The potent allelopathic substances of cogongrass rhizome extracts

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Cogongrass (*Imperata cylindrica* (L.) Raeusch.) is ranked as one of the "100 of the world's worst invasive alien species" reported by the International Union for Conservation of Nature in 2000. This plant invades habitats of other plant species and forms monotypic expanses called the mega-grasslands. In order to clarify the mechanism of the successful invasion of cogongrass, allelopathy of cogongrass has been studied. Several secondary metabolites in leachate and extracts of cogongrass rhizomes were identified as putative allelopathic substances (Donald *et al*. 2013). However, available information about potent allelopathic substances of cogongrass rhizomes is limited. Thus, the objective of this study is isolation and identification of potent growth inhibitory substances of cogongrass rhizome extracts.

METHODOLOGY

The rhizomes of cogongrass were cut into small pieces and extracted with 70% aqueous methanol and filtered. The residues were extracted again with cold methanol and filtered. The two filtrates were combined and evaporated with a rotary evaporator at 40ºC. Aliquots of the extracts were dissolved into methanol and added to filter paper in Petri dish. Then, methanol was evaporated in a fume hood. The filter paper was moistened with 0.05% aqueous solution of Tween 20. The final assay concentrations of the extracts were ranged from 1 to 1000 mg dry weight equivalent extract/mL. These concentrations mean that the extracts obtained from 1 to 1000 mg dry cogongrass rhizomes were contained in 1 mL of the assay solutions. Cress (*Lepidium sativum* L.) seeds were arranged on the Petri dishes and incubated in the dark at 25ºC for 48 hours. Control seeds were incubated under the same condition described above without the extracts. After incubation, hypocotyl and root length of the cress were measured and compared to control. The extracts were then partitioned with water and ethyl acetate. The ethyl acetate fraction was separated by silica gel column. The biological activity of the fractions was determined using a cress bioassay. The growth inhibitory activities were found in two fractions obtained by elution with 70% ethyl acetate in *n*-hexane and methanol.

Purification of the inhibitory substance 1: The growth inhibitory active fraction eluted with 70% ethyl acetate in *n*-hexane was separated by Sephadex LH-20 column, reverse phase C_{18} cartridge and HPLC. The inhibitory activities of all fractions were determined by the cress bioassays after every separation steps. The most active fraction in each step was subjected to a subsequent separation step. The growth inhibitory substance1 was finally purified by HPLC and characterized by ^{1}H-NMR.

Purification of the inhibitory substance 2: The growth inhibitory active fraction eluted with methanol from silica gel column was separated by the same method described above. The inhibitory substance 2 was finally purified by HPLC and characterized by ^{1}H-NMR.The growth inhibitory activitiesof the inhibitory substance 1 and 2 were determined by the cress bioassays. Then, the0concentrations required for 50% growth inhibition were determined by a logistic regression analysis.0

RESULTS

At the concentration of 100 mg dry weight equivalent extract/mL, an aqueous methanol extract of cogongrass rhizomes inhibited the hypocotyl and root growth of cress by 19.1 and 36.6%, respectively. The growth inhibitory activity of the extract was concentration dependent.

Two growth inhibitory substances were isolated from the aqueous methanol extracts of cogongrass rhizomes. The inhibitory substance1 was characterized as abscisic acid. Abscisic acid inhibited the hypocotyl and root growth of cress by 12.7 and 40.3%, respectively, at the concentration of 1.0 ìM. The growth inhibitory activity of abscisic acid was concentration dependent. The concentrations of abscisic acid required for 50% growth inhibition on cress hypocotyls and roots were 0.30 and 0.52 ìM, respectively.

The inhibitory substance 2 was characterized as methyl caffeate. Methyl caffeate inhibited the hypocotyl and root growth of cress by 46.4 and 76.3%, respectively, at the concentration of 1.0 mM. The growth inhibitory activity of methyl caffeate was concentration dependent. The concentrations of methyl caffeate required for 50% growth inhibition on cress hypocotyls and roots were 0.97 and 1.3 mM, respectively.

CONCLUSION

Two potent inhibitory substances, abscisic acid and methyl caffeate, were isolated from the extracts of cogongrass rhizomes. The concentrations of abscisic acid and methyl caffeate required for 50% growth inhibition were ranged from0.30 - 0.52ìM and 0.97 - 1.3 mM, respectively. Those substances may contribute to the allelopathic property of cogongrass rhizomes.

REFERENCES

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