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Allelopathic effects of exotic mangrove species *Laguncularia racemosa* on three native mangrove species in China

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ABSTRACT

To assess the allelopathic potential of exotic mangrove species *Laguncularia racemosa* (L.) Gaertn. F. (Combretaceae) on 3-native mangrove species: *Avicennia marina* (Forsk.), *Aegiceras corniculatum* (L.) Blanco and *Kandelia obovata* (L.) Druce seedlings, 5-concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 g·mL^{-1}) of root aqueous extract were studied. The results showed that high concentrations (0.5 g·mL^{-1}) of *L. racemosa* root extract significantly inhibited the seedling growth and total chlorophyll content in leaves of *A. corniculatum*. In contrast, seedling growth and chlorophyll content in leaves of the *A. marina* and *K. obovata* were stimulated at lower concentrations (0.1 and 0.2 g·mL^{-1}), but were significantly inhibited at higher concentrations (0.3, 0.4 and 0.5 g·mL^{-1}). The allelopathic effects from aqueous extract of *L. racemosa* roots followed the trend: *A. corniculatum* >*A. marina* > *K. obovata*. To identify the allelochemicals in root extract, the N-butanol extract of *L. racemosa* roots aqueous extract was analyzed by GC-MS. Total of 27 compounds were found, including 2 amines (5.95%), 1 acid (0.54%), 2 ketones (14.79%), 11 esters (29.62%), 4 alcohols (3.09%), 1 pyrazole (1.51%), 2 phenols (3.53%), 1 quinone (1.81%), 2 alkanes (1.88%), 1 benzene (1.88%). Further field experiments are needed to investigate the variability of inhibitory and stimulatory effects of *L. racemosa* root extract on native mangrove species in China.

Key words: Allelopathy, *Aegiceras corniculatum*, *Avicennia marina*, *Kandelia obovate*, GC-MS, *Laguncularia racemosa*, roots aqueous extract

INTRODUCTION

Mangroves are productive ecosystems in tropical coastal landscapes and serve as habitats for fisheries (2). However, coastal zones are being over exploited, which caused the loss of contiguous mangrove areas along the coasts. In 1956, mangrove forests were approximately 40,000-42,000 hm^2 in China. Land reclamation in 1970s and 1980s reduced this area to only 15,122 hm^2 of Chinese mangrove forest in early 1990s (6). The existing natural mangrove area in China is currently only 15,000 hm^2 (20). The overuse of natural resources in coastal China has resulted in rapid reduction in mangroves in many areas and there is urgent need for ecological restoration. The establishment of plantations and agroforestry systems are important for restoration of vegetation cover. Exotic plant species may out-compete native plant species, because they have certain characteristics (high growth rates, tolerance to various abiotic conditions), which promote their success in new

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environments (27). To improve the species diversity of mangroves in China and quality of mangroves protection project in estuarine and coastal areas. In 1999, *Laguncularia racemosa* (Figure 1) was first introduced from La Paz city, Mexico, into the Dongzhai Harbour Mangrove Natural Reserve, Hainan Province, China (17). *L. racemosa* is common specie in mangrove forests along the Pacific and Atlantic coasts (13). It is fast-growing and is forest pioneer species of mangroves; recently, it has been used for afforestation along the southeastern coast of China (32). Plantations of exotic, fast-growing, woody species decrease the atmospheric CO_2 concentrations and provide other economic benefits. Currently, *L. racemosa* monocultures are mainly on the southeastern coast of China.

Figure 1. The plantations of exotic mangrove species *Laguncularia racemosa* in Dongzhai Harbour Mangrove Natural Reserve, Hainan Province, China

The exotic fast-growing woody species may negatively impact the ecosystems. For example, the exotic species eucalyptus reduces the biodiversity (3). The exotic plant species can adversely affect the native flora and fauna by affecting the diversity and productivity of ecosystems and changing successional processes. Many problems of exotic species result from their allelopathic effects (31). Allelopathic interactions are important mediators in the establishment of native mangrove specie *L. racemosa* plantations. Allelopathy is an interference mechanism, whereby plants release chemical substances that inhibits or stimulates the growth of other plant species (30). Exotic mangrove plants can produce

allelochemicals that affect the growth of native mangrove plants. Li *et al*. (16) found that *Sonneratia apetala* produced allelopathic effects on native mangroves. Seedling establishment is crucial stage in the life of native plants and determines whether local species can grow successfully in mixed plantations (8,28). As the exotic mangrove specie *L. racemosa* may negatively impacts the growth of native mangrove seedlings, it is critical to assess the potential of native mangrove seedlings to tolerate the allelopathic effects of *L. racemosa*.

This research aimed to investigate the allelopathic potential of *L. racemosa* roots on the native mangrove species on the southeastern coast of China to develop future management practices for mangrove ecosystems and to identify the allelochemicals present in the *L. racemosa* roots aqueous extracts using GC-MS.

MATERIALS AND METHODS

Plant materials

In July 2015, two recipient species of *A. corniculatum* and *K. obovata* were randomly collected from mangrove forests of Jiulong River Estuary, Fujian Province, southeastern coast, China (117°55′E, 24°24′N, subtropical monsoon climate, mean annual temperature is 21.5 °C, Annual rainfall is 1450 mm).

In August 2015, the recipient *A. marina* seeds were randomly collected from the mangrove forests of Haicang Bay, Xiamen City, Fujian Province, southeastern coast, China (118°07' E, 24°49' N, subtropical monsoon climate, mean annual temperature is 20.9 °C and annual rainfall is 1187.4 mm).

In September 2015, the roots of fresh donor mangrove species *L. racemosa* were randomly collected from the forest of Jiulong River tributary, Fujian Province, southeastern coast, China (117°53' E, 24°20' N, subtropical monsoon climate, mean annual temperature is 21.5 °C and annual rainfall is 1450 mm).

Preparation of water extracts

Fresh *L. racemosa* roots were washed with tap water and rinsed with distilled water. Whole fresh roots were cut into small 2 cm pieces and soaked in 2 mL distilled water per one gram root tissue for 72 h at room temperature. This protocol produced fresh *L. racemosa* root aqueous extract as per Li *et al*. method (16). The root extract were filtered using double gauze to achieve a concentration of 0.5 $g \cdot mL^{-1}$ and then stored at 4 °C until further analyses. The 0.5 $g \cdot mL^{-1}$ extract were then diluted to achieve extract concentrations of 0.1, 0.2, 0.3, and 0.4 $g \cdot mL^{-1}$. Water was used as the control treatment.

Experimental design

The experiments were conducted from July 2015 to November 2015, as per the experimental design of Li *et al*. (16) with slight modifications. Relatively uniform seeds of *A. marina* and the hypocotyls of *A. corniculatum* and *K. obovata* seedlings were selected and planted in plastic pots (21 cm diameter and 17 cm depth) with 10 seeds of *A. marina* per pot, 10 hypocotyls of *A. corniculatum* per pot, and 4 *K. obovata* seedlings per pot. The pots were filled with equal proportions of sand collected from the beach near the mangrove

forests of Jiulong River, Fujian Province, southeastern coast of China. The seeds of *A. marina*, and the hypocotyls of *A. corniculatum,* and *K. obovata* seedlings were grown in laboratory corridor, the temperature and light were maintained close to natural conditions. All seedlings were supplied with sufficient water. After 15 days of seed and hypocotyl growth into seedlings, each pot was watered with 500 mL mangrove nutrient solution (14). One month after planting, the seedlings were watered with 50 mL *L. racemosa* root extract at different concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 $g \cdot mL^{-1}$), in addition to control treatments (50 mL water) was added. The treatments were replicated thrice as per Li *et al*. (16).

Plant growth measurements

At the end of experiments, three seedlings from each treatment were randomly chosen and removed from pots. The seedlings roots were then washed and residual moisture was wiped with absorbent paper. The height, root length, and fresh weight of each seedling were measured.

Chlorophyll content measurements

At the end of experiment, mature seedling leaves were chosen randomly from each pot and their chlorophyll content was measured using mixture of leaching method (15). From each treatment, 0.2 g leaves were weighed in triplicate, cut into 2 mm sections and placed in a sealed test tube. Each tube was filled with 1:1 mixture of acetone: ethanol (10 mL) and chlorophyll was extracted at room temperature, in dark conditions for 24 h. The absorbance of *L. racemosa* root extract was measured at wavelength of 645 nm and 663 nm. The chlorophyll content was determined using the Arnon method (1), which showed that chlorophyll a = $[12.71A_{663} - 2.59A_{645}] \times V/W \times 1000$, chlorophyll b = $[22.88A_{645} - 4.67A_{663}] \times V/W \times 1000$ and total chlorophyll content = $[8.04A_{663} + 20.29A_{645}] \times V/W \times 1000$.

Compound Identification

The organic solvent N-butanol of *L. racemosa* roots aqueous extract was extracted by volume ratio of V (mother liquor): V (solvent) =1: 1, and two extracts of the same solvent were combined. Then the N-butanol phase was subjected to GC-MS detection. Firstly, the sample was silanized to testing non-volatile components. Transfer 200 μL of N-butanol phase sample to a sample vial, dried with a stream of N_2, added 200 μL of pyridine and 200 μL of silylation reagent (BSTFA: TMCS=99:1), sealed with lid, and derivatized in 80 ℃ water bath for 1 h, and then dried with a stream of N_2, dissolved in 400 μL of methanol (chromatographically pure) and analyzed by GC-MS injection analysis.

GC-MS conditions: HP-5MS quartz capillary column (30 m × 0.25 mm × 0.25 μm), and the carrier gas was He (purity ≥ 99.999%). Column heating procedure: the oven temperature was held at 60 ℃ and maintained 2 min and programmed to rise to 160 ℃ at the rate of 10 $^{o}C \cdot min^{-1}$ and maintained 1 min; followed by an increase to 235 ℃ at the rate of 5 $^{o}C \cdot min^{-1}$ and maintained 1 min; and then followed by an increase to 280 ℃ at the rate of 30 $^{o}C \cdot min^{-1}$ and maintained 5 min. The injection volume was 1 μL without split injection. The injector and detector temperatures were set to 260 ℃. The ionization method was EI, the electron energy was 70 eV, and the scanning range was 30-600 amu. The NIST11 standard mass spectrometry database was searched by the Agilent Chemstation and

combined with artificial spectral analysis to determine the structure and name of N-butanol phase component. The area normalization method was used to calculate the relative content of each component.

The rate of inhibition (RI) was calculated as under:

RI= (1- Control/Treatment).

C: Control value; T: Treatment value. When RI > 0: indicates promoting effect, when RI < 0, indicates inhibiting effect. The magnitude of its absolute value represents the intensity of allelopathic effect. Finally, a comprehensive evaluation was done of the growth indicators of allelopathic effects of all receptors, the synthesis effect (SE) was also assessed as the mean of allelopathic effect index of the same treatment under the recipient root length index, plant height index, and fresh weight index (29).

Statistical analysis

The data were analysed using two-way analysis of variance (ANOVA) tests with general linear models, followed by Duncan's tests in the SPSS v.17.0 program.

RESULTS AND DISCUSSION

Seedling growth

In this study, the root length of *K. obovata* seedlings increased at lower concentrations (0.1 and 0.2 $g \cdot mL^{-1}$) of *L. racemosa* root aqueous extract, but decreased at higher concentrations (0.3, 0.4 and 0.5 $g \cdot mL^{-1}$) compared to control (Figure 2). In contrast, the root length of *A. marina* seedlings were inhibited at all aqueous extract concentrations compared to control (Figure 2). The 0.4 $g \cdot mL^{-1}$ and 0.5 $g \cdot mL^{-1}$ extract significantly inhibited the root lengths of the three native mangrove seedlings compared to control ($p<0.05$) (Figure 2). This concentration-dependent effect is consistent with previous reports that allelochemicals can promote the growth of treated seedlings at lower concentrations, while inhibit the seedlings growth at higher concentrations (33). The inhibition of root growth decreased the seedling growth (25). The roots were in direct contact with allelochemicals, and root growth of seedling might be more sensitive to their effects due to the inhibition of cell division and elongation in root apical meristems (18).

The plant height and fresh weight of *A. marina* and *K. obovata* were promoted at lower concentrations (0.1 and 0.2 $g \cdot mL^{-1}$) of *L. racemosa* root aqueous extract but inhibited at higher concentrations (0.3, 0.4 and 0.5 $g \cdot mL^{-1}$) compared to control, whereas the plant height and fresh weight of *A. corniculatum* seedlings were inhibited in all concentrations compared to control (Figure 3 and Figure 4). The allelopathic potential depends on the aqueous extract concentrations (4). The 0.3 $g \cdot mL^{-1}$, 0.4 $g \cdot mL^{-1}$and 0.5 $g \cdot mL^{-1}$ extract significantly inhibited the plant height and fresh weight of *A. marina* seedlings compared to control ($p <0.05$). The 0.4 $g \cdot mL^{-1}$ and 0.5 $g \cdot mL^{-1}$ of *L. racemosa* root aqueous extract significantly inhibited the plant height and fresh weight of *A. corniculatum* seedlings compared to control ($p <0.05$). Similar to the plant height of *A. corniculatum*, the 0.4

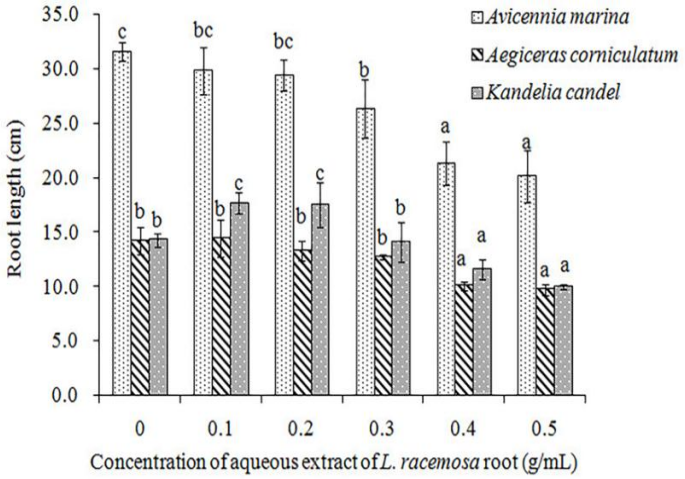

Figure 2. Effects of *L. racemosa* root aqueous extract concentrations on the root length of *A. marina*, *A. corniculatum*, and *K. obovata* seedlings. Mean ± SE values followed by the same letters in each column are not significantly different at the $p < 0.05$ level (ANOVA and Duncan's multiple range tests).

g·mL^{-1} and 0.5 g·mL^{-1} extract also significantly inhibited the plant height of *K. obovata* seedlings compared to control ($p<0.05$), while the 0.1 g·mL^{-1} extracts significantly increased the fresh weight of *K. obovata* seedlings compared to control ($p <0.05$), but the 0.5 g·mL^{-1} extract significantly decreased the fresh weight of the *K. obovata* seedlings compared to the control ($p<0.05$). The reduction in biomass might be due to stunted and reduced seedling growth (27). Reduced plant growth may be a strategy of plants in response to allelopathic stress to save energy for seedling survival (25).

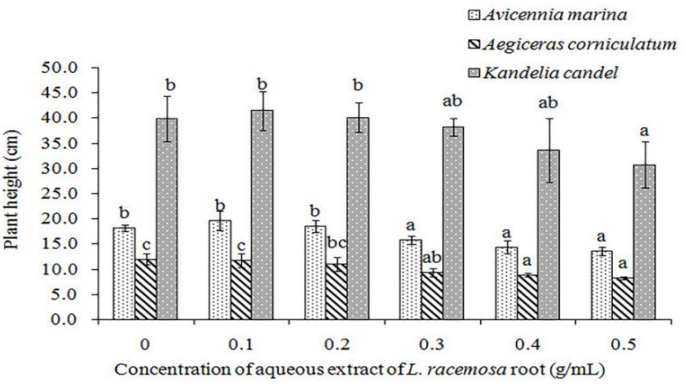

Figure 3. Effects of *L. racemosa* root aqueous extracts concentration on the plant heights of *A. marina*, *A. corniculatum* and *K. obovata* seedlings. Mean ± SE values followed by the same letters in each column are not significantly different at the $p < 0.05$ level (ANOVA and Duncan's multiple range tests).

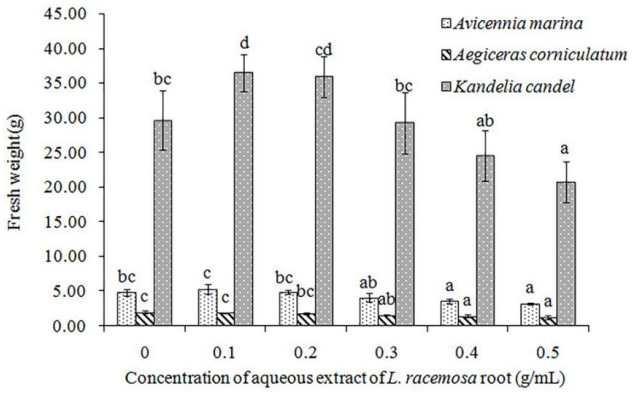

Figure 4. Effects of *L. racemosa* root aqueous extract concentration on the fresh weights of *A. marina*, *A. corniculatum*, and *K. obovata* seedlings. Mean ± SE values followed by the same letters in each column are not significantly different at the $p < 0.05$ level (ANOVA and Duncan's multiple range tests).

The aqueous extract of *L. racemosa* root had variable effects on seedling growth of the three native tested mangrove species (Table 1). The RI index indicated that root length reductions were more in *A. marina* than in *A. corniculatum* and *K. obovata*, whereas the plant height and fresh weight reductions were more in *A. corniculatum* than *A. marina* and *K. obovata*. The SE index for the aqueous extracts of *L. racemosa* roots suggested that the allelopathy followed the order *A. corniculatum* >*A. marina* > *K. obovata*. These results indicated that the inhibitory effects of *L. racemosa* root aqueous extracts were species specific. These results were similar to previous study, which showed that the allelopathic effects of aqueous extract of *Eucalyptus urophylla* were species-specific and could be inhibitory, neutral or stimulatory (19).

Chlorophyll content

The aqueous extract of *L. racemosa* root had different effects on the chlorophyll a, chlorophyll b, and total chlorophyll content in leaves of *A. marina*, *A. corniculatum* and *K. obovata* seedlings (Table 2). The total chlorophyll content of *A. marina* leaves increased significantly ($p<0.05$) by 30.8% and 51.3% at 0.1 g·mL^{-1} and 0.2 g·mL^{-1} treatments compared with control, respectively, but decreased significantly ($p<0.05$) by 15.4% and 33.9% at 0.4 g·mL^{-1}and 0.5 g·mL^{-1} treatments compared with control, respectively. The total chlorophyll content of *K. obovata* leaves increased significantly ($p<0.05$) by 52.9% at 0.1 g·mL^{-1} treatment compared with control, while decreased significantly ($p<0.05$) by 13.5% and 18.9% at 0.4 g·mL^{-1} and 0.5 g·mL^{-1} treatments compared with control, respectively. The total chlorophyll content of *A. corniculatum* leaves decreased significantly ($p<0.05$) by 42.4% and 44.1% at 0.4 g·mL^{-1} and 0.5 g·mL^{-1} treatments compared with controls, respectively. These inhibitory responses were consistent with the concentration-dependent effects that were also observed in the plant height and fresh weight of three native mangrove species.

Table1. Index of allelopathic effects of *L. racemosa* root extract concentrations on seedling growth of 3-native mangrove species.

Test plant species	Concentration ($g \cdot mL^{-1}$)	Rate of inhibition (RI)			Synthesis effect (SE)
		Root length (cm)	Plant height (cm)	Fresh weight (g)	
Avicennia marina	0.0	0.000	0.000	0.000	0.000
	0.1	−0.060	0.076	0.086	0.034
	0.2	−0.075	0.027	0.010	−0.013
	0.3	−0.202	−0.153	−0.120	−0.158
	0.4	−0.484	−0.266	−0.349	−0.366
	0.5	−0.564	−0.341	−0.498	−0.468
Aegiceras corniculatum	0.0	0.000	0.000	0.000	0.000
	0.1	0.014	−0.017	−0.031	−0.011
	0.2	−0.068	−0.082	−0.070	−0.073
	0.3	−0.118	−0.265	−0.245	−0.209
	0.4	−0.420	−0.352	−0.366	−0.379
	0.5	−0.464	−0.451	−0.511	−0.475
Kandelia obovata	0.0	0.000	0.000	0.000	0.000
	0.1	0.192	0.039	0.187	0.139
	0.2	0.183	0.007	0.174	0.121
	0.3	−0.014	−0.045	−0.013	−0.024
	0.4	−0.233	−0.188	−0.209	−0.210
	0.5	−0.430	−0.296	−0.427	−0.384

Table 2. Chlorophyll a, chlorophyll b and total chlorophyll content in leaves of three native mangrove seedlings after exposure to different concentrations of *L. racemosa* root aqueous extract.

Test plant species	Concentration ($g \cdot mL^{-1}$)	Chlorophyll a ($mg \cdot g^{-1}$)	Chlorophyll b ($mg \cdot g^{-1}$)	Total Chlorophyll ($mg \cdot g^{-1}$)
Avicennia marina	0.0	0.565±0.007c	0.197±0.000b	0.762±0.008c
	0.1	0.739±0.027d	0.258±0.017c	0.997±0.044d
	0.2	0.833±0.008e	0.319±0.025d	1.153±0.032e
	0.3	0.493±0.049b	0.179±0.063ab	0.673±0.059b
	0.4	0.493±0.002b	0.151±0.004ab	0.645±0.002b
	0.5	0.378±0.021a	0.125±0.006a	0.504±0.015a
Aegiceras corniculatum	0.0	0.979±0.020f	0.377±0.006d	1.357±0.027e
	0.1	0.712±0.012e	0.230±0.006c	0.943±0.019d
	0.2	0.676±0.014d	0.215±0.010bc	0.891±0.024c
	0.3	0.648±0.007c	0.193±0.005ab	0.842±0.013b
	0.4	0.594±0.013b	0.188±0.008a	0.782±0.006a
	0.5	0.570±0.003a	0.188±0.023a	0.758±0.027a
Kandelia obovata	0.0	0.548±0.007c	0.276±0.005b	0.824±0.013c
	0.1	0.828±0.021d	0.431±0.016c	1.260±0.037d
	0.2	0.564±0.007c	0.279±0.007b	0.843±0.015c
	0.3	0.496±0.032b	0.258±0.021b	0.754±0.054b
	0.4	0.483±0.020b	0.229±0.012a	0.713±0.032ab
	0.5	0.442±0.013a	0.225±0.009a	0.668±0.023a

[a] Different letters within a column represent significant differences at the $p < 0.05$ level (ANOVA and Duncan's multiple range tests).

In this study，the 0.1 $g \cdot mL^{-1}$ extract significantly increased the total chlorophyll content of *A. marina* leaves and *K. obovata* leaves compared with control ($p<0.05$), but the 0.3 $g \cdot mL^{-1}$, 0.4 $g \cdot mL^{-1}$ and 0.5 $g \cdot mL^{-1}$ extract significantly decreased the total chlorophyll content of *A. marina* leaves and *K. obovata* leaves compared with control ($p<0.05$). In contrast, the chlorophyll a, chlorophyll b and total chlorophyll content in the leaves of *A. corniculatum* seedlings were inhibited at all aqueous extract concentrations compared with control. The allelopathic effects of *L. racemosa* root aqueous extract on the chlorophyll content were in accordance with observations on other plants. For example, concentrations of chlorophyll a, chlorophyll b, and chlorophyll a + b from *Eucalyptus grandis* seedlings decreased with increased exposure to aqueous extract from *Cinnamomum septentrionale* leaf litter (10). In this study, the chlorophyll a, chlorophyll b, and total chlorophyll content of three native mangrove seedlings decreased with increasing concentrations of aqueous extract of *L. racemosa* root, which illustrated that the synthesis of photosynthetic pigments were hindered by the root extract. In allelopathic study a reduction in chlorophyll content lead to lower photosynthetic efficiency (23).

Regenerating the native mangrove species plantations within exotic *L. racemosa* mangrove plantations improves the estuarine coastal mangrove protection and maximizes the mangrove ecosystems benefits. Native tree species promote biodiversity, maintains ecological balance and preserve the forest ecosystem (22). Even though the inhibitory effects of *L. racemosa* on some native mangrove species might lessen these beneficial properties. This study also indicated that the exotic mangrove species *L. racemosa* might also promote these ecological functionalities through stimulatory interactions. Therefore, we suggest that optimal mixed plantations of *L. racemosa* and *K. obovata* can be established by transplanting seedlings of *K. obovata* into *L. racemosa* plantations through shoal afforestation along the southeastern coast of China. Because of fast-growing characterstics the exotic mangrove species *L. racemosa* may cause invasion in China, so planting the exotic species in mangrove forest restoration must be very careful.

Identification of the isolated compounds

Twenty-seven compounds were identified in the N-butanol extract of *L. racemosa* roots aqueous extract (Table 3), which primarily included 2 amines (5.95%), 1 acid (0.54%), 2 ketones (14.79%), 11 esters (29.62%), 4 alcohols (3.09%), 1 pyrazole (1.51%), 2 phenols (3.53%), 1quinone (1.81%), 2 alkanes (1.88%), 1 benzene (1.88%). Among them, 2-Pyrrolidinone, 1-methyl- was a major component, with highest relative content of 11.87%, while the lowest relative content of phthalic acid, butyl undec-2-en-1-yl ester was only 0.50%.

The major route release of allelochemicals in the surrounding soil is through root exudation (12). Allelochemicals have usually been considered to be secondary metabolites or waste products of the main metabolic pathways in plants (26). Plant allelochemicals have variety of components. In this study, these isolated and identified compounds, included the amines, acid, ketones, esters, alcohols, pyrazole, phenols, quinine, alkanes and benzene

(Table 3) are secondary metabolites of *L. racemosa* roots aqueous extract. According to the study of Huang *et al*. (11), the primary allelochemicals, included alkanes and ketones, derived from *Cinnamomum septentrionale* leaf litters. Phenols are algicidal to aquatic organisms (24) and low molecular weight phenols are phytotoxic to seed germination and seedling growth of radish and wheat (7). The compounds of *L. racemosa* roots aqueous extract inhibited the plant growth and affected the chlorophyll content of *A. corniculatum*, *K. obovata* and *A. marina* seedlings. These results are similar to the previous study of De Jesus Jatoba *et al*. (5), which showed that the compounds derived from the plant species *Pteridium arachnoideum* inhibited the root and stem growth and affected chlorophyll content of *Sesamum indicum*.

Table 3. Compounds identified in the *L. racemosa* root aqueous extract in N-butanol phase by GC-MS

	Name of the chemical	Retention time (min)	Relative content (%)	Similarity (%)
1	2-Propanamine, N-ethyl-	6.171	4.88	80
2	Acetic acid, trifluoro-	6.346	0.54	50
3	2-Pyrrolidinone, 1-methyl-	6.618	11.87	91
4	4-Pentenoic acid, methyl ester	7.277	1.25	43
5	Triethyl phosphate	7.853	1.29	95
6	2,9-Dimethyl-trans-decahydroquinol-4-ol	8.494	0.70	50
7	1,2-Benzisothiazol-3-amine tbdms	10.033	1.07	52
8	Glycerol, tris(trimethylsilyl) ether	10.117	0.80	74
9	4,5-Dihydro-1-phenyl-1H-furo(2,3-c)pyrazole	10.602	1.51	47
10	1,4-Benzenedicarboxylic acid, dimethyl ester	13.119	0.59	87
11	Phenol, 2,4-bis(1,1-dimethylethyl)-	13.254	0.83	76
12	1,4-Naphthoquinone, 6-acetyl-2,5-dihydroxy-	14.464	1.81	40
13	Hexadecane	14.645	1.18	64
14	8-Dodecen-1-ol, acetate, (Z)-	14.697	5.38	91
15	Hentriacontane	16.379	0.70	86
16	1-Cyclohexyl-2-methyl-prop-2-en-1-one	18.442	2.92	56
17	Phthalic acid, decyl isobutyl ester	19.497	0.99	72
18	Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	20.014	0.68	50
19	Pentadecanoic acid, 14-methyl-, methyl ester	20.499	8.26	98
20	Phthalic acid, butyl undec-2-en-1-yl ester	21.204	0.50	50
21	1-Diphenyl(tert-butyl)silyloxy-4-methoxybenzene	21.405	1.88	47
22	9,12-Octadecadienoic acid, methyl ester	23.533	2.07	99
23	9-Octadecenoic acid, methyl ester, (E)-	23.637	3.21	99
24	Heptadecanoic acid, 16-methyl-, methyl ester	24.096	2.23	98
25	1,3-Benzothiazol-6-ol, 2-(dimethylamino)-2,3-dihydro-	25.513	0.91	50
26	Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	29.057	2.70	97
27	Silicic acid, diethyl bis(trimethylsilyl) ester	29.950	3.85	43

In natural environments, the impact of allelopathic chemicals on native plant species can be lessened by biotic and abiotic interactions with other environmental factors in addition to the competition for growth resources (space, soil, water, light and essential

nutrients) in plants. The laboratory experiments are important to determine the potential effects of allelopathic chemicals on plants in natural estuarine environments (9). For example, Qiu *et al*. (21) found that allelochemical concentrations from *Eucalyptus urophylla* were lower under natural conditions than in laboratory experiments. Under natural conditions, *L. racemosa* root extract were hard to reach the high concentrations in laboratory due to the ocean tidal flow and the substantial dilution of water, thereby decreasing the inhibitory effects of *L. racemosa* root extract. Thus, while this study showed the inhibitory effects of *L. racemosa* on native mangrove species physiological properties, these results may not translate directly to what would be observed in the natural environment. To effectively manage the exotic mangrove species *L. racemosa* and improve the mangrove forests ecosystem along the southeastern coast of China, understanding of the allelopathic capabilities of *L. racemosa* under a range of environmental factors in the field and the influence of resource competition on these effects are needed. Thus, these results should lead to further field study investigating the variability in inhibitory and stimulatory effects of *L. racemosa* extract on native mangrove species in China.

CONCLUSIONS

The exotic mangrove species *L. racemosa* root aqueous extracts were inhibitory to three native mangrove species in China. Lower concentrations of *L. racemosa* root aqueous extract increased the seedling growth of *K. obovata* and *A. marina*, but higher concentrations decreased relative to control. The allelopathic potential of *L. racemosa* root aqueous extracts followed the order: *A. corniculatum* > *A. marina* > *K. obovata*. A total of 27 compounds of N-butanol extract of *L. racemosa* root aqueous extract were found, including amines, acid, ketones, esters, alcohols, pyrazole, phenols, quinine, alkanes and benzene. Most of the allelochemicals of *L. racemosa* might inhibit the growth of other plants. Further experiments involving field conditions are necessary to investigate the variability of inhibitory and stimulatory effects of *L. racemosa* root extracts on the native mangrove species in China.

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