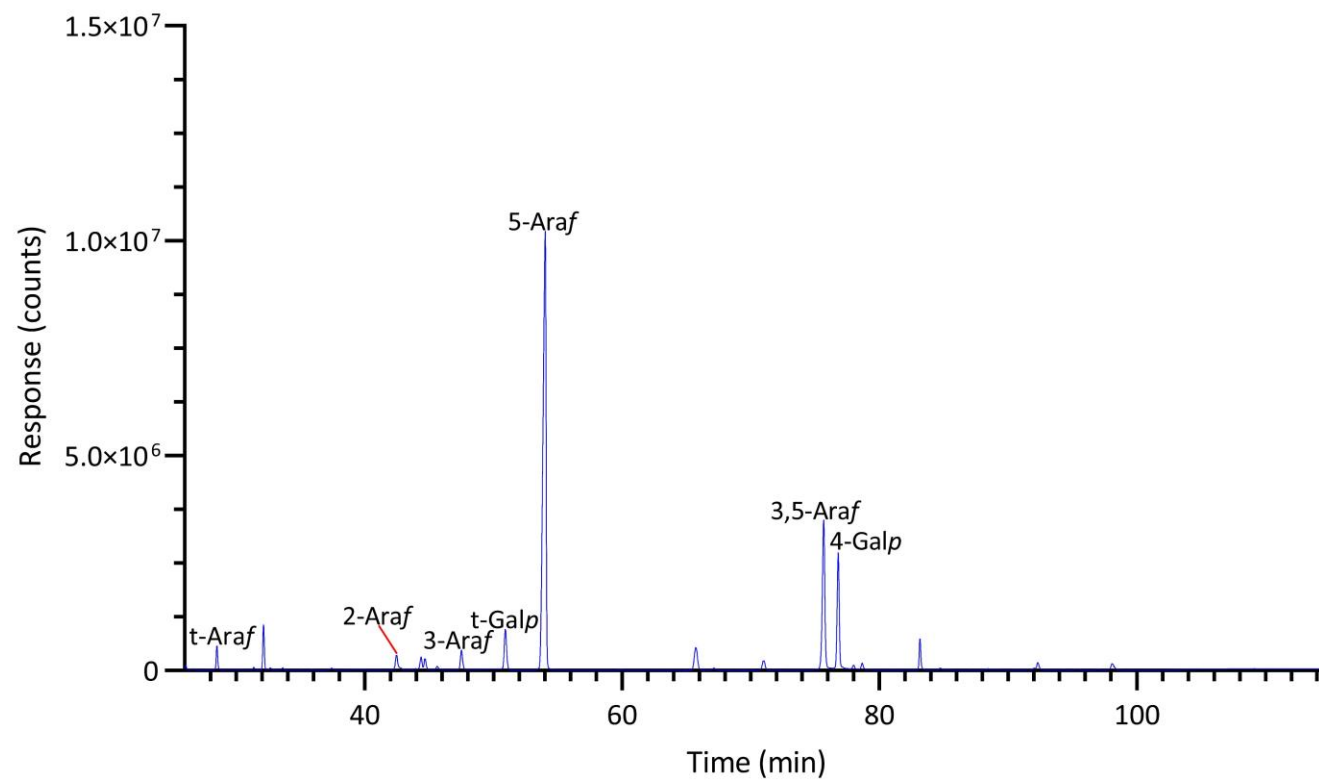


Figure S3. GC-TIC chromatogram of PMAA standards prepared from commercial debranched arabinan.

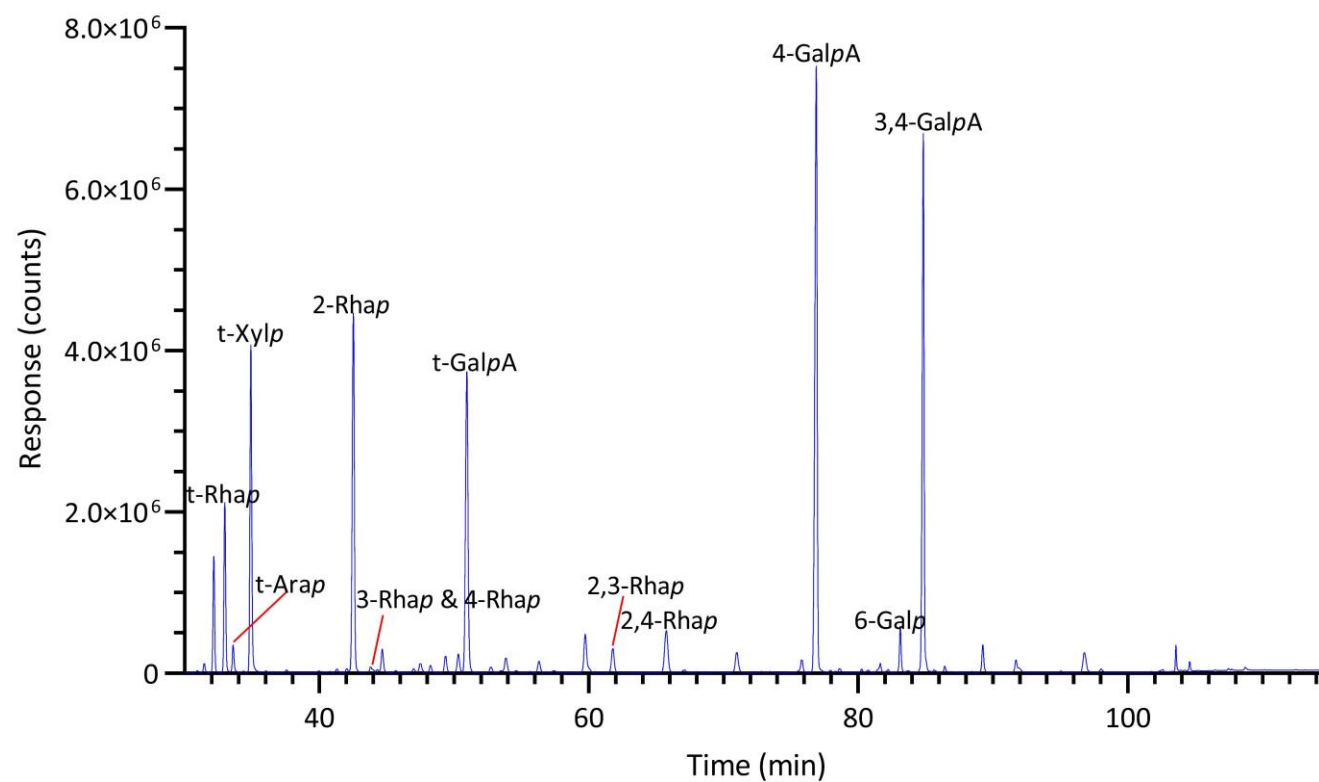


Figure S4. GC-TIC chromatogram of PMAA standards prepared from commercial rhamnogalacturonan I (RG-I)

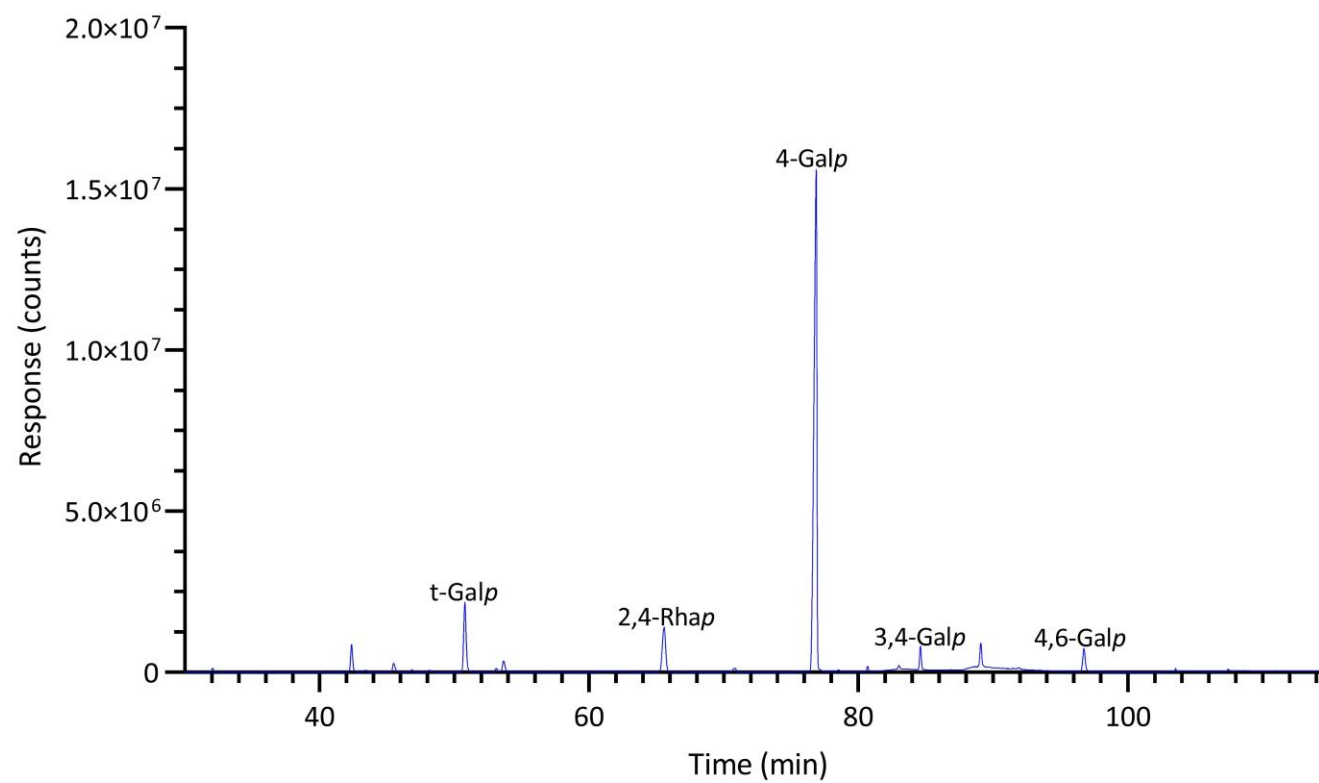


Figure S5. GC-TIC chromatogram of PMAA standards prepared from commercial potato galactan.

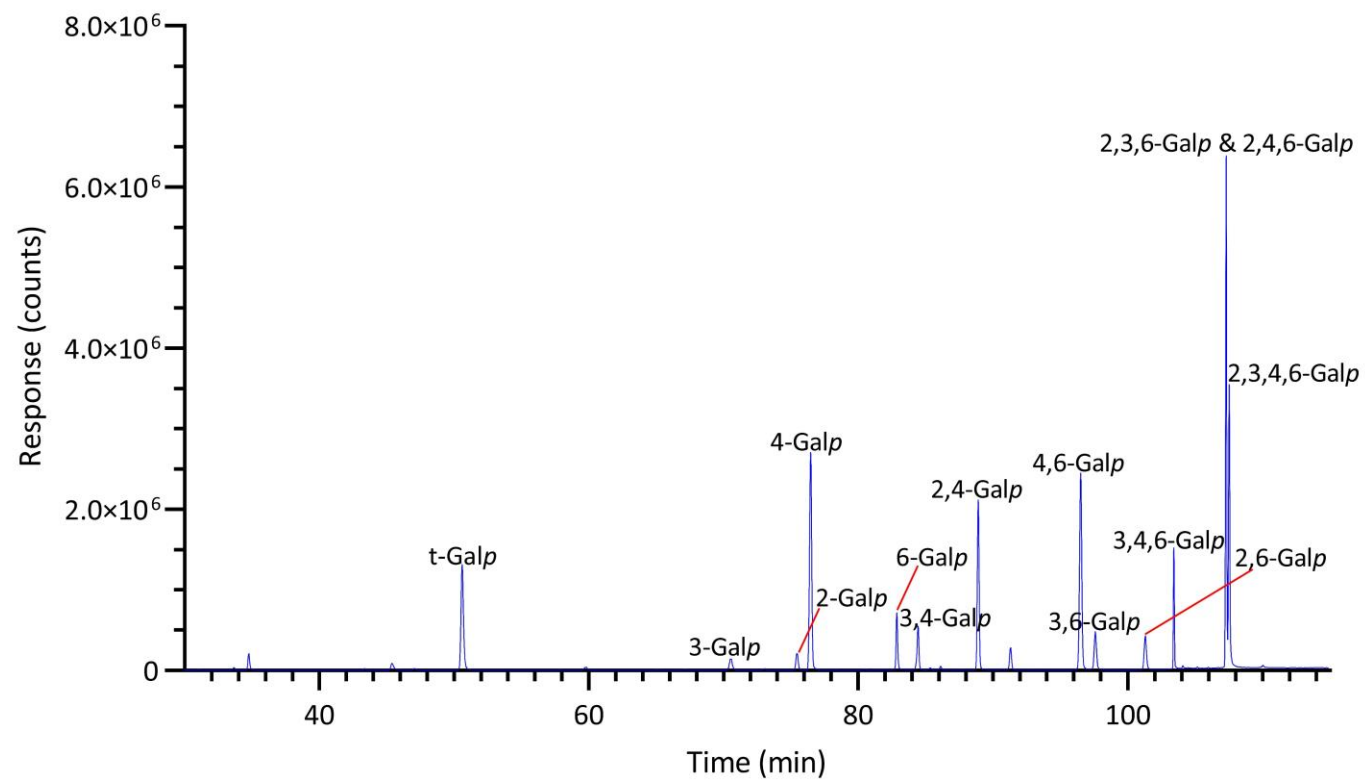


Figure S6. GC-TIC chromatogram of PMAA standards prepared by undermethylation of commercial 1-methyl galactopyranoside.

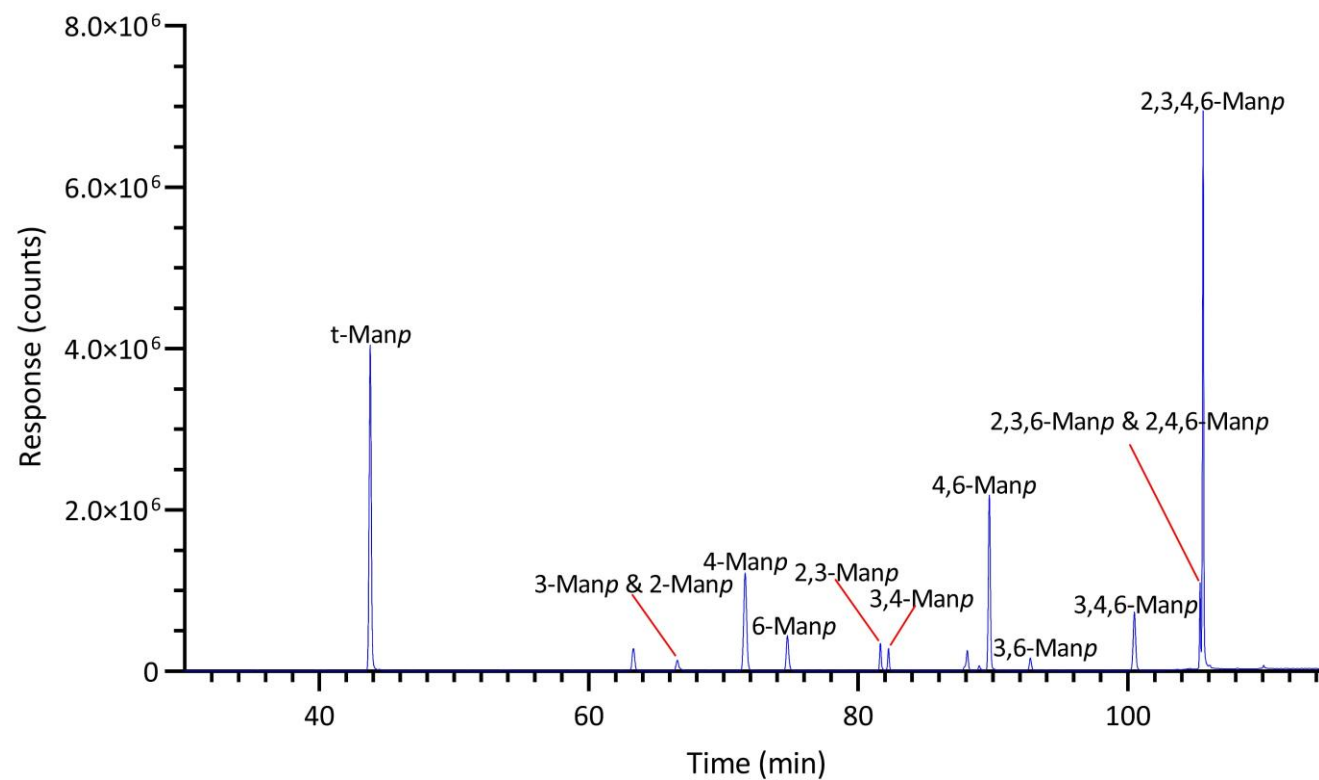


Figure S7. GC-TIC chromatogram of PMAA standards prepared by undermethylation of commercial 1-methyl mannopyranoside.

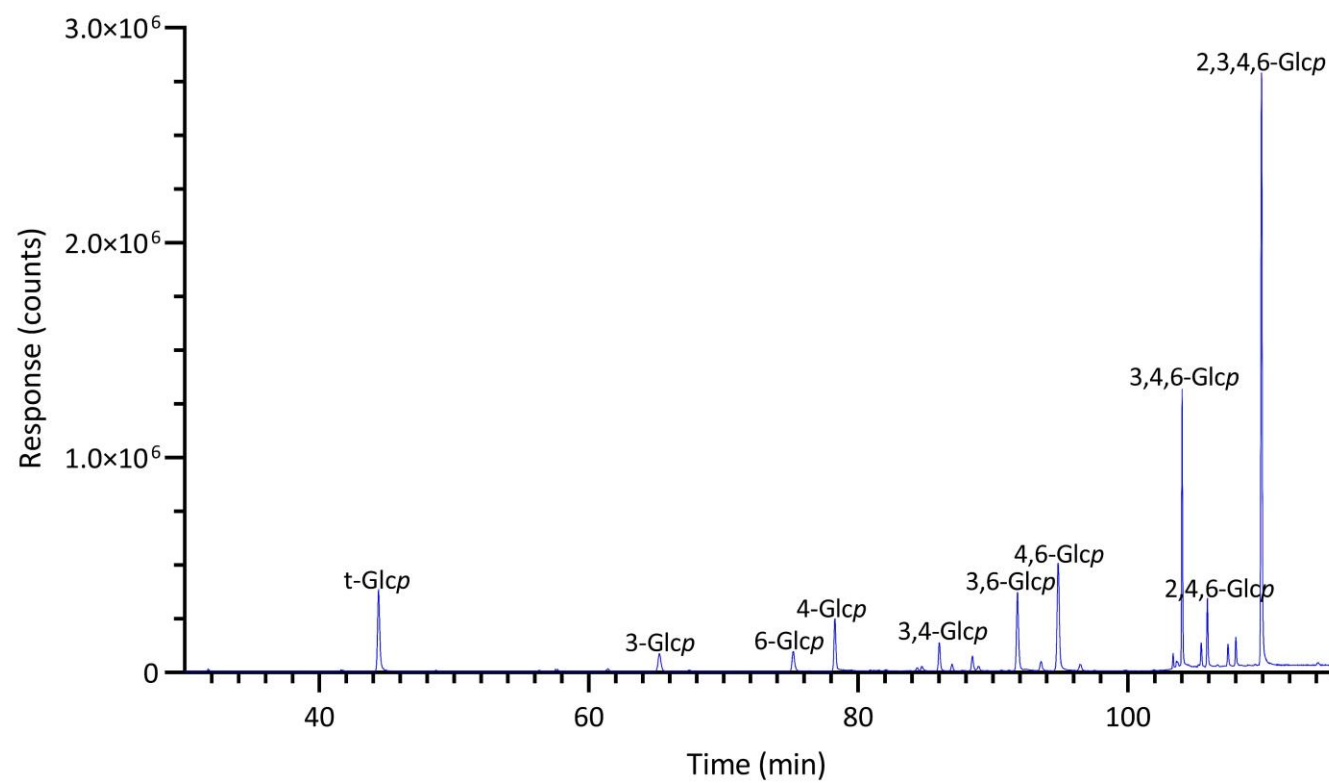


Figure S8. GC-TIC chromatogram of PMAA standards prepared by undermethylation of commercial 1-methyl glucopyranoside.

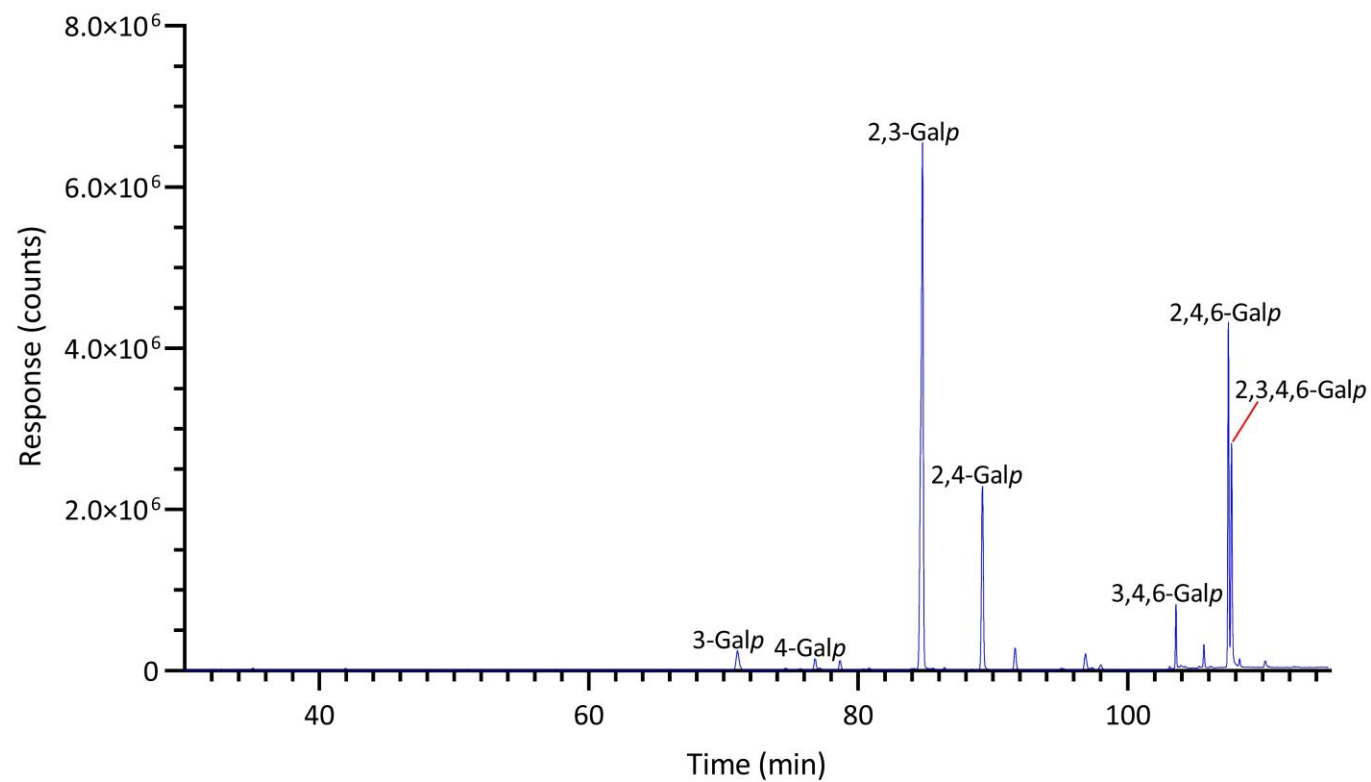


Figure S9. GC-TIC chromatogram of PMAA standards prepared from commercial λ -carrageenan undermethylated by one round of methylation.

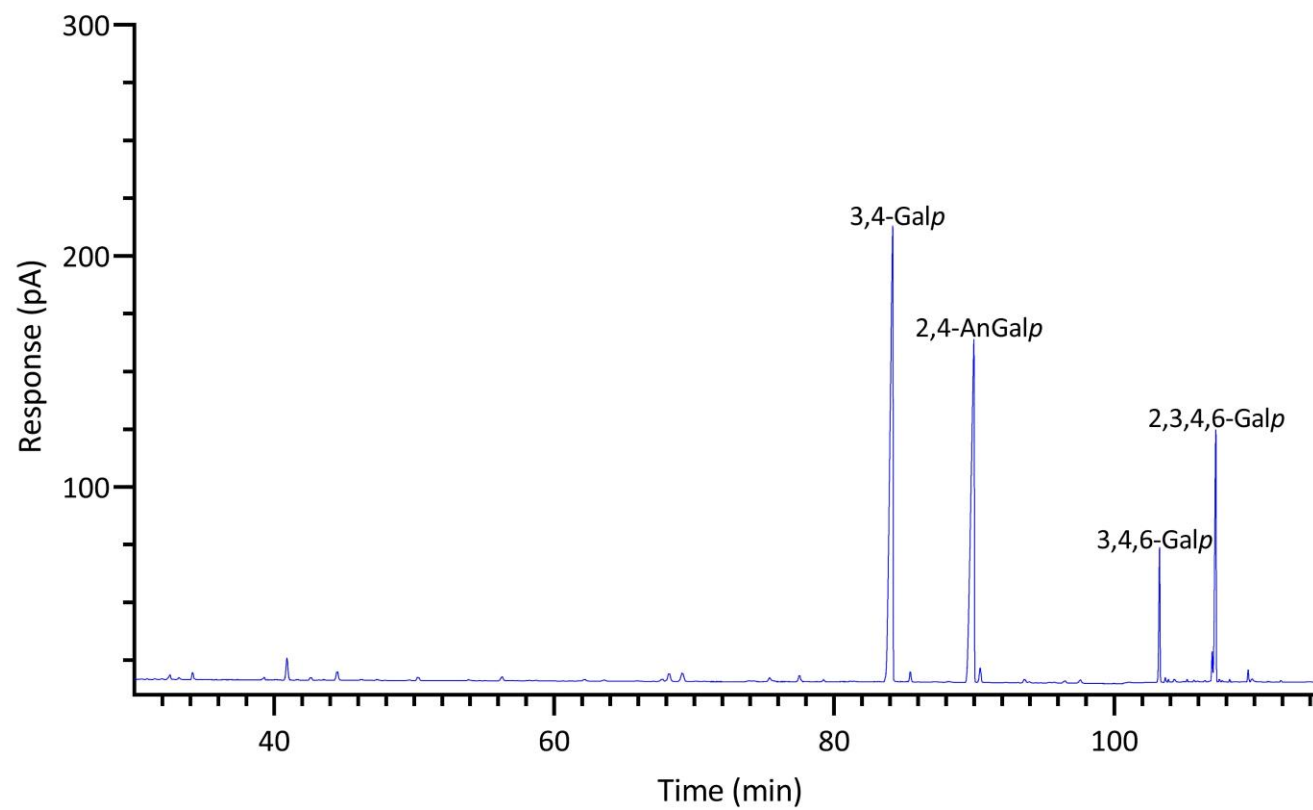


Figure S10. GC-FID chromatogram of PMAA standards prepared from commercial ι-carrageenan undermethylated by one round of methylation.

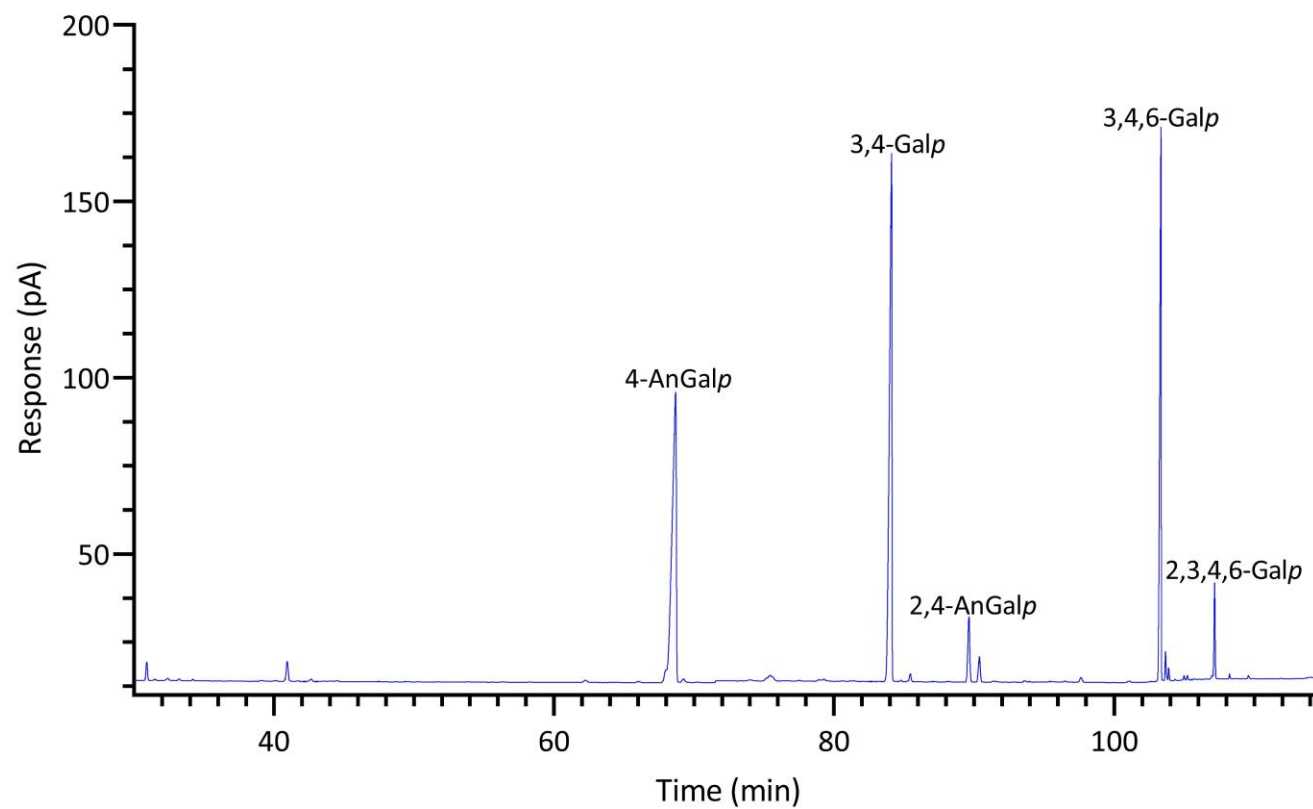


Figure S11. GC-FID chromatogram of PMAA standards prepared from commercial κ -carrageenan undermethylated by one round of methylation.

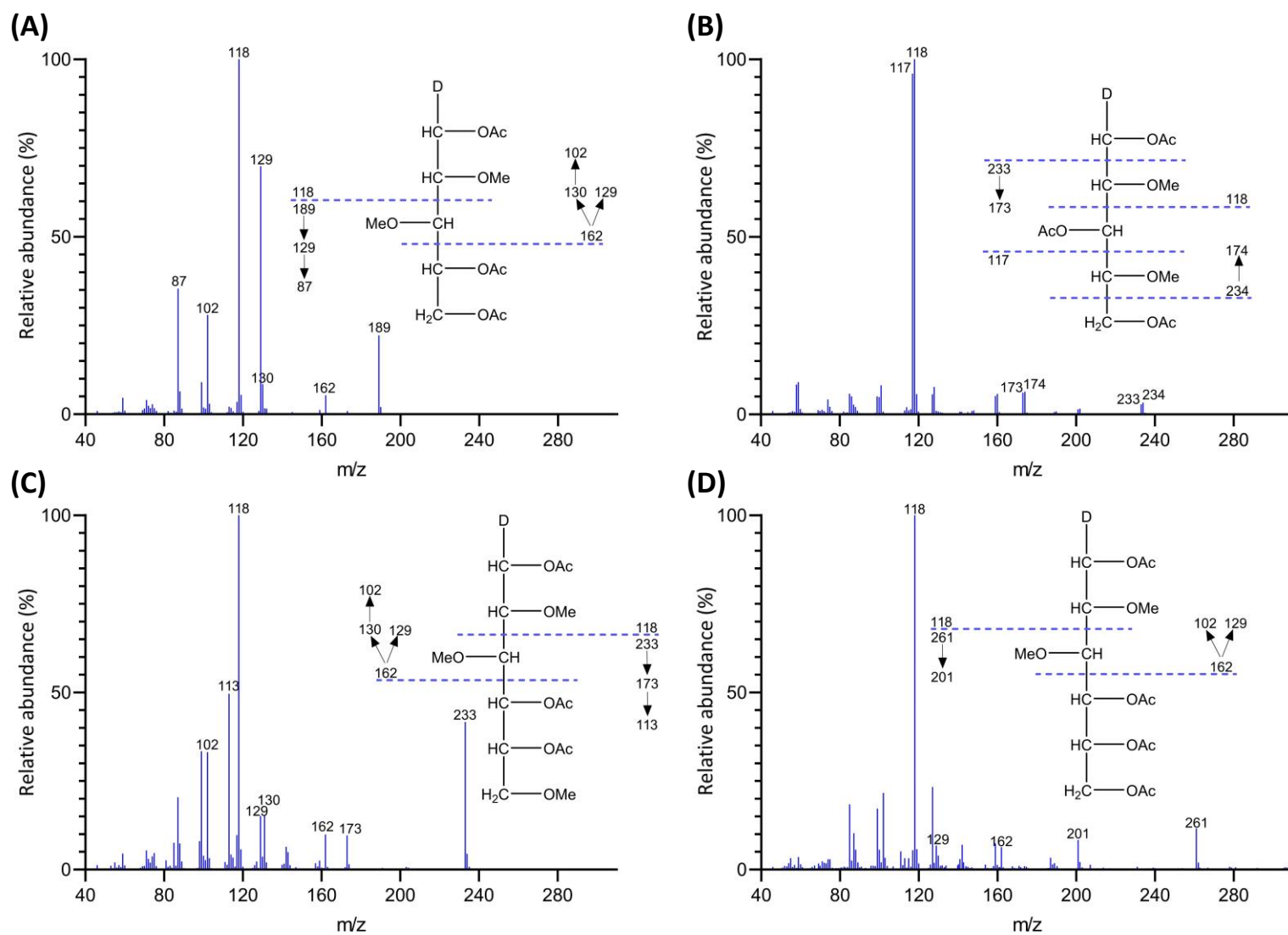


Figure S12. EI-MS spectra and ion fragmentation patterns of PMAAs from (A) 4-Xylp, (B) 3-Xylp, (C) 4-Glcp, and (D) 4,6-Glcp in *Palmaria palmata*.

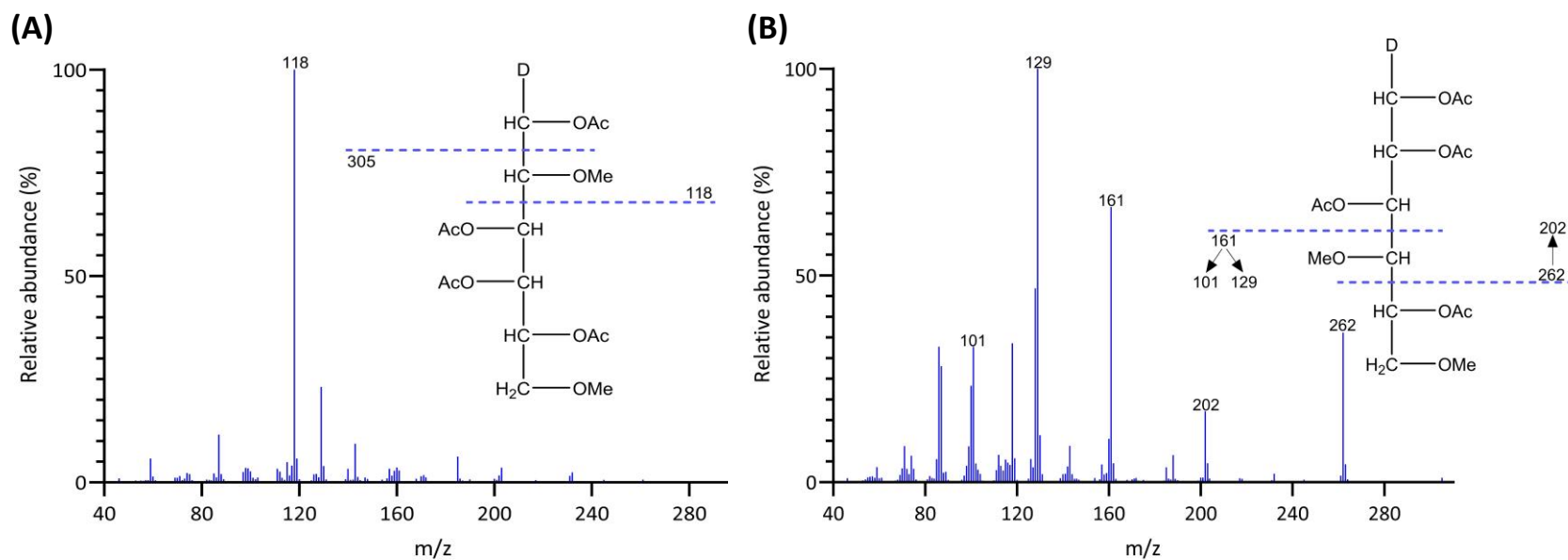


Figure S13. EI-MS spectra and ion fragmentation patterns of PMAAs from **(A)** 3,4-Galp and **(B)** 2,3-Galp.

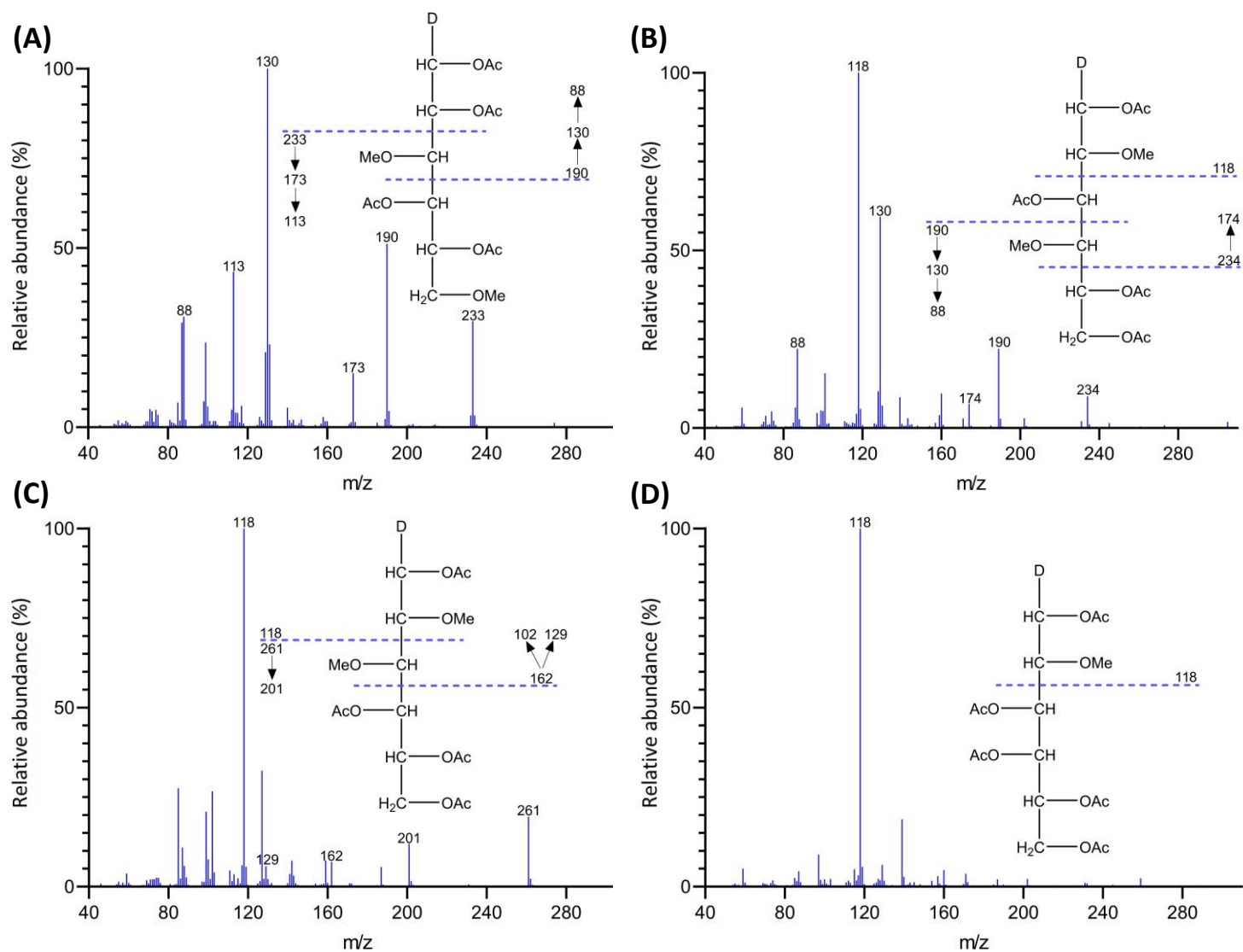


Figure S14. EI-MS spectra and ion fragmentation patterns of PMAAs from **(A)** 2,4-Galp, **(B)** 3,6-Galp, **(C)** 4,6-Galp, and **(D)** 3,4,6-Galp in *Callophyllis* sp.

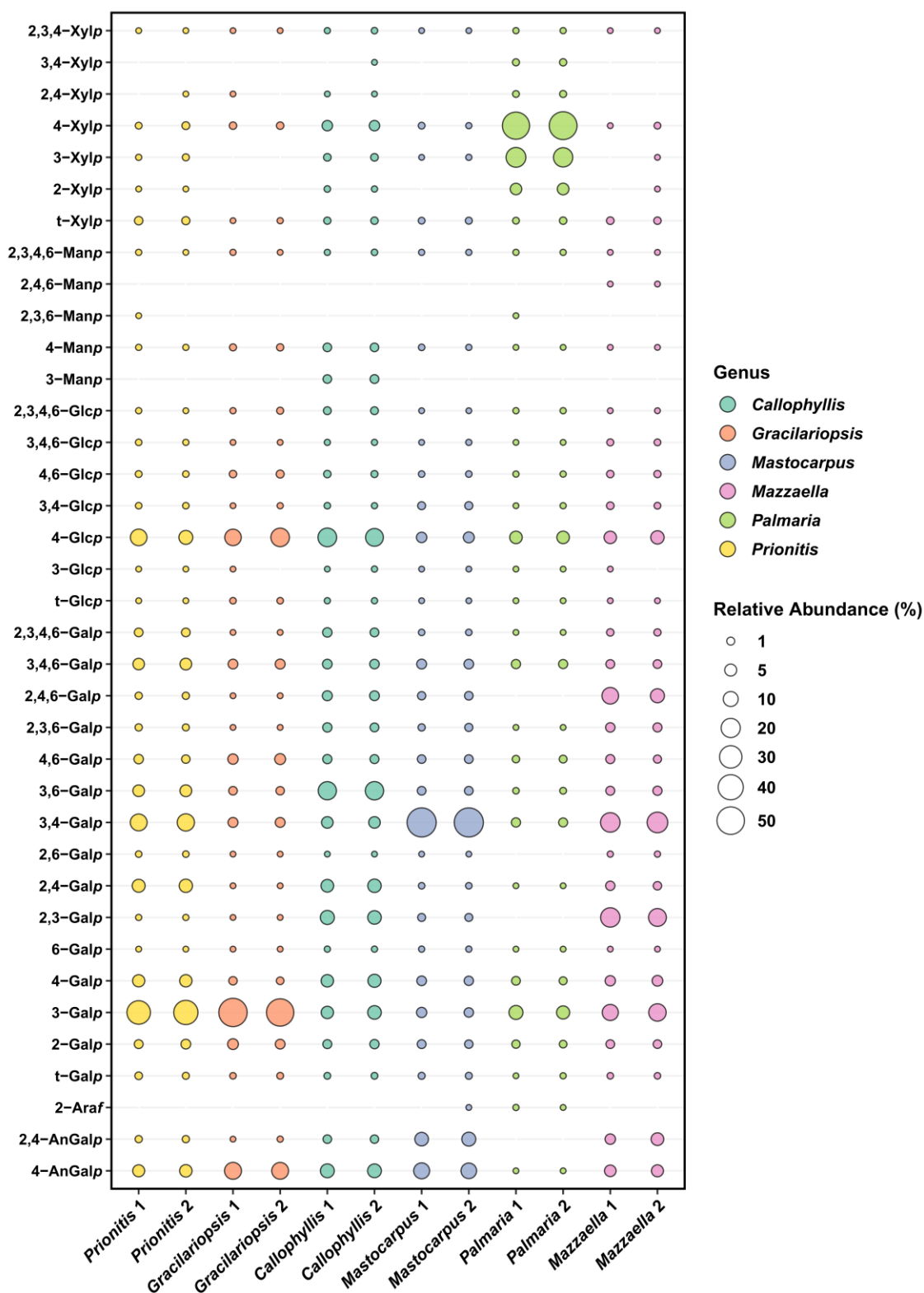


Figure S16. Bubble plot showing the relative linkage composition (Mol%) quantified by GC-MS from each of the two separate experiments conducted on unfractionated polysaccharides from six red seaweed species.

R Script code for bubble plot:

```

# Load required libraries
library(ggplot2) # For creating data visualizations
library(reshape2) # For data reshaping and transformation

# Insert the name of your CSV file here.
# Read data from the CSV file and prevent modification of column names
pc <- read.csv("File Name.csv", header = TRUE, check.names = FALSE)

# Melt the data to long format for ggplot2.
# Replace "Sample" and "Genus" in the entire script with the names of the first 2 columns of your CSV file.
# The 2nd column should be the treatment group, harvest year, disease state, etc.
pcm <- melt(pc, id = c("Sample", "Genus"))
pcm$Sample <- factor(pcm$Sample, levels = unique(pcm$Sample))

# Create the bubble plot
plot <- ggplot(pcm, aes(x = Sample, y = variable)) +
  geom_point(aes(size = value, fill = Genus), alpha = 0.75, shape = 21) +
  scale_size_continuous(limits = c(0.000001, 100), range = c(1, 10), breaks = c(1, 5, 10, 20, 30, 40, 50)) + #limits sets min
and max values for your data set, range sets size of bubbles
  labs(x = "", y = "", size = "Relative Abundance (%)", fill = "Genus") +
  theme(
    legend.key = element_blank(),
    axis.text.x = element_text(colour = "black", size = 10, face = "bold", angle = 45, vjust = 1, hjust = 1), # Adjust angle,
horizontal (hjust), and vertical alignment (vjust) of x-axis labels
    axis.text.y = element_text(colour = "black", face = "bold", size = 9),
    legend.text = element_text(size = 10, face = "bold", colour = "black"),
    legend.title = element_text(size = 11, face = "bold"),
    panel.background = element_blank(),
    panel.border = element_rect(colour = "black", fill = NA, size = 1.2),
    legend.position = "right",
    panel.grid.major.y = element_line(colour = "grey95")
  ) +
  guides(fill = guide_legend(override.aes = list(size = 4)))+ # Set size of upper legend labels
  scale_fill_brewer(palette = "Set2") # Use the Set2 color palette for the bubbles

#Display the plot on RStudio
print(plot)

#Set output file name and save the bubble plot as a PDF or PNG image and change the size of the image
ggsave("File Name.pdf", plot = plot, width = 8, height = 11, units = "in", dpi = 1200)

```

R Script code for PCA score plot:

```

# Load required libraries
library(dplyr) # For data manipulation
library(forcats) # For further data manipulation
library(ggplot2) # For plotting

# Insert the name of your file here
data <- read.csv("File Name.csv")

# Perform principal component analysis (PCA), scale=true will subtract mean and divide by SDev
pca <- prcomp(data[, -1], scale = TRUE)

# Create a data frame with PCA results
pca_data <- as.data.frame(pca$x[, 1:2])
pca_data$Label <- data[, 1]

# Display summary of PCA results
summary(pca)

biplot(pca, scale=0)

# Extract PC scores
str(pca)
pca$x
data2 <- cbind(data, pca$x[, 1:2])
head(data2)

# Extract variance explained by each principal component
variance_explained <- round(pca$sdev^2 / sum(pca$sdev^2) * 100, 2)

# Format variance explained to show 1 significant figure
variance_explained <- sprintf("%.1f", variance_explained)

# Create PCA plot using ggplot2
pca_plot <- ggplot(pca_data, aes(x = PC1, y = PC2, color = Label, fill = Label)) +
  geom_point(size = 5, shape = 16) +
  stat_ellipse(alpha = 0.2) + # Add ellipses
  labs(
    x = paste("PC1, (", variance_explained[1], "%)", sep = ""), # X-axis label
    y = paste("PC2, (", variance_explained[2], "%)", sep = ""), # Y-axis label
    color = expression("Genus"), # Set the legend title for the color aesthetic
    fill = expression("Genus") # Set the legend title for the fill aesthetic
  ) +
  guides(
    color = guide_legend(override.aes = list(shape = 16, size = 4)), #legend label shape and size
    fill = guide_legend(override.aes = list(shape = 16, size = 4)) #legend label shape and size
  ) +
  theme_minimal() +
  theme(
    legend.key.size = unit(0.7, "cm"), # Adjust the size of legend keys
    legend.text = element_text(size = 12, face = "italic"), # Adjust the size of legend text, and make it italic
    legend.title = element_text(size = 14), # Adjust the size of legend title
    plot.background = element_rect(fill = "white"), # Set plot background color

```

```

plot.title = element_text(size = 16, hjust = 0.5), # Adjust plot title size and center it
axis.text = element_text(size = 12), # Adjust the font size for axis tick labels
axis.title = element_text(size = 14), # Adjust the font size for axis labels
panel.grid.major = element_line(color = "gray", size = 0.5), # Add major gridlines
panel.grid.minor = element_blank(), # Remove minor gridlines
panel.border = element_rect(color = "black", fill = NA, size = 1) # Add black border
) +
ggtitle("") # Add a title to the plot

# Update the color palette from ColorBrewer
pca_plot <- pca_plot +
  scale_color_brewer(palette = "Set2") # Change the color palette to any set in ColorBrewer

pca_plot <- pca_plot +
  scale_fill_brewer(palette = "Set2") # Change the color palette for ellipses and legend

# Display the plot
print(pca_plot)

# Set output file name and save PCA plot as PDF or PNG image and change the size of the image
ggsave("File Name.png", plot = pca_plot, width = 10, height = 8, units = "in", dpi = 900)

```