Comparative analysis of flavonoids, polyphenols and volatiles in roots, stems and leaves of five mangroves

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Mangrove plants contain a variety of secondary metabolites, including flavonoids, polyphenols, and volatiles, which are important for their survival and adaptation to the coastal environment, as well as for producing bioactive compounds. To reveal differences in these compounds among five mangrove species' leaf, root, and stem, the total contents of flavonoids and polyphenols, types and contents of volatiles were determined, analyzed and compared. The results showed that *Avicennia marina* leaves contained the highest levels of flavonoids and phenolics. In mangrove parts, flavonoids are usually higher than phenolic compounds. 532 compounds were detected by a gas chromatography-mass spectrometry (GC-MS) method in the leaf, root, and stem parts of five mangrove species. These were grouped into 18 classes, including alcohols, aldehydes, alkaloids, alkanes, etc. The number of volatile compounds in *A. ilicifolius* (176) and *B. gymnorrhiza* (172) was lower than in the other three species. The number of volatile compounds and their relative contents differed among all three parts of five mangrove species, where the mangrove species factor had a greater impact than the part factor. 71 common compounds occurring in more than two species or parts were analyzed by a PLS-DA model. One-way ANOVA revealed 18 differential compounds among mangrove species and nine differential compounds among parts. Principal component analysis and hierarchical clustering analysis showed that both unique and common compounds significantly differed in composition and concentration between species and parts. In general, *A. ilicifolius* and *B. gymnorrhiza* differed significantly from the other species in terms of compound content, while the leaves differed significantly from the other parts. VIP screening and pathway enrichment analysis were performed on 17 common compounds closely related to mangrove species or parts. These compounds were mainly involved in terpenoid pathways such as C10 isoprenoids and C15 isoprenoids and fatty alcohols. The correlation analysis showed that the content of flavonoids/phenolics, the number of compounds, and the content of some common compounds in mangroves were correlated with their salt and waterlogging

tolerance levels. These findings will help in the development of genetic varieties and medicinal utilization of mangrove plants.

Comparative Analysis of Flavonoids, Polyphenols and Volatiles in Roots, Stems and Leaves of Five Mangroves

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Abstract

Mangrove plants contain a variety of secondary metabolites, including flavonoids, polyphenols, and volatiles, which are important for their survival and adaptation to the coastal environment, as well as for producing bioactive compounds. To reveal differences in these compounds among five mangrove species' leaf, root, and stem, the total contents of flavonoids and polyphenols, types and contents of volatiles were determined, analyzed and compared. The results showed that *Avicennia marina* leaves contained the highest levels of flavonoids and phenolics. In mangrove parts, flavonoids are usually higher than phenolic compounds. 532 compounds were detected by a gas chromatography-mass spectrometry (GC-MS) method in the leaf, root, and stem parts of five mangrove species. These were grouped into 18 classes, including alcohols, aldehydes, alkaloids, alkanes, etc. The number of volatile compounds in *A. ilicifolius* (176) and *B. gymnorrhiza* (172) was lower than in the other three species. The number of volatile compounds and their relative contents differed among all three parts of five mangrove species, where the mangrove species factor had a greater impact than the part factor. 71 common compounds occurring in more than two species or parts were analyzed by a PLS-DA model. One-way ANOVA revealed 18 differential compounds among mangrove species and nine differential compounds among parts. Principal component analysis and hierarchical clustering analysis showed that both unique and common compounds significantly differed in composition and concentration between species and parts. In general, *A. ilicifolius* and *B. gymnorrhiza* differed significantly from the other species in terms of compound content, while the leaves differed significantly from the other parts. VIP screening and pathway enrichment analysis were performed on 17 common compounds closely related to mangrove species or parts. These

32 compounds were mainly involved in terpenoid pathways such as C10 isoprenoids and C15
33 isoprenoids and fatty alcohols. The correlation analysis showed that the content of
34 flavonoids/phenolics, the number of compounds, and the content of some common compounds
35 in mangroves were correlated with their salt and waterlogging tolerance levels. These findings
36 will help in the development of genetic varieties and medicinal utilization of mangrove plants.

37 **Subjects** Bioinformatics, Marine Biology, Plant Science

38 **Keywords** Mangrove plant, Volatiles; Metabolomic analysis, Terpene metabolism, Tolerance

39

Introduction

41 The growth and development of plants are often affected by incompatible environments such as
42 drought, salt, cold, frost, and elevated temperatures that result in low yields and, in worse cases,
43 the death of the plants (*Chen et al., 2022*). Abiotic stresses such as flooding, heat, drought, cold,
44 etc., and biotic stresses such as pathogenic attacks lead to the formation of some secondary
45 metabolites, which play important roles in plant survival and create ecological connections
46 between other species (*Jan et al., 2021*). Many secondary metabolites produced by medicinal
47 plants have anti-microbial properties, high antioxidant levels, cytotoxic properties, as well as
48 other properties that are medically quite significant (*Punetha et al., 2022*).

49 Mangrove is a unique forest ecosystem distributed in tropical and subtropical coastal areas
50 (*Dahibhate et al., 2019*). To adapt to harsh natural environments such as high salinity, high
51 temperature, and low oxygen, mangrove plants have evolved into highly developed
52 morphological and physiological adaptability (*Dahibhate et al., 2019*). The secondary metabolites
53 of mangroves are unique and novel with diverse bioactive functions (*Das et al., 2016*), which
54 enable them to endure biotic and abiotic stresses and adapt to harsh environmental conditions
55 (*Dahibhate et al., 2019*; *Sivaramakrishnan et al., 2019*). In addition, mangrove plants are rich in
56 flavonoids, steroids, terpenes, alkaloids, and other chemical compounds (*Dahibhate et al., 2019*;
57 *Karim et al., 2021*). These bioactive and natural compounds may be used as precursors for
58 pharmaceuticals and industrial raw materials (*Dahibhate et al., 2019*). As one of the important
59 medicinal plants, mangrove plants are widely used as traditional (ethnic) in the world (*Karim et
60 al., 2021*). Mangrove plants have been reported to contain active chemical ingredients useful for
61 medicinal purposes in the past few decades. Structural types and the biological activities of
62 natural products found in true mangroves and semi-mangroves worldwide have been
63 summarized (*Wu et al., 2008*; *Li et al., 2009*). The current reports on the activity detection of
64 compounds from mangrove plants indicate that mangrove plants are a valuable source of
65 pharmacologically active substances with broad prospects (*Okla et al., 2019*).

66 However, plants have developed several mechanisms to counteract the effects of abiotic stress
67 at the morphological, anatomical, biochemical, and molecular levels, including changes in
68 secondary metabolite production due to exposure to environmental stress (*Punetha et al., 2022*).
69 Metabolomics is considered a fundamental branch of systems biology (*Rosato et al., 2018*). It
70 provides a powerful tool for understanding abiotic stresses in plants and developing resistance
71 strategies at the metabolite level (*Carrera et al., 2021*). Gas chromatography-mass spectrometry
72 (GC-MS) is one of the most effective, reproducible, and widely used analytical platforms because
73 of its robustness, repeatability, and selectivity of the technology and a large number of mature
74 commercial and metabolite databases. GC-MS has become an important method of choice for
75 metabolomic analysis and answering various biological questions in metabolomics (*Feizi et al.,*
76 *2021*). Recently, studies on mangrove ecosystems have focused on microorganisms associated
77 with mangroves rather than on flora (*Wu et al., 2022*). Although GC-MS has also been applied to
78 the detection of different bioactive compounds in mangrove plant extracts (*Bidve et al., 2018*;
79 *Joel & Bhimba, 2010*; *Lalitha et al., 2021*; *Dahibhate & Kumar, 2022*;_*Swaraiah et al., 2020*;
80 *Huang et al., 2019*), fewer studies have applied metabolomics to analyze and compare metabolic
81 differences among root, stem, and leaf parts of different mangrove species. Under stress
82 conditions, different metabolites are allocated or synthesized in different parts of plants; however,
83 little is known about how genotypic differences affect these processes (*Kang et al., 2019*). In this
84 study, the GC-MS techniques of volatile compounds were established for five mangrove species.
85 The flavonoids, polyphenols, and volatiles of their leaves, stems, and roots were analyzed and
86 compared, aiming to understand the structures, biosynthesis, and resources of these
87 metabolites in five mangrove species with different adaptations.

Materials & Methods

Plant Material

90 The seedlings of five mangroves, *Acanthus ilicifolius* L. (*genus* Acanthus)*, Bruguiera*
91 *gymnorrhiza* L. (*genus Bruguiera*)*, Aegiceras corniculatum* L. (*genus* Myrsinaceae)*, Kandelia*
92 *candel* L. (*genus* Kandelia)*,* and *Avicennia marina* F. (*genus* Avicennia) were collected from
93 Tongming river, which is located in Zhanjiang Mangrove National Nature Reserve (ZMNNR),
94 Guangdong (E110.1667°, N 20.9765°). These species are the most common native mangrove
95 species in the ZMNNR area, and have been identified and recorded in the national mangrove
96 resource survey in 2001 (*Gao et al., 2009*). Field experiments were approved by Guangdong
97 provincial science and technology department and Guangdong provincial forestry bureau
98 (project number: 20208020214001). The seedlings of the five mangrove species were 1.5 years
99 old and 25-50 cm high (Figure 1), and were identified by ZMNNR Professor Yuechao Chen.

100   Three plants of each mangrove species were used for biological duplication, and their leaves,
101   roots, and stems were taken. The combinations of species and organ parts from the leaves,
102   roots, and stems were coded and named species_part. Leaves, roots, and stems of *A. ilicifolius*
103   (AI), *B. gymnorrhiza* (BG), *A. corniculatum* (AC), *K. candel* (KC), and *A. marina* (AM) were
104   simplified as AI_leaf, AI_root, AI_stem, BG_leaf, BG_root, BG_stem, AC_leaf, AC_root,
105   AC_stem; KC_leaf, KC_root, KC_stem, AM_leaf, AM_root, AM_stem, respectively.

106   **Determination of flavonoids and polyphenols**

107   The total flavonoid content was determined by the protocol described previously (Kim et al.,
108   2003). The absorbance of flavonoids was determined at 506 nm using a visible
109   spectrophotometer (PE Lambda-6, USA). Based on the linear equations [y = 1.2051x-0.0066 (R^2
110   = 0.9988, SEE (The sum of squares due to error) = 0.0119)] from catechin (purity >97.50%,
111   Macklin Inc, Shanghai) standard solutions of different concentrations, flavonoid concentrations
112   were calculated. Spectrophotometric analysis was performed to determine the content of total
113   phenolics/polyphenols using Folin-Ciocalteu's phenol reagent (*Kim et al., 2003*). The absorbance
114   of polyphenols was also determined using a PE Lambda-6 spectrophotometer at 765 nm, and
115   the polyphenol content was calibrated using a linear equation [y = 7,1266x + 0.0095 (R^2 = 0.9993,
116   SEE = 9,070)] from gallic acid (purity >99.00%, Macklin Inc, Shanghai) reference solution. As a
117   percentage of the sample's dry mass, the final content of flavonoids or phenols was obtained.

118   **Sample preparation**

119   In April 2020, healthy leaves, stems and roots from the whole plant of each mangrove species
120   were picked, washed and dried in the shade and crushed. 5 g of leaves, roots, and stems,
121   respectively, were weighed accurately and put into a triangular flask. 100 mL of 70% ethanol was
122   added and extracted at 25 ℃ for 48 h (shaken for 5 min every 12 h), and then centrifuged for 10
123   min at 4000 R/min. The supernatant was filtered with quantitative filter paper and concentrated to
124   dryness under reduced pressure, then 1 mL of ethyl was added to dissolve and prepared to test.

125   **GC-MS analysis**

126   1.0 µL of the sample solution was used in a Shimadzu GC-2010 gas chromatograph (Shimadzu
127   Scientific Instrument, Inc.).

128   The GC-MS system was equipped with an HP-5ms (30 m × 250 µm × 0.25 µm) chromatographic
129   column, made of (5%-phenyl) -methylpolysiloxane.

130   In the heating procedure, the initial temperature was set at 40 ℃ for 2 minutes, increased at 5
131   ℃/minute to 230 ℃ and held at 230 ℃ for 2 minutes, then increased to 250 ℃ at 20 ℃/minute
132   and held at 250 ℃ for another 2 minutes. Samples were injected in splitless mode; Injection time

133 was 1.00 min. The carrier gas was helium with a flow rate of 1 mL/min. The temperature of the

134 GC injector was 250 ℃.

135 The GC MS-QP2010 SE mass spectrometer was operated in EI mode at 70 eV of electron

136 energy; Ion source temperature was equal to 230 ℃, and the interface temperature was equal to

137 250 ℃. The solvent delay time was 1.00 min; The scan interval was 0.30 sec with a 2000

138 amu/sec scan speed, and the scan Mas range was 50~550 *m/z*. The maximum length of

139 retention time was 43.00 min. All measurements were repeated three times.

140 **Calibration curve of standard solutions for GC-MS**

141 Three methyl esterified standards (Sigma-Aldrich, USA) were used for the qualitative analysis by

142 external standard method. Separately, a certain amount of methanolic linoleic acid, methanolic

143 octadecanoic acid and methanolic linolenic acid standards were weighed and prepared as 0.1,

144 0.2, 0.3, 0.4 and 0.5 mg/ml of n-hexane, concentration gradient solution each. Six standards,

145 including 3-octanol, limonene, 3-cyclohexen-1-ol, hexadecane, cedrol and heptadecane, were

146 supplied by Sigma-Aldrich and were selected for validation of study findings. Six standards were

147 prepared as 0.04, 0.06, 0.08%(v/v) n-hexane concentration gradient solution (where cedrol

148 concentration is 0.04, 0.06, 0.08mg/mL) with reference to the above method. Each solution was

149 used for GC-MS analysis, and the measurements were repeated three times. The mean values

150 were calculated. The regression equations between the peak areas and the concentrations of

151 the standards solutions were obtained as follows:

152 $y_{\text{linolenic acid}} = -3.65547 \times 10^6 + 7.66475 \times 10^9 x$, ($R^2 = 0.99336$)

153 $y_{\text{octadecanoic acid}} = 9.84569 \times 10^5 + 9.65142 \times 10^8 x$, ($R^2 = 0.98193$)

154 $y_{\text{linoleic acid}} = 2.60555 \times 10^8 + 6.71208 \times 10^{10} x$, ($R^2 = 0.99835$)

155 $y_{\text{3-octanol}} = -1.74715 \times 10^7 + 1.07282 \times 10^9 x$, ($R^2 = 0.9131$)

156 $y_{\text{limonene}} = -8.13324 \times 10^6 + 1.83422 \times 10^9 x$, ($R^2 = 0.9995$)

157 $y_{\text{3-cyclohexen-1-ol}} = -3.79158 \times 10^7 + 2.37430 \times 10^9 x$, ($R^2 = 0.8630$)

158 $y_{\text{hexadecane}} = 7.03236 \times 10^7 + 4.06271 \times 10^9 x$, ($R^2 = 0.9364$)

159 $y_{\text{cedrol}} = 4.94046 \times 10^6 + 4.65335 \times 10^8 x$, ($R^2 = 0.9379$)

160 $y_{\text{heptadecane}} = 5.02313 \times 10^7 + 5.02804 \times 10^9 x$, ($R^2 = 0.9905$)

161 **Data Analysis**

162 The mass spectral fragmentation patterns of the compounds detected by GC-MS were

163 compared with those in the NIST 2014, Wiley (version 9) libraries. Those with a mass spectral

164 similarity of over 90% were selected for identification. The relative content (%) of each compound

165 was calculated by comparing the peak area, expressed as % from total peak areas, in GC-MS

166 analysis. PLS-DA (partial least squares discriminant analysis) is a statistical technique for feature
167 extraction and supervised discriminant analysis (*Barker & Rayens, 2003*), which produces a nice
168 separation to distinguish the observed values between groups (*Barker & Rayens, 2003*). A
169 relationship model between the metabolomic data block and the respective labelled groups was
170 established by PLS-DA, which could detect the variables that influence the differences between
171 groups and classify and predict samples. VIP (Variable important in projection) is a PLS-DA
172 weight value (*Banerjee et al., 2013*), which can be used to measure the influence intensity and
173 explanatory ability of the accumulation difference of each metabolite for the classification and
174 discrimination of each group of samples (*Tang et al., 2021*) . In the PLS-DA models, the VIP
175 parameter was used to identify metabolites that make the most contribution to diagnostic group
176 discrimination, and threefold cross-validation of the models was conducted to evaluate their
177 predictive ability (*Kaddurah-Daouk et al., 2013*). The prompt function of R language (R version
178 4.2.0, (2022-04-22), R Core Team) and PCA of the ggplot2 package was used for research and
179 visualization. Scatterplot and pheatmap packages were used to prepare the corresponding to
180 scatter plots and heatmaps of compounds. The upset analysis diagram was performed using
181 TBtools software (*Chen et al., 2020*). MetaboAnalyst (http://www.metaboanalyst.ca) is a network
182 application platform for metabolomic data analysis and interpretation and other omics
183 association analysis (*Kaddurah-Daouk et al., 2013*). The data were processed successively as
184 follows, first removing the features with more than 50% missing values and then the missing
185 values replaced by LoDs (1/5 of the minimum positive value of each variable). To eliminate
186 batch-to-batch differences, the data were normalized by Pareto scaling. The statistical module of
187 MetaboAnalyst 5.0 was used to carry out a one-way statistical analysis of mangrove compounds
188 from species and parts, IDs of the metabolic compound were searched in an HMDB database
189 (Human Metabolome Database, https://hmdb.ca/) and a PubChem database
190 (https://pubchem.ncbi.nlm.nih.gov/), and obtained the corresponding KEGG (Kyoto
191 Encyclopedia of Genes and Genomes, https://www.genome.jp/kegg/) IDs, and pathway
192 enrichment analysis was performed in 1072 subchemical class metabolome or lipome dataset.

193 ## Results

194 **Flavonoid and polyphenol contents of five mangroves**

195 Figure 2 shows that AM_leaf contains the highest level of flavonoids with a value of 12.19%,
196 followed by BG_root and KC_root. Additionally, AM_leaf contains the highest concentration of
197 phenolics, reaching 7.87%. However, the average distribution of phenolics was about 2% in
198 other parts of mangrove species. In general, the flavonoid content of different parts of mangroves

199 was higher than the phenolic content. As a result of clustering based on flavonoid and phenolic

200 content, AM_leaf and BG_root clustered into one group, which markedly distinguished different

201 parts of other mangroves. However, AM_root and AM_stem exhibited relatively low flavonoid

202 and phenolic contents, which were also noticeably different from those of other mangrove

203 species. The same organ parts, such as the leaf, were generally clustered into one subclass.

204 **Volatile comparison of five mangrove species**

205 The total ion current (TIC) diagrams of leaves, stems, and roots of five mangrove species

206 detected by GC-MS, were shown in Figure 3 and Figure S1. There are 532 different compounds

207 in the mangrove parts, involving 18 classes, including alcohols, aldehydes, alkaloids, alkanes,

208 etc. (Figure 4, Table S1, S2). From Figure 4, it can be seen that some mangrove parts contained

209 large amounts of alkane-like compounds, followed by acids, then alcohols, ketones and esters,

210 especially alkanes were widely distributed and abundant in the whole plant of *A. marina*, *A.*

211 *corniculatum,* and *B. gymnorrhiza* species. In addition, the roots and stems of *K. candel* and *B.*

212 *gymnorrhiza* were rich in monocyclic aromatic compounds.

213 Figure 5-8 showed that a minimum of 41 compounds (in BG_stem) and a maximum of 86

214 compounds in AC_leaf and AM_root could be determined in each part of five mangrove species.

215 Furthermore, the more compound parts were the *K. candel* root with 85 compounds detected,

216 and *A. corniculatum* root with 84 compounds detected.

217 A total of 247 compounds were found in *A. corniculatum*, 244 in *K. candel*, and 240 in *A. marina*

218 (Figure 5 a). The number of compounds in three mangrove species was significantly more than

219 that in *A. ilicifolius* (176) and *B. gymnorrhiza* (172). The roots of mangrove plants contained the

220 greatest number of compounds (399), followed by the stems (341), and finally the leaves (339).

221 Upset analysis of these compounds was studied, and unique and common compounds among

222 different sample groups were identified (Figure 5 b). There were 40 unique compounds found in

223 *A. corniculatum* leaves, 39 in *A. corniculatum* stems, 33 in *A. ilicifolius* roots and leaves, and just

224 three in *B. gymnorrhiza* stems. There was a 30.16% high concentration of 3-hexen-1-ol, acetate

225 (z) - in *B. gymnorrhiza* leaves (BG_leaf), followed by acetic acid (E) -non-3-enyl ester (9.17%) in

226 *A. marina* roots (AM_root) and hexanoic acid, ethyl ester (8.1%) in *A. corniculatum* leaves

227 (AC_leaf), followed by endo-borneol (AC_root) and 2-heptenoic acid, ethyl ester (AI_leaf) (Figure

228 6).

229 The term "common compound" refers to a chemical compound found in multiple parts of 2 or

230 more mangrove species (Figure 5b). The most common compounds were found in the *A. marina*

231 roots and stems, with five common compounds.

232 The total content of the compounds detected (Figure 7a) and five mangrove species' unique

233   compounds (Figure 7b) were compared. The highest levels of detected compounds in *B.*
234   *gymnorhiza* leaves (92.67%), followed by *A. corniculatum stems* (90.39%), and *B. gymnorhiza*
235   stems (87.54%). *B. gymnorrhiza* had the highest percentage at 88.58% among mangrove
236   species. Leaves had the highest content among the three parts.
237   Two factors were used to estimate the total compound content and unique compound content of
238   mangrove species (factor A) and parts (factor B). R^2 of the two models was greater than 0.999
239   with P values less than 0.001 (Table S3, S4). The regression equations of the two models were
240   shown as follows:
241   Total content of detected compounds = 70.74 - 26.57 $A_{[1]}$ + 17.85 $A_{[2]}$ + 7.54 $A_{[3]}$ + 5.36 $A_{[4]}$ +
242   0.8581 $B_{[1]}$ - 1.35 $B_{[2]}$ - 0.4955 $A_{[1]}B_{[1]}$ + 3.23 $A_{[2]}B_{[1]}$ - 2.88 $A_{[3]}B_{[1]}$ - 1.12 $A_{[4]}B_{[1]}$ - 0.9991 $A_{[1]}B_{[2]}$ -
243   1.71 $A_{[2]}B_{[2]}$ - 8.76 $A_{[3]}B_{[2]}$ + 8.44 $A_{[4]}B_{[2]}$ (1)
244   Total content of unique compounds = 12.86 + 0.28$A_{[1]}$-0.84$A_{[2]}$ + 6.66$A_{[3]}$-3.59$A_{[4]}$ + 6.64$B_{[1]}$-
245   1.45$B_{[2]}$ + 3.83$A_{[1]}B_{[1]}$ + 14.01$A_{[2]}B_{[1]}$-4.23$A_{[3]}B_{[1]}$-2.39$A_{[4]}B_{[1]}$ + 0.54$A_{[1]}B_{[2]}$ - 7.39$A_{[2]}B_{[2]}$ - 0.55$A_{[3]}B_{[2]}$
246   - 2.09$A_{[4]}B_{[2]}$ (2)
247   The regression equation (Equation 1) of the total content of the detected compounds indicates
248   that the maximum coefficient for mangrove species is 26.57 from $A_{[1]}$, which suggests that
249   mangrove species (factor A) had the greatest influence. A[3] and B[3] in equation 2 have
250   maximum coefficients of 6.66 and 6.64, respectively, which indicates that mangrove species and
251   parts have almost equal factor effects on the total content of unique compounds.
252   **Differences of compounds in three parts of mangrove plant**
253   71 common compounds in five mangrove species were detected and used for further analysis.
254   Figure S2 shows that *K. candel* leaves had the fewest common compounds, whereas their stems
255   and roots had more common compounds. The content of most common compounds in the *A.*
256   *ilicifolius* roots and stems, and *A. marina* leaves and stems were concentrated in a particular
257   range, indicating no significant difference in their contents. It was noted that *B. gymnorrhiza* and
258   *A. corniculatum* leaves contained a few common compounds with high concentrations, which
259   were primarily distributed at the 5-15 minute retent time (Figure S2), and the top five compounds
260   were methoxy phenyl oxime, 1-hexanol, 3-octanone, 3-octanol, and 3-hexen-1-ol in order of their
261   content.
262   71 common compounds were further analyzed using the metaboanalyst platform. 18 different
263   compounds were found among mangrove species and nine among parts (Figure 8). α-
264   Amorphene, furan 2-pentyl-, naphthalene 1-methyl-, 1-hexanol, and 3-octanol were the
265   differential compounds in the two models. Statistically significant differences were found
266   between species and parts with log10(P)>1.3 and FDR (false discovery rate) less than 0.05.

267 These indicated significant differences in the contents of common compounds between
268 mangrove plants.

269 **Statistical and pathway enrichment analysis of common compounds**

270 VIP ≥ 1 was taken as the screening criterion, and the differences among groups were
271 preliminarily screened out (*Tang et al., 2021*). The model prediction accuracy Q^2 (the cross-
272 validated R^2) is calculated by cross-validation (CV) and has a high level of predictive ability when
273 Q^2 is greater than 0.5 (*Triba et al., 2015*). When PCs (principal component) equals five in the
274 PLS-DA model (Figure 9), the accumulated Q^2 of species factor and part factor is 0.81 and 0.78,
275 respectively, which indicates that the two models had high reliability (Figure 9). The species
276 factor model (Figure 10a) has a contribution rate of 32.1% and 22.4% for component1 and
277 component2, while the other part factor has a contribution rate of 42.4% and 7.4% for
278 component1 and component2 is 42.4% and 7.4% (Figure 10b). The samples of five mangrove
279 species could be clearly separated on the two-dimensional plot of PLS-DA (Figure 10a). There is
280 a clear separation between the light green (*A. ilicifolius*) and the cool blue (*B. gymnorrhiza*)
281 groups along the PC1 direction along the horizontal of Figure 10a. PC1 is closely related to
282 mangrove species because intra-species differences in common compounds are relatively minor
283 among the three other mangrove species. Leaves (pink group) and stems (blue group) in Figure
284 10b could be distinguished, despite the overlap of stems and roots. The findings of the clustering
285 analysis of common compounds (Figure S3) indicated that the leaves of *B. gymnorrhiza, A.*
286 *corniculatum,* and *A. ilicifolius* formed a distinct class. Another big class consisted of three
287 subclasses.

288 Further, VIP scores of 71 common compounds were discriminated against and sorted by two
289 PLS-DA models (Figure 11), with the top 5 being hexanol, 3-octanone, trans-caryophyllene,
290 trans-alpha-octanone, and oximemethox in the species factor model, and L-limonene, (+) -
291 isomenthol, 3-hexen-l-ol, 1-hxanol, and 3-octanone, naphthalene in the part factor model.
292 Functional pathway enrichment analysis (Table 1 and Table S5) was performed with 17
293 differential common compounds identified by two PLS-DA models. As shown in Table 1, the
294 results showed that 11 of the differential common compounds were enriched on six pathways,
295 according to the enrichment ratio (-log10(P) value). These pathways were C10 isoprenoids, C15
296 isoprenoids, fatty alcohols, hydrocarbons, diterpenoids, and monoterpenoids.

Discussion

298 **Role and Differences of flavonoids and phenolics**

299 Plant secondary metabolites, mainly phenolics, flavonoids, alkaloids, and terpenoids, are

300   involved in environmental adaptation and stress tolerance (*Boncan et al., 2020*). Flavonoids and
301   polyphenols are the most important secondary metabolites commonly distributed in the kingdom
302   of plants (*Panche et al., 2016*) and play an essential role in plant growth, development, stress
303   resistance, and other biological processes (*Panche et al., 2016*). Flavonoids participated in some
304   plants' stress response process (*Samanta et al., 2011*; *Frank et al., 2021*), which involved
305   flavonoid accumulation (*Samanta et al., 2011*). Mangrove plants are potential sources of
306   medicines due to the presence of bioactive compounds. The antioxidant activity of mangrove
307   plants was related to their phenolic and flavonoid contents (*Krishnamoorthy et al., 2011*).
308   *Rhizophora apiculata* and *A. ilicifolius* root extracts were rich sources of phenolic compounds
309   and flavonoids (*Asha et al., 2012*). In this study, the flavonoid and phenolic contents of the five
310   mangrove species were rich and different in the parts of the mangrove species. Flavonoid
311   content was greater than phenolics, which differed significantly from the stem barks of *Bruguiera*
312   *cylindrica* and *Ceriops decandra* in India (*Krishnamoorthy et al., 2011*). The results in Figure 2
313   showed that among the five mangroves, AM_leaf contained the highest levels of flavonoids
314   (12.19%) and the highest concentration of phenolics (7.87%). The flavonoid and phenolic
315   contents of five mangrove species and three parts were further correlated. The results (Figure 12)
316   showed that the flavonoid content and phenolic content of the same part were significantly
317   correlated with large correlation coefficients (absolute values), such as flavonoids and phenolics
318   in leaves with 0.99 correlation coefficients, and strong correlation between mangrove stems and
319   leaves in flavonoid content or phenolic content. These suggested that the flavonoid and phenolic
320   content of mangrove plants varied with different species and parts, which was influenced by age,
321   origin, and ecological environment (*Amirav et al., 2013*). Thus, we assumed that these
322   differences were not only from mangrove species and parts, but also from the mangrove
323   environment. In natural conditions, mangrove species are zoned according to their adaptability,
324   especially tolerance to flooding (*Chen et al., 2017*). Mangrove species in Leizhou Peninsula,
325   Guangdong are *A. marina*→ *A. corniculatum* → *K. candel* →*B. gymnorrhiza* →*A. ilicifolius* in
326   order of distribution from the coast to the inner shore (*Chen et al., 2017*). Four mangrove species
327   showed a decrease in flooding tolerance in the following order: *A. marina* > *A. corniculatum* >
328   *Rhizophora stylosa* > *B. gymnorrhiza* (*He et al., 2007*). Compared to *K. candel, B. gymnorrhiza*
329   was not as waterlogging tolerant (*Ye et al., 2003*). Therefore, we classified five mangrove
330   species into five levels of salt and waterlogging tolerance from weak to strong. The correlation
331   between the flavonoid or phenolic content of mangroves and their levels of salt and waterlogging
332   tolerance was analyzed (Figure 12). The results showed that salt and waterlogging tolerance
333   levels were negatively correlated with the flavonoid/phenolic content of roots and stems, while

334 the flavonoid/phenolic content of leaves was positively correlated. According to these results,

335 flavonoids and phenolic content is highly correlated with the adaptation level of mangrove

336 species.

Differences in volatiles between mangrove species and their part

338 As a powerful and unique analytical method, GC-MS has become increasingly popular to

339 analyze medicinal plants in recent years (*Al-Rubaye et al., 2017*). We have developed and

340 optimized GC-MS conditions to detect volatiles from the leaves, stems, and roots of five

341 mangrove species based on previous studies (*Wu et al., 2012*). In this study, 532 compounds in

342 three organ parts were identified. The number of compounds in each part identified by GC-MS

343 ranged from 41 to 85, and was higher than that reported in some literature on mangroves (*Kumar*

344 *Dinesh & Rajakumar, 2016*; *Joel & Bhimba*, 2010; *Lalitha et al*., 2021; *Dahibhate & Kumar, 2022*;

345 *Swaraiah et al., 2020*). Furthermore, we observed that GC-MC rarely detected common fatty

346 acids. There were two reasons for this; the first was that GC-MS was limited in its ability to

347 analyze a limited number of volatile, thermally stable chemicals, and the second was that

348 mangrove species rarely contain fatty acids. Only *A. ilicifolius* and *A. marina* have been reported

349 to contain fatty acids (*Nabeelah Bibi et al., 2019*).

350 In this study, five mangroves contained 532 compounds, of which 341 compounds were found in

351 stems, 339 in leaves, and 399 in roots (Figure 5), and these differences were not significant

352 among roots, stems, and leaves. There were also differences in the number of compounds

353 among the five species. However, the number of compounds in all three parts and the total

354 mangrove plant was also positively correlated with their salt and waterlogging tolerance levels

355 (Figure S4). Among these species, the total content of detected compounds differed significantly

356 (Table S3), indicating that different tolerance mangrove species contain varying levels of

357 compounds. The one-way analysis of 71 common compounds revealed that 18 metabolites that

358 differed were found between mangrove species, while nine metabolites were found between

359 parts. In summary, mangrove species had greater factorial effects on metabolite differences than

360 part factors.

Differential compounds involved in metabolic pathways and their role

362 Among these 532 compounds, 114 compounds with KEGG ID (Table S1) were mainly involved

363 in the following three metabolic pathways (Figure 13): monoterpenoid biosynthesis (Figure 13

364 and Figure S5), sesquiterpenoid and triterpenoid biosynthesis, and α-Linolenic acid metabolism

365 (Figure 13). The metabolic pathway enrichment analysis of different common compounds was

366 mainly involved in the metabolism of C10 isoprenoids, C15 isoprenoids, fatty alcohols, etc.

367 (Table 1).

368   Terpenes, also known as terpenoids or isoprenoids, comprise the most chemically and
369   structurally diverse family of natural products (*Christianson, 2017*). Their main skeleton
370   comprises five-carbon isopentyl units, known as 2-methyl-1,3-butadiene or isoprene (*Yeshi et al.,*
371   *2022*). Monoterpenoids are composed of two isoprene units (C-10 carbon atoms) and
372   sesquiterpenoids of three isoprene units (C-15 carbon atoms) (*Yeshi et al., 2022*). The results of
373   the above two analyses are consistent. Thus, our study suggested that the main differences in
374   the GC-MS metabolites of the five mangroves and their parts were related to the isoprenoids or
375   terpenoids.
376   Plant volatiles are involved in plant-environment interactions and, to some extent, in abiotic
377   stress responses (*Dudareva et al., 2004*; *Dudareva et al., 2006*). The volatile emission profile of
378   plants may be one of the signature responses to stress conditions as plant volatiles mitigate the
379   effects of oxidative stress (*Boncan et al., 2020*). Terpenoids play a defensive role in plants
380   responding to biotic and abiotic stresses (*Yeshi et al., 2022*). Isoprene can aid plant performance
381   under abiotic stresses (*Frank et al., 2021*). The isoprene can improve thermotolerance or reduce
382   oxidative stress, preventing herbivores and parasitoids from attracting plants (*Sharkey et al.,*
383   *2008*). The triterpenoid content played an important role in mangrove plants for protection from
384   salinity in both salt-secretors and non-secretors (*Basyuni et al., 2012*). *A. marina'*s metabolites
385   and metabolic pathways were important factors contributing to its salinity and drought stress
386   tolerance (*Ravi et al., 2020*). Concerning the tolerance levels of the five species mentioned
387   above, the common compounds that differed in the one-factor statistical analysis of mangrove
388   species (Figure 11a) were analyzed for correlation between the compound content and the
389   tolerance levels of the species (Figure S6). It was found that seven compounds in leaves had
390   significant correlation with >0.6 absolute values of correlation coefficients, three of which
391   reached significant levels. Among the three parts, the mean value of correlation coefficients
392   (absolute values) was the largest in leaves. Again, these indicated that some compound content
393   correlated with the tolerance level of mangrove species, especially the content of compounds in
394   the leaves is more correlated.
395   Meanwhile, terpenoids have different biological activities (*Mitić et al., 2017*), including good
396   antibacterial activity (*Patra & Mohanta, 2014*), cytotoxic and antiviral activity (*Gong et al., 2017*),
397   and moderate cytotoxic and antimicrobial activities (*Cerri et al., 2022*). Terpenes and terpenoids
398   are the main bioactive compounds of essential oils (*Masyita et al., 2022*), which have been
399   comprehensively studied and reported to play critical roles in human health (*Perveen, 2018*).
400   Mangrove metabolites have potential applications in the discovery of novel medicinal properties.
401   The above results suggested that different mangrove species have various secondary metabolite

402 profiles associated with different tolerance levels of salt and waterlogging. Further related studies

403 should be conducted to understand the precise mechanisms behind this relationship and identify

404 specific secondary metabolites that contribute to mangrove resistance to salt and waterlogging.

405 For bioactive secondary metabolites in mangroves, more attention should be paid to the abiotic

406 stresses affecting their pharmacological properties.

Conclusions

408 Mangrove plants grow in harsh coastal areas where interaction with the surrounding environment
409 is inevitable. Their production of secondary metabolites, especially organic compounds and
410 terpenes, is related to their ability to withstand biotic and abiotic stresses. In this paper,
411 flavonoids, polyphenols, and volatiles in mangroves' roots, stems, and leaves were tested,
412 analyzed, and compared. Flavonoid and phenolic contents varied among mangrove species and
413 parts, which are highly correlated with the adaptation levels of mangrove species to salt and
414 waterlogging. And 114 compounds were involved in the biosynthesis pathways of
415 monoterpenoid, sesquiterpene and triterpenes, and α-linolenic acids. Seventeen common
416 compounds were involved in the metabolic pathways of isoprenes, fatty alcohols, and others.
417 GC-MS metabolites from the five mangroves and their parts mainly differed in their isoprenoids
418 or terpenoids; their number and content correlated with the tolerance level of mangrove species.
419 In terms of volatile content and type differences, *A. ilicifolius* and *B. gymnorrhiza* were
420 distinguished from the other three species, while leaf was clearly distinguished from the other
421 parts, and the mangrove species had a greater factor effect than their part. Our study highlights
422 the distribution and importance of secondary metabolites in mangroves and their role in
423 mangrove tolerance. The study's findings will serve as a reference for future fundamental
424 research on the molecular biology of mangrove plants and the development and use of
425 pharmaceuticals.

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Author Contributions

430 Zhihua Wu conceived and designed the experiments, performed the experiments, authored or

431 reviewed drafts of the paper and approved the final draft.

432 Xiuhua Shang analyzed the data and approved the final draft.

433 Guo Liu analyzed the data and approved the final draft.

434 Yaojian Xie conceived and designed the experiments, and approved the final draft.

Supplemental Information

436 Supplemental information for this article is available in the Supplemental Files.

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Figure 1

Morphological characteristics of the seedlings of five mangrove species used in this study.

(**a**): *A. ilicifolius*; (**b**): *B. gymnorrhiza*; (**c**): *A. corniculatum*; (**d**): *K. candel*; (**e**): *A. marina.*The pot with a square mouth has the following specifications: the upper pot side length is 4.2 cm, the bottom side length is 3.8 cm, and the pot height is 4.0 cm. The cultivation substrate is mainly composed of rice husk, coconut shell shreds, vermiculite, and mixed with sea mud. During the cultivation period, the seedlings are watered every 2-3 days with a salinity of about 0.3 % seawater.

Figure 2

Content heatmap of flavonoids and phenols of five mangrove species and parts

In the figure, FL_sd represents the flavonoid standard deviation, and PH_sd represents the polyphenol standard deviation. The color scale in the legend is on a log2 scale from 0-4. The data in the heatmap are the percentages in the dry sample. The clustering dendrogram of samples was based on their Euclidean distance using the complete clustering method.

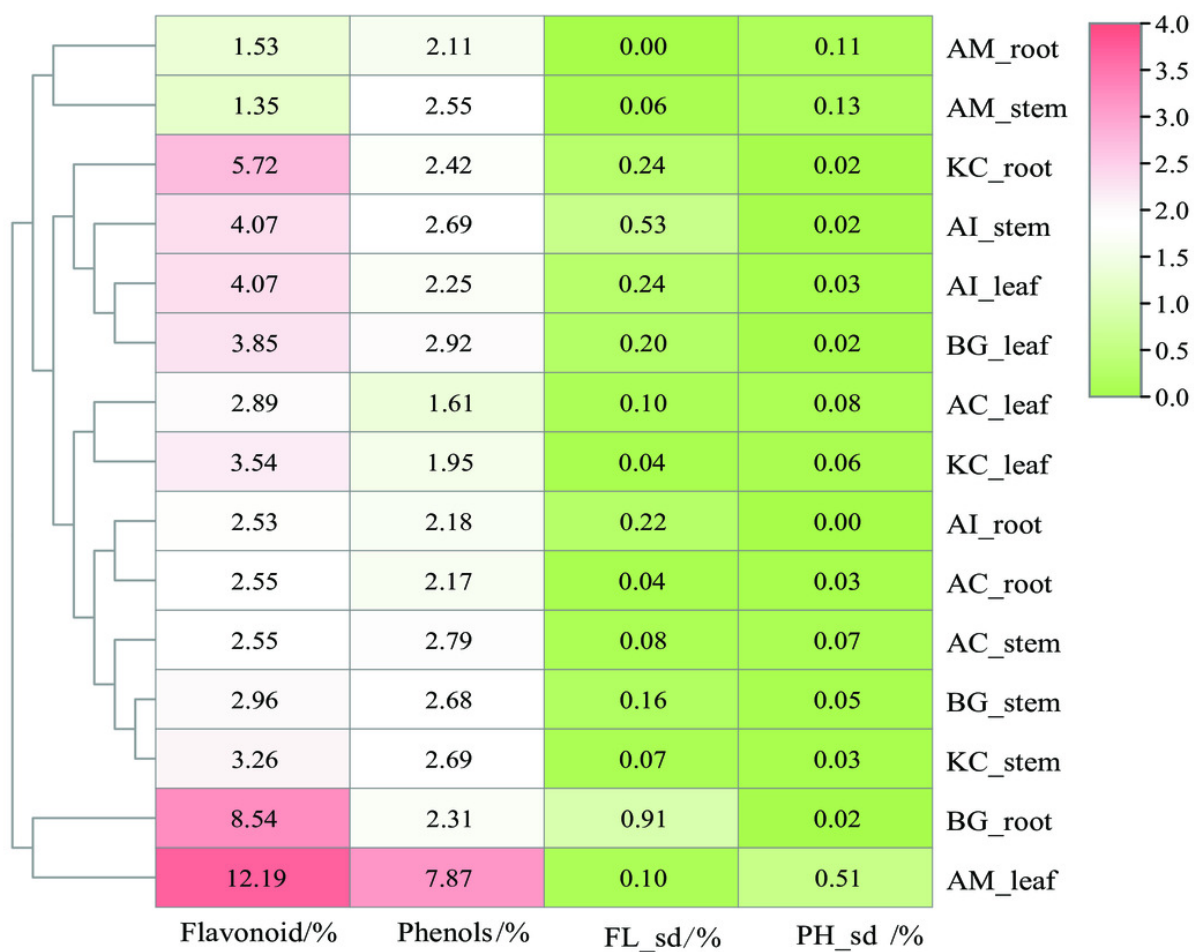



Figure 3

The total ion current (TIC) diagram of two mangrove leaves, stems, and roots by GC-MS.

The number and compound marked next to the peak in the figure indicate the retention time of the compounds, respectively. At 3-4 min of retention time, a certain amount of column loss was present.

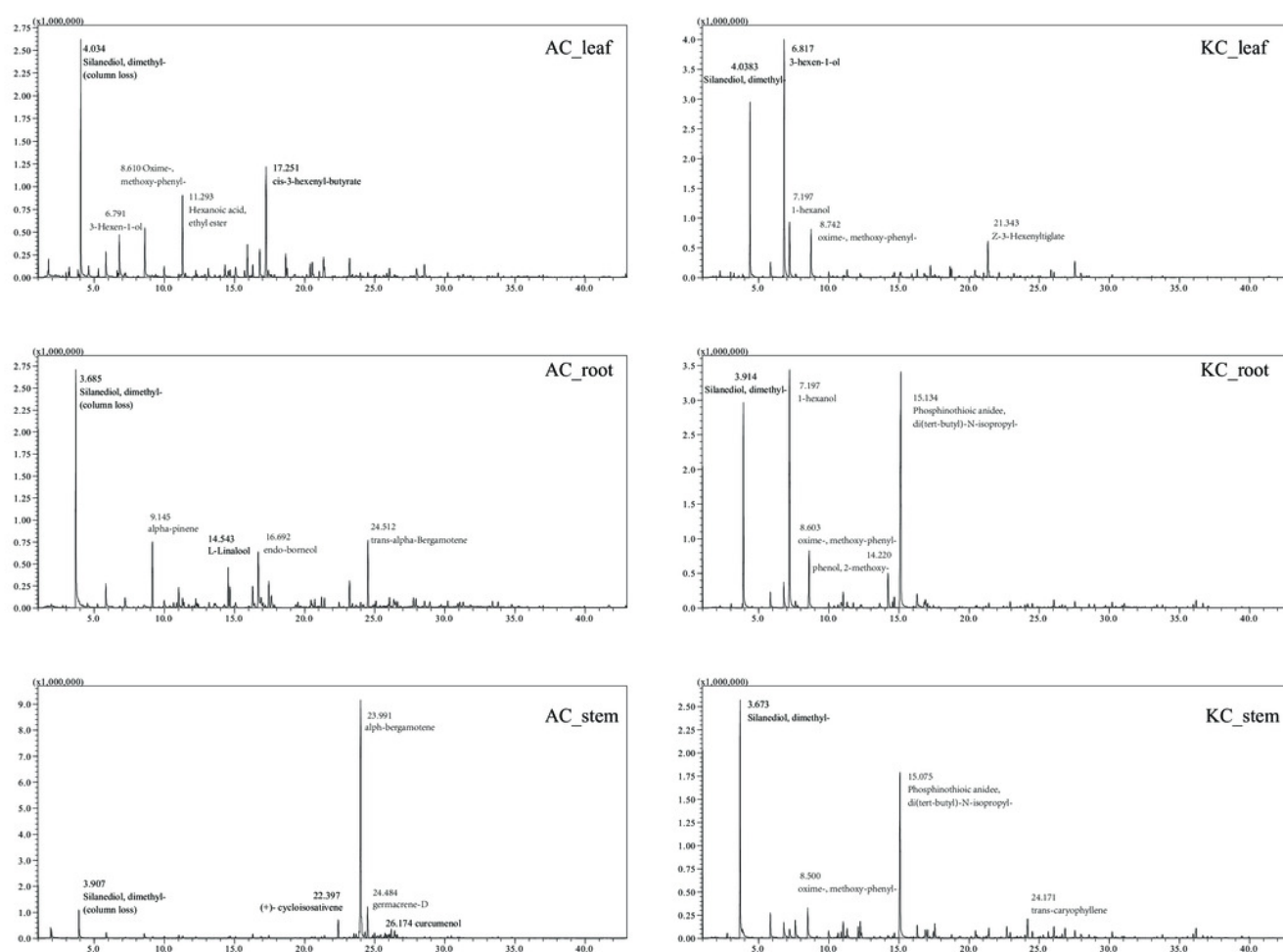



Figure 4

The distribution and content of 18 classes of compounds in different mangrove species and parts.

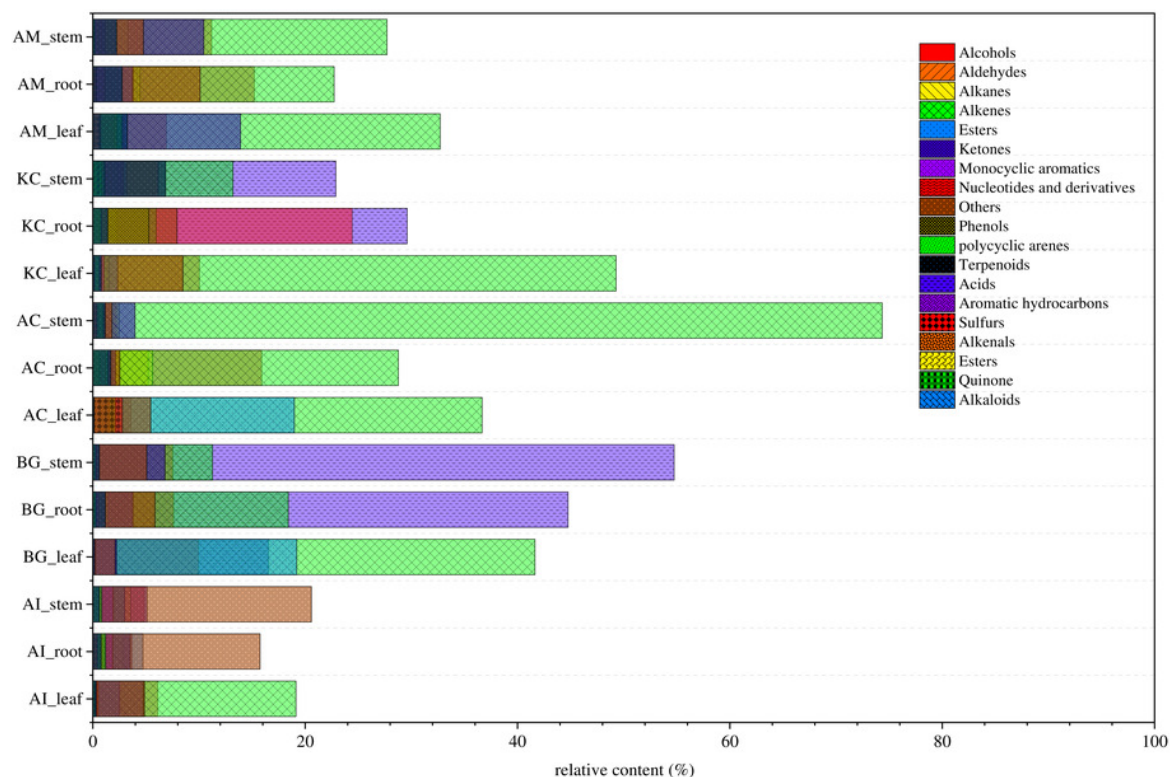



Figure 5

The number of chemical compounds in leaves, stems, and roots of five mangrove species by GC-MS.

(**a**): the number of compounds tested in different species and parts. The number on the left side of the column is the number of compounds tested, (**b**): Distribution of unique or common compounds on different mangrove species and parts. The number indicates the number of unique compounds in this column (only one marked black dot in the unique compound group) or the number of common compounds containing $> _= 2$ in this column (multi-dot connected by a line in the common compound group). The details of the compounds in each combination of mangrove species and parts are referred to the Dryad database (https://doi.org/10.5061/dryad.ffbg79cz8).

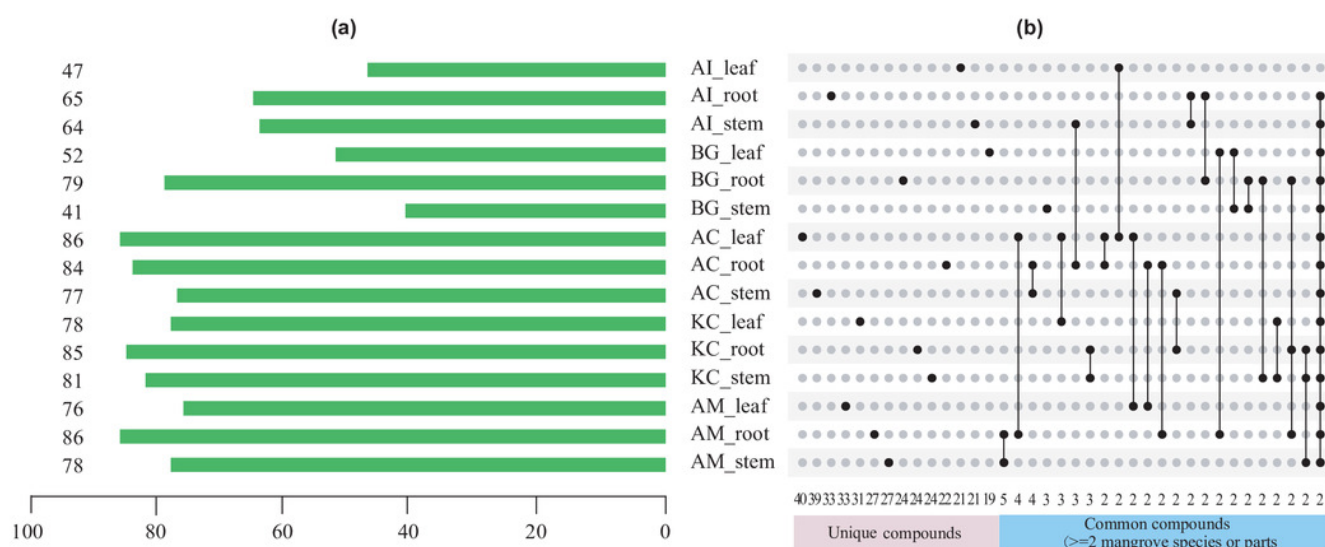



Figure 6

Top 5 unique compounds ranked by content in five mangroves and parts.

The left vertical coordinate in the plot indicates the metabolite unique compound name. The horizontal coordinates indicate the relative content (%) and the right vertical coordinates indicate the relative content of the unique compounds and their retention time. The different colored bars in the figure indicate the different mangrove species and parts.

Unique compounds

Content/% (Time/min)

Compound	Content/% (Time/min)
2-ethyl-1-hexanol	4.19(12.255)
Octadecanoic acid, ethenyl ester	1.78(1.092)
Hexanoic acid, 3-hexenyl ester, (z)-	2.62(22.919)
2-heptenoic acid, ethyl ester, (e)-	6.26(29.599)
Cycloheptanone, 2-methyl-	2.89(1.170)
Oxacyclohexadecane-2,13-dione 13-oxime	0.64(29.596)
Pyrazine, 2-methoxy-3-(1-methylethyl)-	1.02(14.388)
Phosphine oxide, triphenyl-	1.20(39.285)
Neryl acetate	0.79(12.253)
Tetradecanal	0.94(31.296)
Hexane, 3,3-dimethyl-	0.22(25.079)
2-pentadecanone, 6,10,14-trimethyl-	0.20(34.189)
2-oxononane	0.22(29.600)
Pentadecane	0.20(20.619)
1,1-dimethoxy decane	0.25(31.365)
7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.94(35.888)
1-octen-3-yl acetate	0.29(14.954)
E2-dodecenylacetate	0.48(20.477)
Acetic acid, decyl ester	0.68(23.691)
Cyclobutanecarboxylic acid, octyl ester	0.21(15.315)
3-hexen-1-ol, acetate, (z)-	30.16(11.583)
Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-, (1.alpha.,3.alpha.,4.beta.,6.alpha.)-	0.12(25.965)
.alpha.-gurjunene	0.64(23.646)
2-decyn-1-ol	0.36(12.253)
Cembrene	0.59(36.309)
.beta.-ylangene	0.44(36.182)
Cyclohexane, cyclopropylidene-	0.19(16.395)
Diazene, bis(1,1-dimethylethyl)-	0.03(2.980)
Benzene, methyl-	0.01(4.450)
3-tetradecene, (z)-	1.27(20.386)
1-hexene, 3,5,5-trimethyl-	0.89(14.551)
1,3,3-trimethyl-6-(3-nitro-benzenesulfonyl)-6-aza-bicyclo[3.2.1]octane	1.58(15.076)
Hexanoic acid, ethyl ester	8.01(11.293)
Butanoic acid, 2-pentenyl ester, (z)-	1.24(14.322)
2-dodecenal, (e)-	0.62(22.452)
Endo-borneol	7.52(16.692)
1,e-8,z-10-tetradecatriene	0.93(27.756)
Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde, 6,6-dimethyl-	2.11(17.623)
Cis-7-tetradecen-1-ol	1.05(27.928)
Spiro[5.5]undeca-1,8-diene, 1,5,5,9-tetramethyl-, (r)-	1.21(23.897)
.beta. bisabolene	1.53(26.408)
(+)-cycloisosativene	3.52(22.397)
(3as,8as)-6,8a-dimethyl-3-(propan-2-ylidene)-1,2,3,3a,4,5,8,8a-octahydroazulene	1.90(26.174)
(1s,2e,6e,10r)-3,7,11,11-tetramethylbicyclo[8.1.0]undeca-2,6-diene	0.87(26.612)
Hydrazinecarbodithioic acid, methyl ester	0.92(15.119)
1,6,10-dodecatriene, 7,11-dimethyl-3-methylene-, (e)-	1.11(25.838)
2,5,5,8a-tetramethyl-3,4,4a,5,6,8a-hexahydro-2h-chromene	1.66(20.424)
3-hexen-1-ol, 2,5-dimethyl-, formate,(z)-	0.67(21.034)
Z-3-hexenyl tiglate	4.75(21.343)
Dihydro linalool	1.67(16.294)
2-heptenal, (e)-	0.34(12.268)
Hexanediamide, n,n'-di-benzoyloxy-	0.57(16.805)
Phenol	0.99(10.875)
1,1,7,12-tetramethyl-1,2,3,4,9,10,11,12-octahydrophenanthrene	0.32(35.987)
Undecanone	0.93(20.459)
.alpha.-muurolene	1.57(22.691)
O-cymene	1.12(12.100)
.delta.-cadinene	1.10(26.845)
Alloaromadendrene	0.43(25.286)
Cyclopropane, propyl-	0.45(7.205)
Benzene, 1-(1,1-dimethylethyl)-3-methyl-	0.29(20.126)
2-propenoic acid, 3-phenyl-, ethyl ester	0.57(25.265)
Ethanone, 1-(3-methylphenyl)-	0.29(15.186)
L-(-)-menthol	0.53(16.899)
Acetic acid, non-3-enyl ester, cis-	9.17(20.469)
Tricyclo[2.2.1.0(2,6)]heptane, 1,7-dimethyl-7-(4-methyl-3-pentenyl)-	1.15(24.105)
Acetic acid, hexyl ester	1.00(11.744)
7-oxabicyclo[4.1.0]heptane, 3-methyl-	0.56(13.178)
Trans,trans-nona-2,4-dienol	1.75(21.913)
2-cyanomethyl-tetrahydrofuran	0.17(20.084)
1,6-octadien-3-ol, 3,7-dimethyl-	1.92(14.544)
6-tridecanone	2.51(25.358)
Zingiberene	0.22(23.987)
2-heptyn-1-ol	0.31(16.420)

Samples

- AI_leaf
- AI_root
- AI_stem
- BG_leaf
- BG_root
- BG_stem
- AC_leaf
- AC_root
- AC_stem
- KC_leaf
- KC_root
- KC_stem
- AM_leaf
- AM_root
- AM_stem

Content /%

Figure 7

Total content of various compounds in three parts of five mangrove species.

(**a**): total content of detected compounds; (**b**): Total content of unique compounds. In the figure, the numbers on the histogram are the total contents of compounds tested in different parts of five species, followed by different lowercase letters, indicating that the tukey comparison between different parts in the same species is significantly different at the level of 0.05. The color numbers and lowercase letters indicate the total means of species and their differences at the level of 0.05.

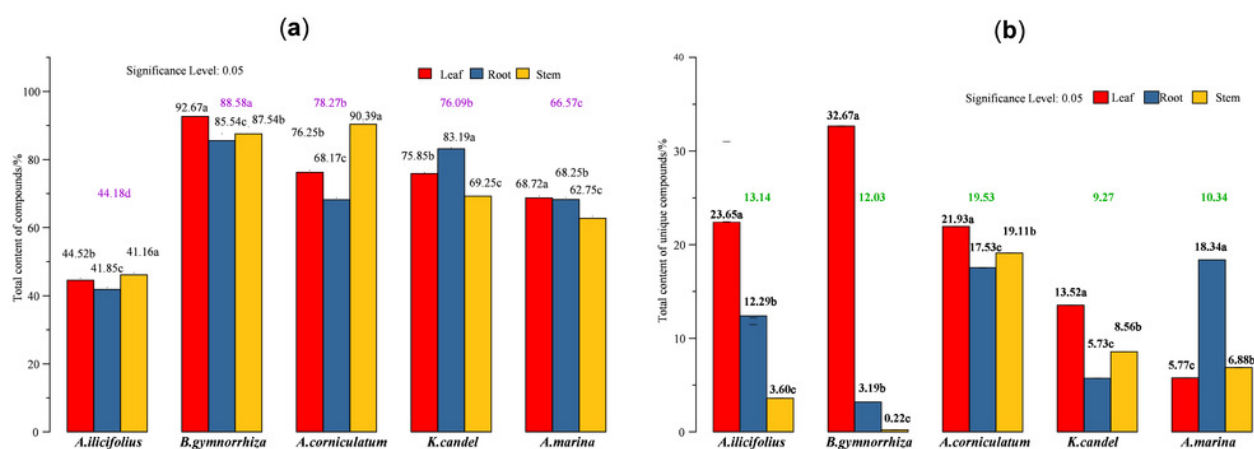



Figure 8

Bubble diagrams of common compounds with significant differences based on species and parts.

F.value, an F-statistic value computed by Fisher's LSD test, tested whether the effects of the different treatments were all equal, in the one-way ANOVA. FDR is an adjusted p.value calculated by Benjamini-Hochberg method. The size of the circles in the plot indicates that the FDRs were processed by -log10 transformation, similarly, different colors and numbers in the color scale indicate the p.value after -log10 preprocessing.

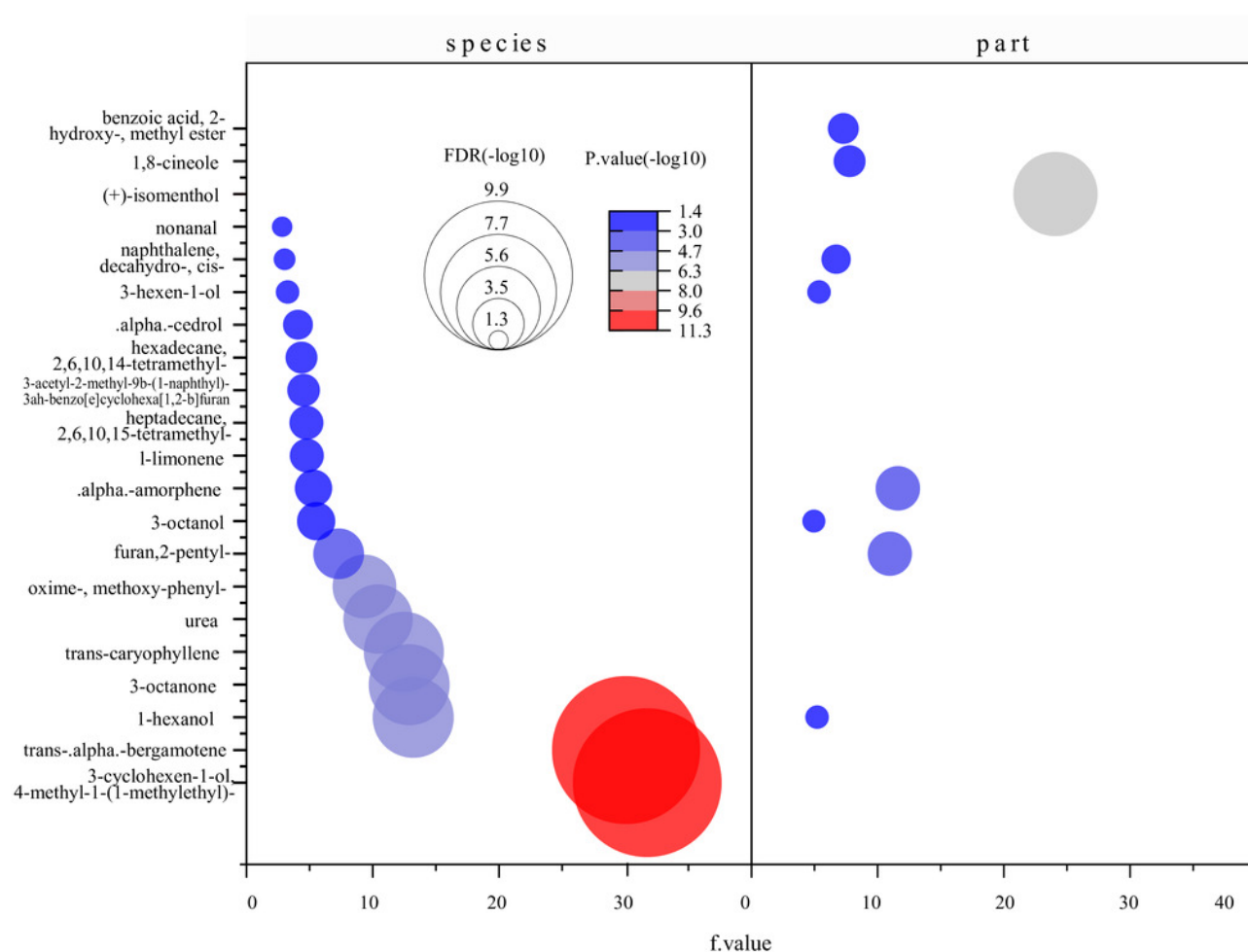



Figure 9

A column chart of the PLS-DA cross-validation result.

The selected performance measure—Q^2 showed that the five-component model was best (indicated by a red star).

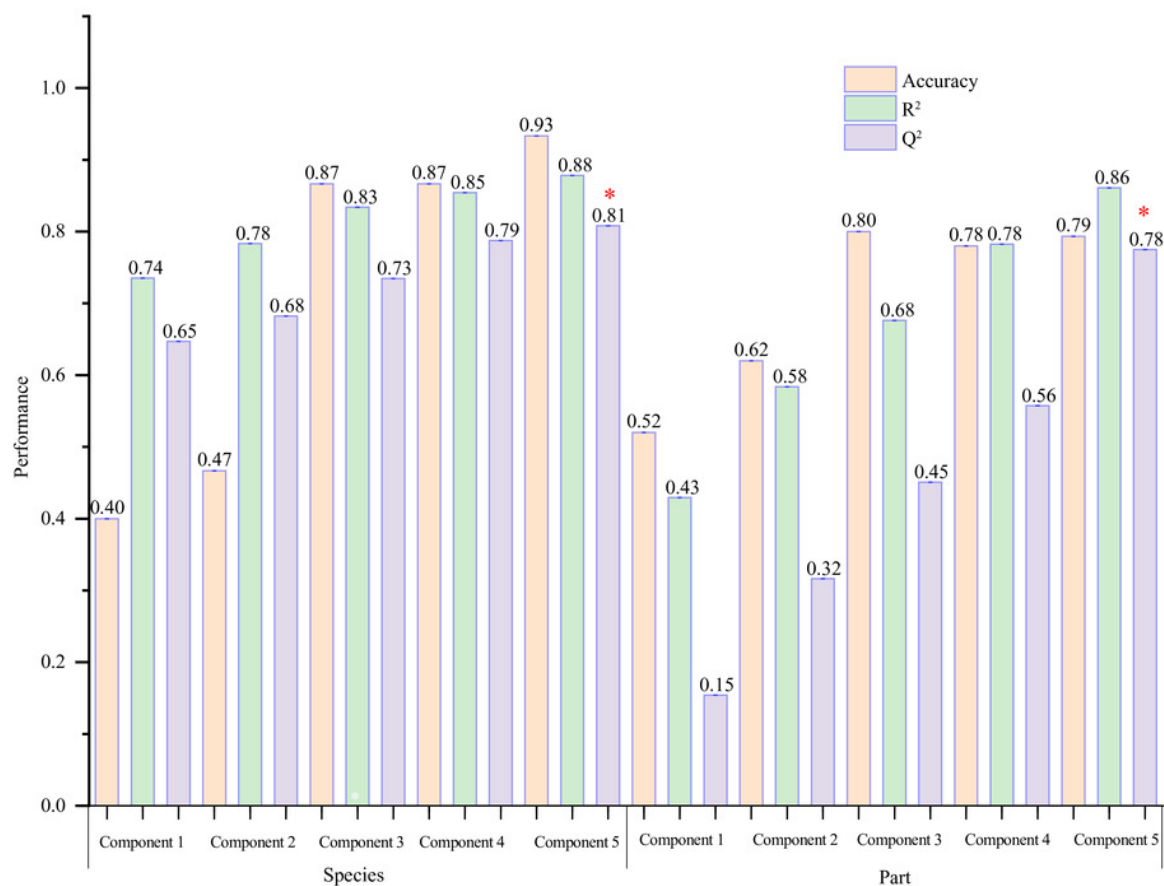



Figure 10

2D score plot of the first two components from common compounds.

(**a**): species factor; (**b**): part factor. The circles in the figure represent the various samples.

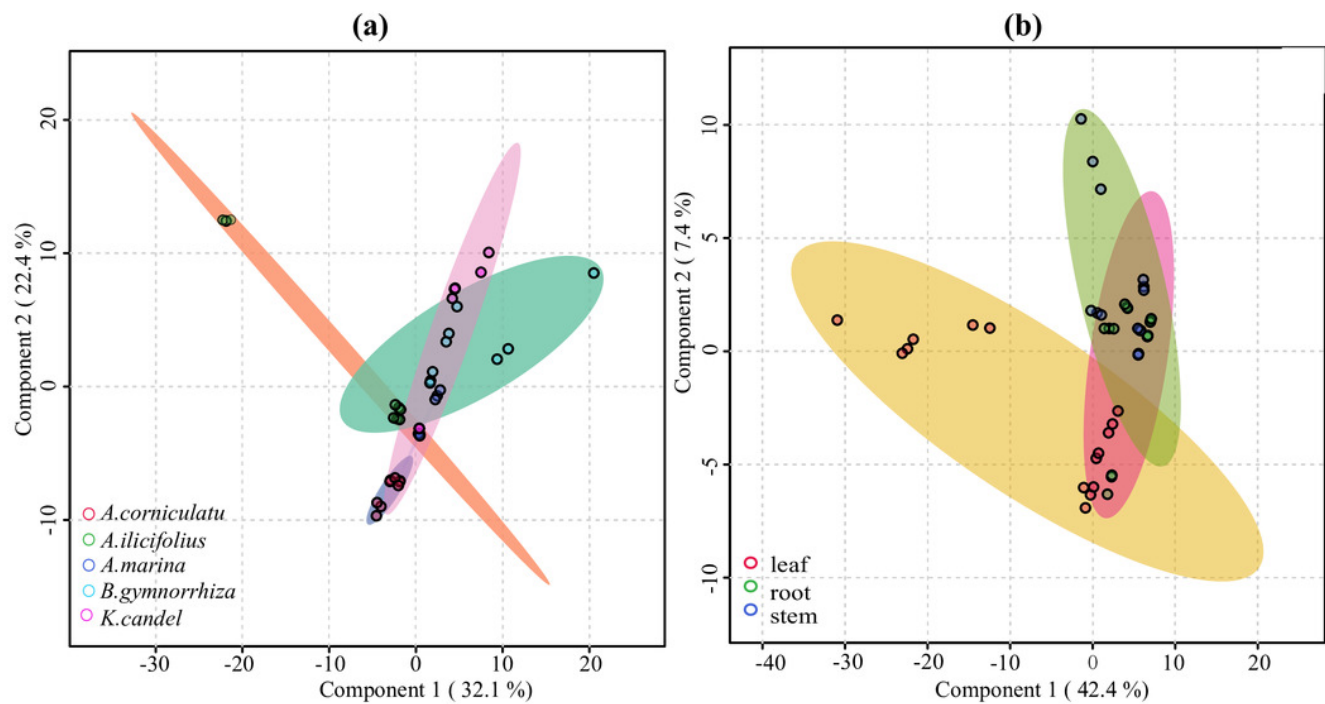



Figure 11

The VIP scores plot of the top 15 important compounds ranked by VIP coefficient.

(**a**): species factor, and (**b**): part factor

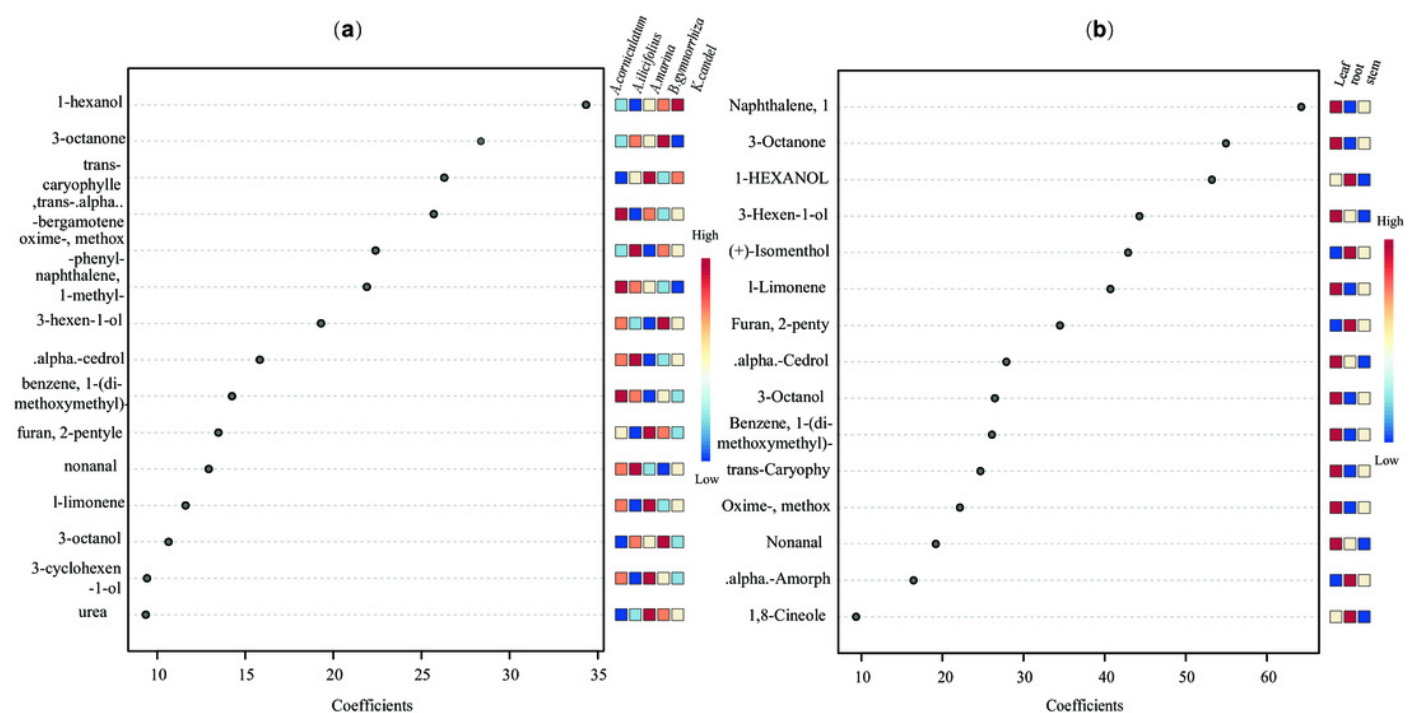



Figure 12

The relationship between flavonoid or phenolic contents of mangroves and their levels of salt and waterlogging tolerance.

The mean values of flavonoid or phenolic contents in each part or whole plant of the five mangrove species. Variables of flavonoid or phenolic contents represented as compound name plus parts / whole plant, e.g. Flavonpid_roots and Flavonpid_plant. Tolerance levels were evaluated from weak to strong on a scale of 1-5 level as follows: AI (level 1), BG (level 2) , KC(level 3) , AC (level 4), and AM (level 5) .

Figure 13

The volatile compounds with KEGG ID involved in secondary metabolic pathways in iPath3.0 (http://pathways.embl.de).

Red dots indicate compounds in the metabolic pathways. Seven compounds were found in the pathway of monoterpenoid biosynthesis, and two compounds each in the sesquiterpene and triterpene biosynthesis pathway, as well as in the α-linolenic acid metabolism synthesis pathway. C1: 1,8-cineole(KEGG ID: C09844); C2: (-)-alpha-Terpineol(C11393); C3: (-)-3-Carene(C09839); C4: (-)-alpha-Pinene (C06308); C5: alpha-Pinene (C09880); C6: (-)-Limonene(C00521); C7: cis-3-Hexenyl acetate (C19757); C8: 3-Hexen-1-ol (C08492); C9: (-)-Linalool (C11388); C10: Myrcene (C06074); C11: (2E,6E)-Farnesol (C01126); C12: beta-Caryophyllene (C09629); C13: Cembrene (C11893).

Metabolism of Terpenoids and Polyketides

Monoterpenoid biosynthesis

Starch and sucrose metabolism

Glycerophospholipid metabolism

Ascorbate and aldarate metabolism

Zeatin biosynthesis

MEP DOXP pathway

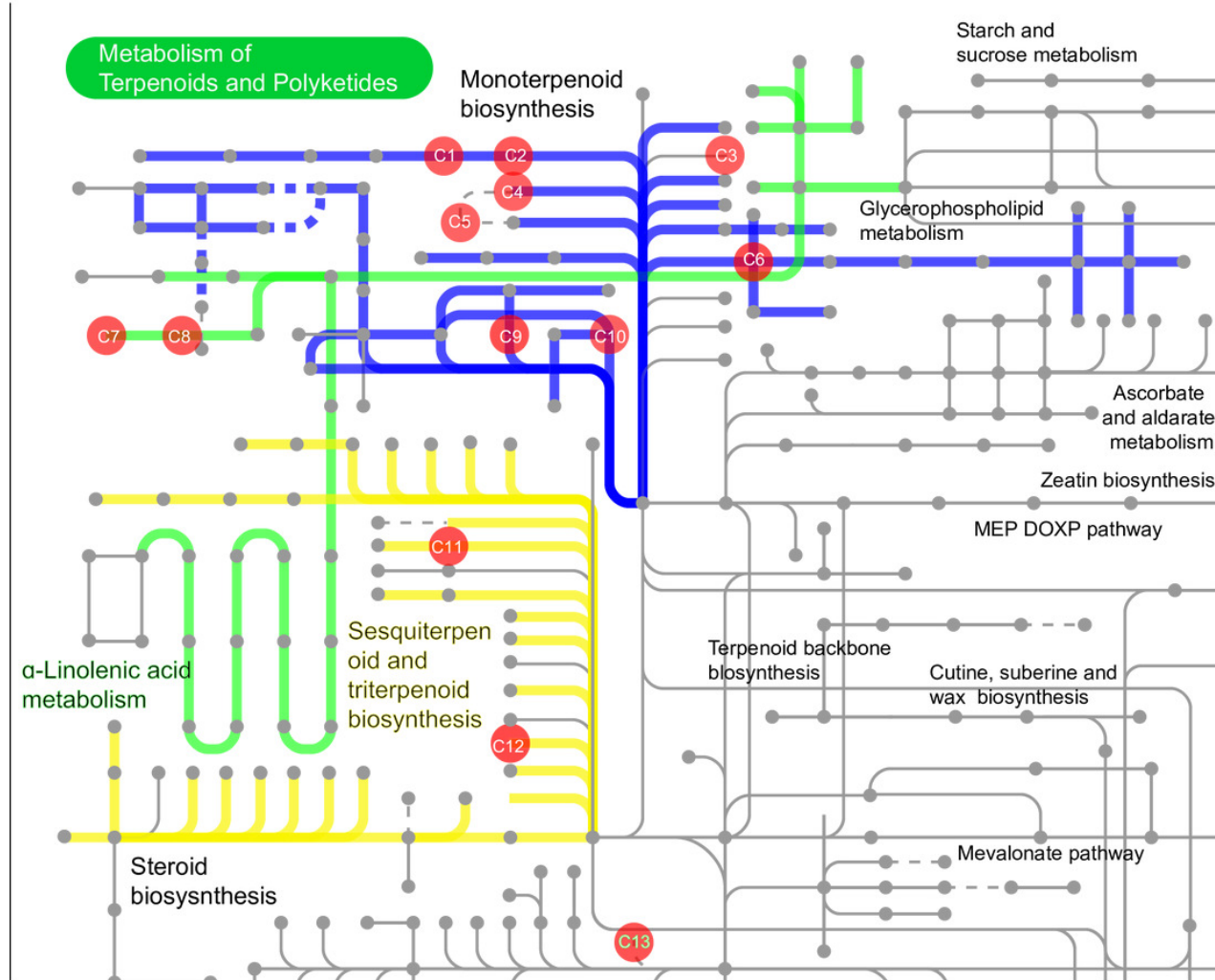
Terpenoid backbone biosynthesis

Cutine, suberine and wax biosynthesis

α-Linolenic acid metabolism

Sesquiterpenoid and triterpenoid biosynthesis

Mevalonate pathway

Steroid biosysnthesis

Metabolic pathway enrichment analysis of 17 common compounds in three parts of five mangrove specie.

Enrichment ratio(-log10(P))	Metabolite set	Total	Hits	P value	Holm correction P value	FDR	Compounds
5.9	C10 isoprenoids	242	3	1.20×10^{-6}	0.00126	0.00126	eucalyptol, isomenthol, D-Limonene
5.5	C15 isoprenoids	333	3	3.12×10^{-6}	0.00326	0.00163	α-Copaene, α-amorphene, α-cedrol
3.2	fatty alcohols	452	2	6.87×10^{-4}	0.717	0.24	3-hexen-1-ol, 3-octanol
2.4	hydrocarbons	52	1	0.00444	1	1	heptadecane, 2, 6, 10, 15-tetramethyl
1.6	diterpenoids	276	1	0.0234	1	1	hexadecane, 2, 6, 10, 14-tetramethyl
1.5	monoterpenoids	348	1	0.0294	1	1	3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)