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Original article

Thin Layer Chromatography Screening and Profiling of Terrestrial Aroids (Araceae) Lipophilic Extracts from Saiyok Forest, Thailand

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ABSTRACT

Terrestrial aroids belong to the family Araceae and are abundant in tropical rainforest, especially in Southeast Asia. Little data have been available on the chemical characteristics of these plants in Thailand. We determined the chemical profiles of lipophilic extracts from aroid plant species, all of which are distributed in natural habitats in Saiyok forest. Thin layer chromatography (TLC) profiling and preliminary phytochemical investigations were carried out on modified stem extractions of 11 species of terrestrial aroids in seven genera. Detection using different specific reagents showed the presence of diverse groups, chemical characteristics and relative front values in methanol extract. The lipophilic extracts from the aroid plants revealed terpenoids, phenolic compounds and alkaloids. TLC showed similarities in the chemical profiles of the modified stem extracts and this study is the first known published report of TLC profiles of secondary metabolites from aroids. In conclusion, our findings scientifically described the chemical profiles. We also revealed comparable profiles from the 11 species of aroids. Thus, these findings support the chemical characters and suggest that it might be possible to utilize this information in taxonomical work and further product development of these medicinal plants in ethnobotany.

Keywords: Terrestrial Aroids, Araceae, chemical character, TLC profile

INTRODUCTION

Terrestrial aroids are rhizomatous herbs in the family Araceae. In Thailand, they can be edible native plants, ornamental plants or medicinal plants, where they are commonly known as “Buke Bon”. They are widely distributed in the tropical zone, especially in tropical rain forest (Boyce *et al*., 2012). In Thailand, they can be found throughout many regions, especially in wet areas, among

deciduous and evergreen forest and along streams and ditches. Botanical characteristics of the family are highly diverse in life forms, leaf morphology and inflorescence character. The life forms range from submerged or free-floating aquatics to terrestrial and epiphytic or hemi-epiphytic plants or climbers. Leaves range from simple and entire to compound and highly divided, and may be basal or produced from an aerial stem. The family is best characterized by its distinctive inflorescence a spadix with bisexual or unisexual flowers (sometimes with sterile region) and subtended by a solitary spathe on a long or very short peduncle (Huntington, 2014). The value of terrestrial aroids is not limited to ornamental plants. In South East Asia, various aroids can potentially be cultivated for food and medicinal uses. Growers have selected numerous varieties over the centuries, together with different methods of cultivating them. Some species also have potential in medicine and ritualistic purposes (Sangnin and Sookchaloem, 2008). A previous phytochemical investigation in the rhizomes of terrestrial aroids reported the isolation of β sitosterol acetate and stigmasterol compounds i.e. flavones, *C*-glycosides, and proanthocyanidins, which are chemicals that are characteristics of the subfamily Lasioideae (Dinda *et al*., 2004) and flavonol 3'-methyl quercetin-3-*o*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside,which can be used as chemotaxonomic markers of the genus *Lasia* from *Lasia spinosa* (L.) Thw. (Williams *et al*., 1981). Other alkaloids groups and terpenoids have been reported in *Aglaonema treubii* Engl., *Amorphophallus paeoniifolius* (Dennst) Nicolson, *Arisaema decipiens* Schott., *Arisaema erubescens* (Wall.) Schott., *Typhonium flagelliforme* (Lodd.)Blume and *Typhonium trilobatum* (L.) Schott (Asano *et al.,*1997; Zhao *et al.,*2010; Jeelani *et al.*, 2010; Kandhasamy and Arunachalam, 2008).

Moreover, isolation of phenolic compounds has been reported from *Alocasia indica* (Roxb.) Schott, *Alocasia maccorhiza* (L.) Schott. and *Amorphophallus paeoniifolius* (Dennst) Nicolson. (Wang and TB, 2003; Champagne *et al*., 2011; Chan and Kao-Jao, 1997) Thin layer chromatography (TLC) has been successfully integrated into the analysis of chemical compounds with high mass, such as lipids and saccharides (Nimptsch *et al.,*2010). In addition, TLC is the preferred method of rapid separation needed at low cost and can be used in parallel with highly sensitive instrumental analysis (Kanai *et al.,* 2008). Natural products, such as plant extracts, either as pure compounds or standardized extracts, provide extensive opportunities for drug discoveries because of the chemical diversity in plants. Therefore, the present study aimed to select a suitable mobile phase system for the separation analysis of modified stem extracts of terrestrial aroids. We compared the extracts of 11 species of terrestrial aroids in the most appropriate solvent system for TLC profiling analysis.

MATERIALS AND METHODS

Plant materials

In May-October 2012, modified stems of terrestrial aroids were collected from Saiyok National Park, Kanchanaburi province, Thailand. Each voucher specimen of terrestrial aroid was deposited at Kasetsart University, Faculty of Forestry.

Preparation of plant extracts

The fresh modified stems of each terrestrial aroid (500 g for each species) were separated, cleaned and chopped into small pieces, and then powdered using an electronic mill. The powder was macerated with methanol (CH_3OH) for 7 days in darkness at room temperature.

Extractions

After 7 days, the extracts were filtered through filter paper (Whatman No.1), and then subsequently concentrated by rotary evaporator at 37-39^{O}C, with the crude extract appearing semi-solid. The concentrated crude extract was partitioned into two parts hydrophilic extract (distilled water) and lipophilic extract (chloroform).The lipophilic extract was stored at -75^{O}C and analysis of all extracts followed Laungsuwon and Chulalaksananukul (2013) using TLC on silica gel 60 F_{254} aluminum support plates, and then detection using UV irradiation (254 and 365 nm).

Phytochemical analysis by thin layer chromatography

Phytochemical analysis of the lipophilic extracts of terrestrial aroids was performed on TLC pre-coated silica gel 60 GF_{254} plates (20×20 cm; Merck) using a solvent system, hexane:ethyl acetate (9:1, v/v). The TLC plate start lines were drawn using a carbon pencil at 1.5 cm and the relative front (R_f) values were calculated to be 15 cm. With capillary, at 30 mg/ml in each extract, a suitable concentration from a trial-and-error method was found to be 20 drops/spot on the base line. Then, the extract was developed in a solvent system in a chromatographic chamber that was pre-saturated in the mobile phase. Care was taken to see that the level of solvent was slightly below the level of the spots. After development, the TLC plate was air-dried and exposed to UV light for the detection of fluorescence compounds and for Rf values to be determined.

Thin layer chromatography screening

The TLC plates were sprayed with detecting reagent for screening major secondary metabolites using different reagents consisting of anisaldehyde-sulfuric acid reagent followed by heating on a TLC hot plate at 110^{O}C for 10 minutes for terpenoids detection and iodine fuming for organic compounds detection (Merck, 1980), vanilline sulfuric acid reagent followed by heating on a TLC hot plate at 110^{O}C for 10 minutes for phenolic compounds detection, 10% NaOH (10% (w/v) aqueous solution; Ricca Chemical Company, USA) for coumarin detection, Dragendorff's reagent for alkaloids detection, Kedde's reagent and Raymond's reagent for unsaturated lactones ring detection (Farnsworth,1966), and UV light for fluorescence compounds detection.

RESULTS AND DISCUSSION

Qualitative analyses of thin layer chromatography screening

The phytochemical screening for major secondary metabolites can be investigated by color detection after spraying TLC plates with different reagents. The results of the qualitative tests performed on lipophilic extracts from rhizomes are shown in Table 1. The results on the TLC plates appeared as violet and grey-green bands for detection of terpenoids using anisaldehyde-sulfuric acid as the spraying reagent. The dark violet bands were used for

detection of phenolic compounds using vanillin sulfuric acid spraying reagent. The yellow-green fluorescent band, under long wavelength (365 nm) UV light, was used for detection of coumarin after being sprayed with 10% NaOH in ethanol. The orange band was used for detection of alkaloids after spraying with Dragendorff's reagent and the orange-brown bands show up a light brown background for detection of organic compounds iodine vapor in a tank. However, compounds like alcohols, acids and halides can give a negative stain (white bands on a light brown background) initially (Merck, 1980; Kurian and Sankar, 2007). The R_f values of the lipophilic extracts from the rhizomes in the current study are shown in Table 2 and the TLC profile is shown in Figure 1.

Table 1 Results of qualitative tests performed on lipophilic extracts from rhizomes of terrestrial aroids.

Botanical name	Organic compounds	Alkaloids	Coumarin	Phenolic compounds	Terpenoids	Unsaturated lactone ring
Aglaonema simplex	+	+	+	+	+	-
Alocasia acuminata	+	-	+	+	+	-
Alocasia hypnosa	+	-	-	+	+	-
Amorphophallus maxwellii	+	+	+	+	+	-
Amorphophallus muelleri	+	+	-	+	+	-
Amorphophallus paeoniifolius	+	-	-	+	+	-
Arisaema maxwellii	+	+	+	+	+	-
Colocasia esculenta	+	-	-	+	+	-
Colocasia gigantea	+	+	+	+	+	-
Lasia spinosa	+	+	+	+	+	-
Typhonium trilobatum	+	+	+	+	+	-

Note: (+) indicates positive test of phytochemical, (−) indicate absence of phytochemical

Information on qualitative phytochemical screening for the presence of secondary metabolites in modified stem extract of terrestrial aroids (Araceae) can be investigated by color detection after spraying the TLC plates with different reagents. A preliminary separation of the investigated extract was possible by using TLC with a suitable mobile phase. Lukasz and Monika (2009) used TLC for ensuring the identity, purity and quality of botanical materials, then focused on chemotaxonomy work for analysis of plant extracts.

In this study, we used a single solvent system, hexane:ethyl acetate (9:1 v/v), as a suitable mobile phase based on the trial-and-error method. Then we sprayed with detecting reagent for screening major secondary metabolites using different reagents, and observed the presence or absence of secondary metabolites on the TLC plate. After that, R_f values were calculated and chemical characteristics were compared for each extract from the bands present (positive

test) in the single solvent system. Besides, brought the TLC platesput in tank together with crystal iodine for organic compounds detection.. The iodine vapor is more quickly generated by gently warming the tank. Many organic compounds showed orange to brown spots on the TLC plate (Merk, 1980), as shown in Figure 1 (c). The secondary metabolites present included alkaloids, coumarin, phenolic compounds, and terpenoids. The results are shown in Table 1.

Table 2 Relative front (R_f) values of lipophilic extracts in single solvent system.

Botanical name	R_f values			
	Terpenoids	Coumarin	Phenolic compounds	Alkaloids
Aglaonema simplex	0.03, 0.10, 0.30, 0.40, 0.55, 0.91	0.56	0.08, 0.20, 0.33, 0.43, 0.95	base line
Alocasia acuminata	0.30, 0.40, 0.91		0.20, 0.33, 0.95	base line
Alocasia hypnosa	0.30, 0.40, 0.91	0.56	0.20, 0.33, 0.95	-
Amorphophallus maxwellii	0.30, 0.40, 0.53, 0.91	- 0.56	0.20, 0.33, 0.95	-
Amorphophallus muelleri	0.30, 0.40, 0.43, 0.91	-	0.20, 0.33, 0.95	base line
Amorphophallus paeoniifolius	0.40		0.20, 0.33, 0.95	-
Arisaema maxwellii	0.30, 0.91	-	0.20, 0.33, 0.95	base line
Colocasia esculenta	0.30, 0.40, 0.91	0.56	0.20, 0.33, 0.95	-
Colocasia gigantea	0.30, 0.40, 0.91	-	0.20, 0.33, 0.95	base line
Lasia spinosa	0.03, 0.10, 0.30, 0.40, 0.91	0.87 0.56	0.08, 0.20, 0.33, 0.95	base line
Typhonium trilobatum	0.03, 0.10, 0.30, 0.40, 0.67, 0.91	0.57	0.20, 0.33, 0.50, 0.95	base line

The relative front (R_f) values of lipophilic extracts using different reagents present as follows. When the TLC plate is sprayed with anisaldehyde-sulfuric acid reagent for terpenoids detection, and after heating the TLC plate at 110^{O}C until maximal visualization of spots, the color ranged from violet (terpenes), blue (sugar) and red (steroids) to grey-green (phenol) (Merck, 1980).

The results agreed with the report of terpenoids and showed that all lipophilic extracts produced a color change to violet at R_f values of 0.03, 0.10, 0.30, 0.40, 0.43, 0.53, 0.55, 0.67 and 0.91, as shown in Figure 1 (d).

For coumarin detection, the TLC plate was sprayed with 10% NaOH and exposed to UV light at a long wavelength (365 nm). The results showed that adventitious root extracts of *Aglaonema simplex, Alocasia acuminata, Amorphophallus maxwellii, Arisaema maxwellii, Colocasia gigantea, Lasia spinosa* and *Typhonium trilobatum* produced green fluorescence. The reagent detected coumarin at R_f values 0.56, 0.57 and 0.87, as shown in Figure 1(i). Moreover, this study presents

a first reported detection of coumarin in the adventitious root of terrestrial aroids by using the TLC technique, and produced positive test results for seven species.

The results of phenolic compound detection by spraying vanillin sulfuric acid reagent and heating the TLC plate at $120^{O}C$ until maximal visualization of the spots showed that the color ranged from violet and blue to green (Merck,1980), and conformed with the results of isolated chemical compounds in Araceae (Champagne *et al*., 2011; Chan and Kao-Jao,1997; Huang *et al*., 2004). The results showed that all lipophilic extracts produced a change of color to violet at R_f values of 0.08, 0.20, 0.33, 0.43, 0.50 and 0.95, as shown in Figure 1 (e).

After spraying Dragendorff's reagent for alkaloids detection, the results showed that *Aglaonema simplex, Alocasia acuminata, Amorphophallus muelleri, Arisaema maxwellii, Colocasia gigantea, Lasia spinosa* and *Typhonium trilobatum* produced positive test results as orange spots at the base line, as shown in Figure 1(f).The positive test of alkaloids showed in 7 extracts of aroids plant in this study.. Asano *et al*.(1997), Ramalingam *et al.* (2010) and Zhao *et al.*(2010) detected and isolated alkaloids from many aroids, such as *Aglaonema treubii*, *Amorphophallus paeoniifolius*, *Arisaema decipiens*, *Arisaema erubescens* and *Typhonium flagelliforme*. These results differed slightly from this study, as alkaloids in *Amorphophallus paeoniifolius* extracts were not included.

There was no presence of a purple or blue color when the TLC plate was sprayed with Kedde's reagent and Raymond's reagent, as shown in Figure 1(g) and (h). This test from TLC screening gave similar results to those from the phytochemical screening test appearing negative to Kedde and Raymond reaction.

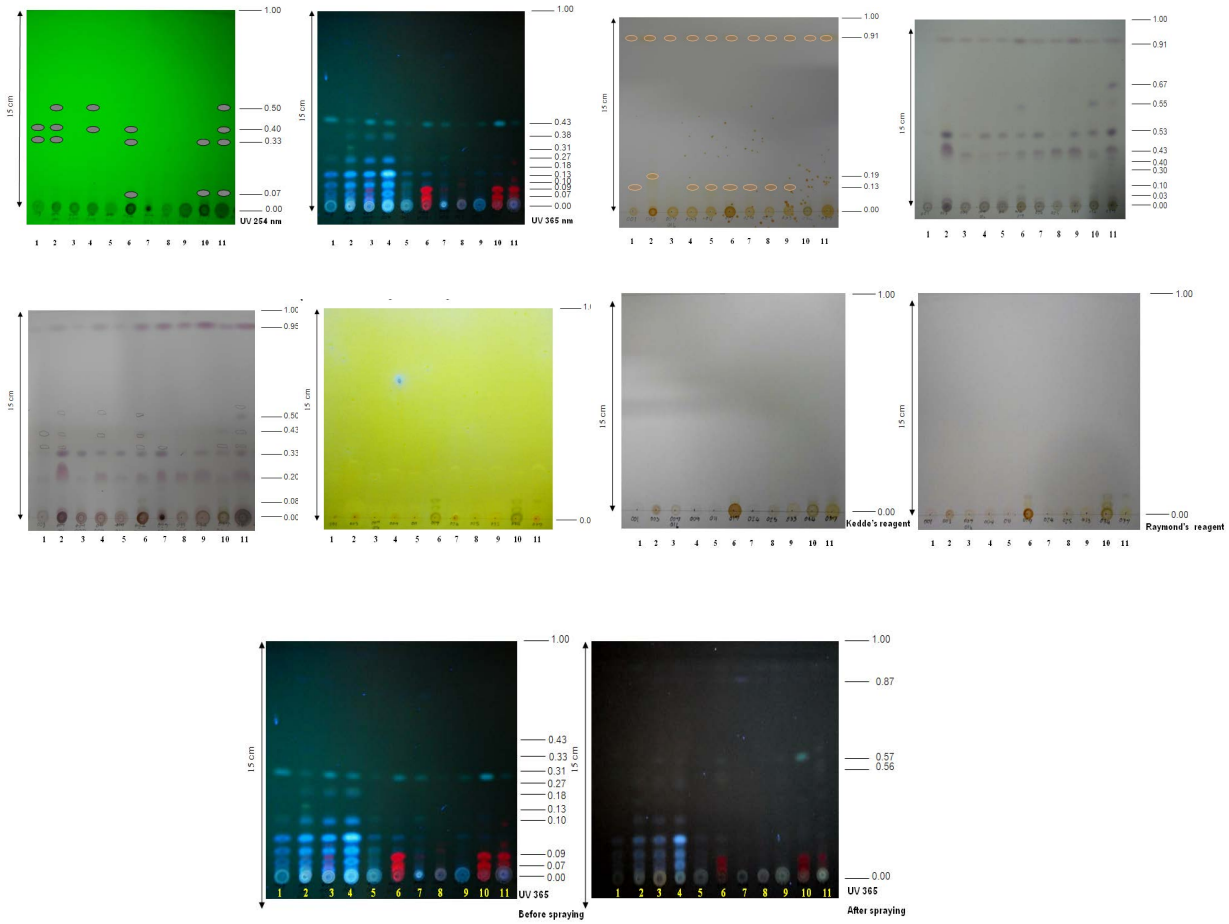

Figure 1 TLC profiling and relative front (R_f) values of terrestrial aroids using different reagents: (a) Detection under short wavelength UV of 254 nm, (b) Detection under long wavelength UV of 365 nm, (c) Iodine as general detection reagent, (d) Anisaldehyde-sulfuric acid reagent, (e) Vanillin sulfuric acid reagent, (f) Dragendorff's reagent (g) Kedde's reagent, (h) Raymond's reagent and (i) 10% NaOH in ethanol and for terrestrial aroids labeled: 1 = *Amorphophallus paeoniifolius* (Dennst.) Nicolson, 2 = *Amorphophallus muelleri* Blume, 3 = *Alocasia acuminata* Schott, 4 = *Amorphophallus maxwellii* Hett. & Gusman, 5 = *Alocasia hypnosa* J.T. Yin, Y.H. Wang & Z.F. Yu, 6 = *Lasia spinosa* (L.) Thwaites, 7 = *Colocasia gigantea* (Blume) Hook.f., 8 = *C. esculenta* (L.) Schott, 9 = *Arisaema maxwellii* Hett. & Gusman, 10 = *Aglaonema simplex* (Blume) Blume, 11 = *Typhonium trilobatum* (L.) Schott.

Chemical characteristics of modified stem extracts from TLC analyses

The mobilities of all compounds separated from the 11 extracts in each species of terrestrial aroids in a suitable single solvent system were compared. The match in R_f values, color, size and shape of detection zones under 365 nm UV light among samples was evidence for the identification of characteristics of the samples (Aloisi *et al*., 1990). The results from this study present a first report of the chemical characteristics and TLC profiling of terrestrial aroids (Araceae) in Thailand.

From Figure 1(b), a comparison of the R_f values of detected compounds between extract No.3 (*Alocasia acuminata*) and extract No.4 (*Amorphophallus maxwellii*) found that the results were similar, with even the same color and shape. In addition, extract No.5 (*Alocasia hypnosa*) and extract No.7 (*Colocasia gigantea*) were similar. Only at R_f values 0.31 of extract No.2 (*Amorphophallus muelleri*), which appeared different from other extracts.. Hence, it could be assumed that adventitious root extract of *A. acuminata*, and *A. maxwellii* showed distinctly similar profiles.

After spraying anisaldehyde-sulfuric acid reagent and heating the TLC plate at 120^{O}C until maximal visualization of spots, the profile and R_f values of extract No.6 (*Lasia spinosa*) were similar to extract No.10 (*Aglaonema simplex*) and R_f values 0.67 specifically appeared in No.11 (*Typhonium trilobatum*) extract only were shown in Figure 1 (d) and Table 2. For detection of phenolic compounds by spraying with vanillin sulfuric acid reagent and heating the TLC plate at 120^{O}C until maximal visualization of the spots, the profiles of all extracts were similar. However, only extract No.11 (*Typhonium trilobatum*) had an R_f value of 0.50, which was different from other extracts (Figure 1(e) and Table 2).

Furthermore, considering the profile of coumarin detection after spraying with 10% NaOH and exposing to UV light at a long wavelength (365 nm), extracts of No.3 (*Alocasia acuminata*), No.4 (*Amorphophallus maxwellii*), No.6 (*Lasia spinosa*), No.9 (*Arisaema maxwellii*) and No.10 (*Aglaonema simplex*) appeared to test positive with R_f values of 0.56. Moreover, the profiles of extract No.7 (*Colocasia gigantea*) and No.11 (*Typhonium trilobatum*) were specific and appeared at R_f 0.57 and 0.87, respectively.

CONCLUSION

Qualitative analysis using TLC screening of lipophilic extract from the modified stem of terrestrial aroids in seven genera consisting of 11 species showed groups of secondary metabolites such as terpenoids, phenolic compounds, coumarin and alkaloids.

TLC profiling of the modified stem extracts of terrestrial aroids showed distinctly similar profiles for *Alocasia acuminata* and *Amorphophallus maxwellii* when detected using a UV wavelength of 365 nm. Only the extract of *Amorphophallus muellerii* appeared different from the other extracts. Detection with anisaldehyde-sulfuric acid reagent and vanillin sulfuric reagent, both had similar R_f values, specifically for *Typhonium trilobatum.* Coumarin produced a positive test result in seven species. There were distinct chemical characteristics identified by using TLC technique in this study, hence, performing separation of these compounds in the future.

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