Phenolic acids from Imperata cylindrica (L.) Raeusch. var. Major (Nees) c.e. Hubb.

NIKODEMUS T.W. *, PADMAWINATA K. **, SOETARNO S. **
* Dept. of Pharmacy, Faculty of Science, Padjadjaran University, Bandung, Indonesia
** Dept.of Pharmacy, Faculty of Science, Bandung Institute of Technology, Bondung, Indonesia

INTRODUCTION

Alang-alang rhizomes, *Imperata cylindrica* (L.) Raeusch. var. *major* (Nees) C.E. Hubb., is widely used traditionally as a diuretic, antipyretic, anti-inflammatory, and haemostatic agent[1,2,3,4].

Alang-alang is know by various local names *e.g. eurih* (West Java), *langalang*, *alang-alang*, *kambengan* (Central and East Jabva), *kebut*, *lalang* (Madura)[1,2].

Imperata cylindrica var. *major* is an annual grass with long creeping, branched, scaly rhizomes; stem of vertical, simple (flowering) shoots not elongating and remaining underground until shortly before anthesis; basal leaves vigorous, with coriaceous sheaths, blades erect, linear-lanceolate, gradually attenuate downwards, upwards gradually tapering into acute, usually hard and sharp point, inflorescence a silky panicle, fruit caryopsis[5,6].

For man, the *Poaceal Gramineæ* is the one of the most important family in the plant kingdom because it is a source for food, building material etc., so that most of the chemical investigations are directed to compounds which are important economically *e.g.* carbohydrates, proteins, vitamins, volatile oils, while the secondary metabolites are still relatively unknown[7].

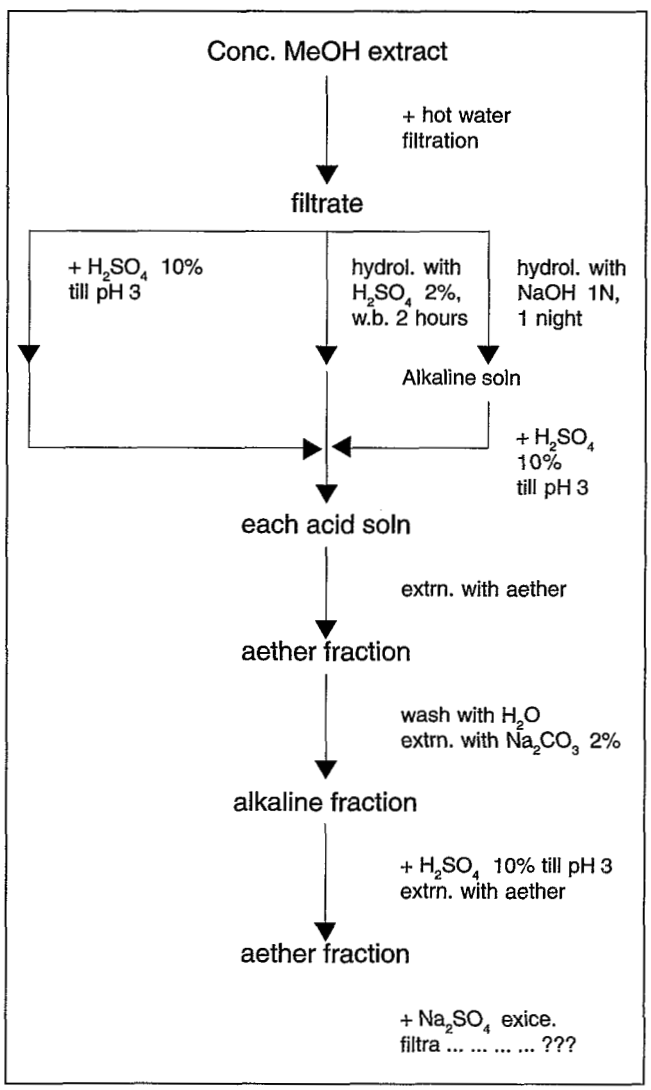
To support the governmental program in promoting the use of traditional drugs, an investigation on the chemical contents of *alang-alang* rhizomes, especially an analysis of the phenolic acids, was carried out.

MATERIALS AND METHODS

The methanol extract of *alang-alang* rhizomes was obtained by soxhletation, evaporated under pressure at a temperature below 60 °C using a rotary evaporator.

Free phenolic acids, phenolic acids as glycosides and esters were investigated. The isolation schema of the phenolic acids can be seen in Fig. 1.

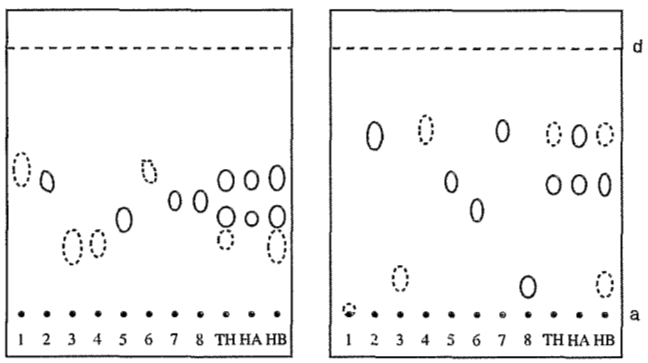
Fig. 1
Isolation scheme of phenolic acids



Identification of the phenolic acids is done by paper chromatography on Whatman 1 paper, with HOAc-water 2% and benzene-HOAc-water (60 : 22 : 1, 2) as eluents, together with the reference standards.

For detection of the acids, the papers are sprayed with a diazo-paranitroaniline solution, followed by a 15% sodium carbonate solution[8] (Fig. 2).

Fig. 2
Paper chromatography of phenolic acids on Whatman 1



A: Eluent 1% – B: HOAc + water 2%

Analysis of the phenolic acid components is done directly using the methanol extract and after acid and alkaline hydrolysis of the methanol extract, using two-dimensional paper chromatography, with the same eluents mentioned before (Fig. 3, 4, 5).

Isolation of the various phenolic acids is done with preparative paper chromatography on Whatman 3 paper.

Identification of each phenolic acid is confirmed by ultraviolet spectral measurements and comparing with the ultraviolet spectra of the reference standards (Fig. 1.).

RESULTS AND DISCUSSION

According to the results of the two-dimensional paper chromatography together with the reference standards, from the position, the fluorescence under UV-light and the colour of the spots after spraying, it can be concluded that:

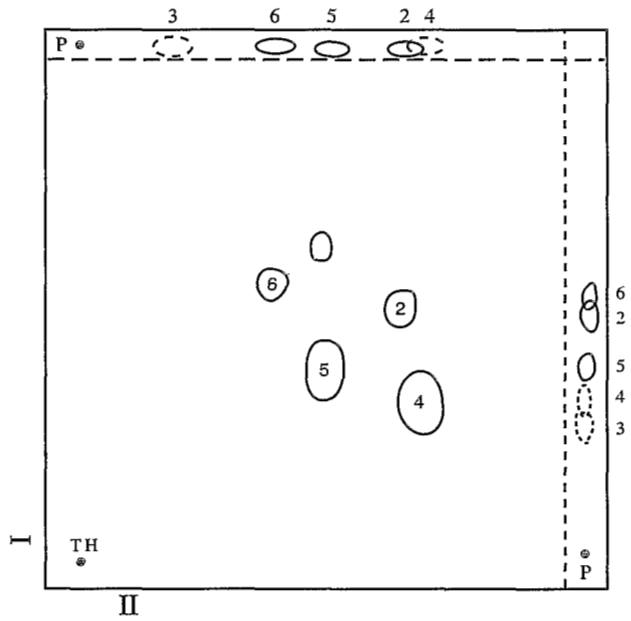
– the methanol extract (before hydrolysis) contains vanillic-, ferulic-, p-coumaric-, and p-hydroxybenzoic acids.

– the methanol extract after acid hydrolysis contains vanillic-, p-coumaric-, and p-hydroxybenzoic acids.

– the methanol extract after alkaline hydrolysis contains vanillic-, p-coumaric-, p-hydroxybenzoic and caffeic acids.

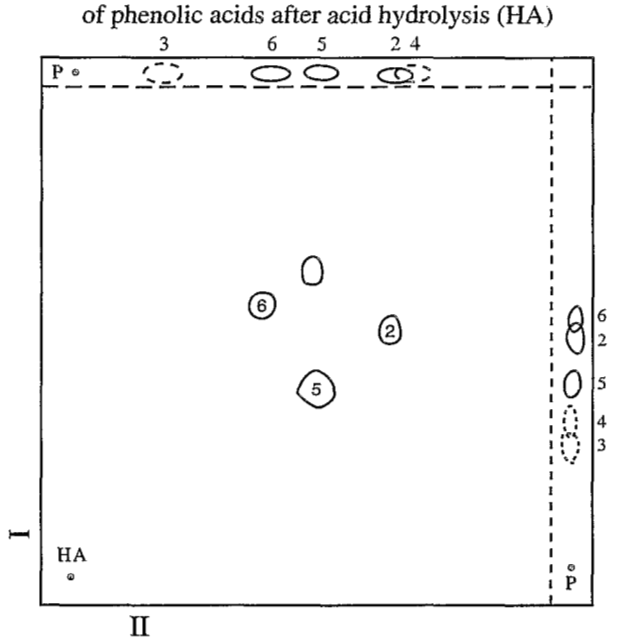
The ultraviolet spectra of vanillic-, ferulic-, p-coumaric, and caffeic acids show differences with the ultraviolet spectra of the reference standards, because the isolated phenolic acids,

Fig. 3
Two-dimensional paper chromatography of free phenolic acids (TH)



I.: Eluent HOAc-water 2% – II.: Eluent benzene-HOAc-water (60:22:1,2)
Relative position and colour of spot 2 (violet), 4 (blue-brown, blue fluor.), 5 (blue-brown), and 6 (rose) corresponds with vanillic-, ferulic-, p-coumaric, and p-hydroxybenzoic acid.

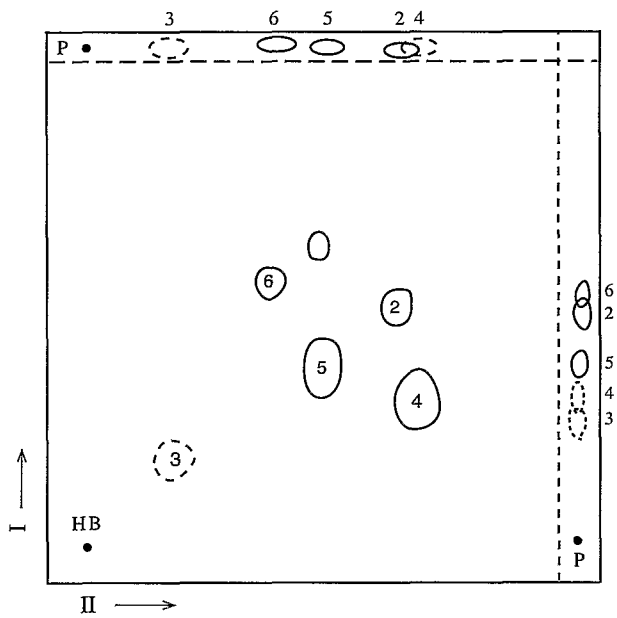
Fig. 4
Two-dimensional paper chromatography of phenolic acids after acid hydrolysis (HA)



I.: Eluent HOAc-water 2% – II.: Eluent benzene-HOAc-water (60:22:1,2)
Relative position and colour of spot 2 (violet), 5 (blue-brown), and 6 (rose) corresponds with vanillic-, p-coumaric, and p-hydroxybenzoic acid.

especially ferulic-, p-coumaric, and caffeic acids, are always obtained as mixtures of *cis*- and *trans*-isomers, while the isolated vanillic acid is probably still mixed with the *cis*-isomer of ferulic acid[9], p-Hydroxybenzoic acid cannot be obtained in the pure form because it is always mixed with the *cis*-isomer of p-coumaric acid, and due to its very small amount no ultraviolet spectrum could be made.

Fig. 5
Two-dimensional paper chromatography of phenolic acids after alkaline-hydrolisis (HB)



I.: Eluent HOAc-water 2% – II.: Eluent benzene-HOAc-water (60:22:1,2) Relative position and colour of spot 2 (violet), " (brown, blue fluor.), 4 (blue-brown, blue fluor.), 5 (blue-brown), and 6 (rose) corresponds with vanillic-, caffeic-, ferulic-, p-coumaric, and p-hydroxybenzoic acid.

CONCLUSION

From the results it can be concluded that *alang-alang* rhizomes contain vanillic-, ferulic-, p-coumaric-, and p-hydroxybenzoic acids in the free form, and caffeic acid in the ester form.

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