

เอกสารอ้างอิง

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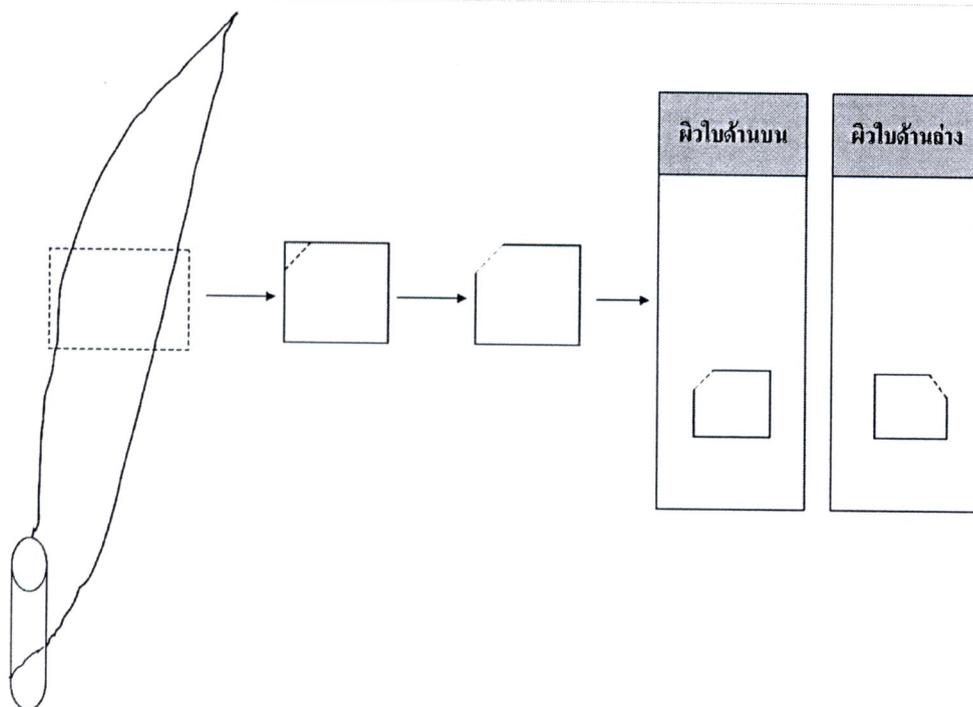
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ภาคผนวก

วิธีการตัดชิ้นตัวอย่างในการศึกษาจุลลักษณะด้วยวิธีทำให้ใส

เนื่องจากในการศึกษาด้วยวิธีนี้ ตัวอย่างเนื้อเยื่อที่ใช้ในการศึกษาจะต้องผ่านขั้นตอนต่าง ๆ จนตัวอย่างใสแล้วจึงนำไปย้อมสี จากนั้นจึงนำชิ้นส่วนตัวอย่างที่ได้ไปผนึกเป็นสไลด์ถาวร ซึ่งในขั้นตอนสุดท้ายนี้จะเกิดความสับสนในการที่จะระบุว่าชิ้นเนื้อด้านใดเป็นด้านบนหรือด้านล่าง เนื่องจากเมื่อผ่านขบวนการต่าง ๆ แล้วเนื้อเยื่อแผ่นใบทั้งสองด้านจะมีความคล้ายคลึงกันมากการแยกด้วยตาเปล่าอาจทำให้เกิดความผิดพลาดได้ ดังนั้นการตัดเนื้อเยื่อด้วยเทคนิคช่วยจำตั้งแต่เริ่มต้นก่อนที่จะนำมาผ่านขั้นตอนต่าง ๆ นั้นจะสามารถช่วยให้การนำชิ้นตัวอย่างมาผนึกเป็นสไลด์ถาวรทำได้สะดวกรวดเร็วขึ้นและยังช่วยให้เกิดความถูกต้องแม่นยำ เมื่อนำตัวอย่างสไลด์ไปศึกษารายละเอียดจุลลักษณะด้วยกล้องจุลทรรศน์ โดยอาศัยเทคนิคในการตัดตัวอย่างดังนี้



ผลงานทางวิชาการ

1. ผลงานที่นำเสนอในงานประชุมวิชาการระดับนานาชาติ

1. **Kaewsart S, Preeprame S, Thitimetharoch T. Preliminary Thin-Layer Chromatography (TLC) Identification of Four Cyanotis species (Family Commelinaceae).** ในงานประชุมวิชาการระดับนานาชาติ The 3rd Sino-Thai Conference on Traditional Medicine and Natural Products; 29 - 31 Oct 2008; Guangxi Traditional Chinese Medical (TCM) University. Nanning, ประเทศสาธารณรัฐประชาชนธิปไตยประชาชนจีน
2. **Kaewsart S, Porasuphatana S, Thitimetharoch T. Total Phenolic Content and DPPH[•] Radical Scavenging Activity from Aerial Vegetative Part of *Murdannia bracteata*, *M. gigantea*, *M. macrocarpa* and *M. simplex* (Family Commelinaceae).** ในงานประชุมวิชาการระดับนานาชาติ Flore du Combodge, du Laos et du Vietnam; 6-8 December 2010 – Hanoi, Vietnam, ประเทศเวียดนาม
3. **Kaewsart S, Chaichomporn O, Porasuphatana S, Thitimetharoch T. Total Phenolic Content and DPPH[•] Radical Scavenging Activity in the Genus *Murdannia* (Family Commelinaceae).** ในงานประชุมวิชาการระดับนานาชาติ XVIII International Botanical Congress 23-30 July 2011, Melbourne Australia.

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Preliminary Thin-Layer Chromatography (TLC) Identification of Four *Cyanotis* species (Family Commelinaceae)

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Abstract: Thin layer chromatograms of *Cyanotis arachnoides*, *C. axillaris*, *C. thwaitesii* and *C. vaga* are useful for identification. The patterns of *Cyanotis arachnoides* generally similar to *C. thwaitesii* and *C. vaga*, whereas *C. axillaris* is different. The best chromatographic system is shown to be solvent system of ethyl acetate - formic acid - water (32.5 : 7.5 : 10, v/v).

Introduction

Asian herbs from genus *Cyanotis* (Family Commelinaceae) have been used as medicines such as Indian *C. axillaris* is for typanitis (Malabar Coast) and ascites (Behar) ¹. Moreover Philippine *C. vaga* is for breast cancer². Eight taxa of the genus *Cyanotis* were enumerated for the Flora of Thailand³. The general occurrences of some species are similar. They are difficult for the distinguishing, especially from sterile parts.

Flavonoids were the major compounds for Family Commelinaceae⁴. Thin layer chromatograms of three *Murdannia* species (Family Commelinaceae) were valuable for the diagnosis^{5,6}. The aim of this preliminary study is to apply TLC study for the identification.

Materials and Methods

The whole plants of four species in genus *Cyanotis* (Family Commelinaceae): *C. arachnoides* Wight, *C. axillaris* (L.) Sweet, *C. thwaitesii* Haussk. and *C. vaga* (Four.) Schult. f. were collected for voucher specimens and experimental materials. The vouchers were deposited at Herbal Herbarium, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen and Forest Herbarium Bangkok (BKF), Bangkok, Thailand. Solvent systems [system I: ethyl acetate - formic acid - water - methanol (30 : 6 : 6 : 3, v/v), system II : ethyl acetate - formic acid - water (32.5 : 7.5 : 10, v/v), system III : ethyl acetate - formic acid - acetic acid - water (40 : 3.4 : 3.4 : 4.2, v/v)] were modified for methanol extraction of the samples and standard markers (quercetin and ferulic acid) on silica gel 60 F254. The chromatograms were investigated by using 10% sulfuric acid in ethanol and ultraviolet light 254 nm and 366 nm^{3,7} in respectively.

Results

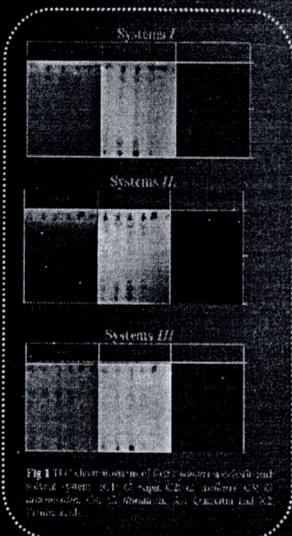
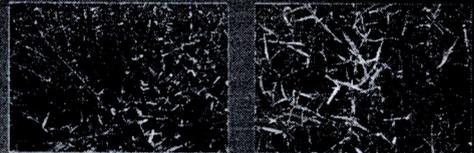
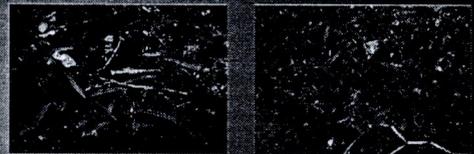


Fig. 1. TLC chromatograms of *Cyanotis* species and standards. System I: *C. vaga*, *C. axillaris*, *C. arachnoides*, *C. thwaitesii*, *M. murdannia*, *M. murdannia* and *M. murdannia*.



C. axillaris



C. vaga

R _f	Quercetin				Ferulic acid			
	Q	F	V	A	Q	F	V	A
0.15	+	+	+	+	+	+	+	+
0.25	+	+	+	+	+	+	+	+
0.35	+	+	+	+	+	+	+	+
0.45	+	+	+	+	+	+	+	+
0.55	+	+	+	+	+	+	+	+
0.65	+	+	+	+	+	+	+	+
0.75	+	+	+	+	+	+	+	+
0.85	+	+	+	+	+	+	+	+
0.95	+	+	+	+	+	+	+	+

Q: Quercetin, F: Ferulic acid, V: *V. vaga*, A: *A. axillaris*. P: pink, R: red, N: white, Y: yellow.

Discussion & Conclusion

The solvent systems are preliminarily modified for the separation. Flavonoids are main chemical constituents. The chromatograms show the different occurrences among four *Cyanotis* species. In general, the contents in each sample display stronger polarity than the markers, whereas the *Murdannia* species are weaker polarity^{6,7}. The samples are well separated in solvent system II [ethyl acetate - formic acid - water (32.5 : 7.5 : 10, v/v)]. However, the suitable systems need to be performed for valuable identification and future researches.

Acknowledgements

We would like to thank Graduate School, Center for Research and Development of Herbal Products, Faculty of Pharmaceutical Sciences, Khon Kaen University and Thailand Research Fund (TRF-MRG-5080006) for their supports.

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Total Phenolic Content and DPPH[•] Radical Scavenging Activity from Aerial Vegetative Part of *Murdannia bracteata*, *M. gigantea*, *M. macrocarpa* and *M. simplex* (Family Commelinaceae)

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Introduction

Phenolic compounds are secondary metabolites of plants that have various biological activities. They are known to have antioxidant, anti-inflammatory, and anticancer properties. The total phenolic content (TPC) and DPPH[•] radical scavenging activity (DPPH[•] RSA) are two important parameters used to evaluate the antioxidant potential of plants. In this study, we investigated the TPC and DPPH[•] RSA of the aerial vegetative parts of four species of Murdannia: *M. bracteata*, *M. gigantea*, *M. macrocarpa*, and *M. simplex*.

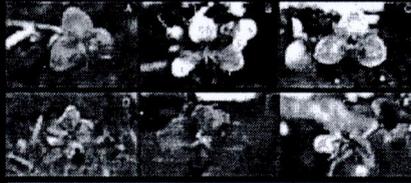


Figure 1 Murdannia bracteata (A), M. gigantea (B – D): B, white flowers, Mg (40), C, purple white flowers, Mg (20), D, violet flowers, Mg (60); M. macrocarpa (E) and M. simplex (F)

Materials and Methods

The aerial vegetative parts of *Murdannia bracteata*, *M. gigantea*, *M. macrocarpa*, and *M. simplex* were collected from various locations in Chiang Mai province, Thailand. The plants were washed with distilled water and dried in a vacuum oven at 40°C for 48 hours. The dried plant material was ground into a fine powder and stored in a dark, dry place until use. The TPC and DPPH[•] RSA were determined using the Folin-Ciocalteu assay and the DPPH[•] assay, respectively. The results were expressed as mean ± SD (n = 3). The data were analyzed using one-way ANOVA and Tukey's post-hoc test (p < 0.05).

Acknowledgements

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2. ...
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4. ...
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6. ...

Results

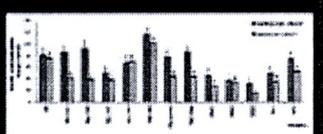


Figure 2 Comparative TPC of the samples in different extracting solvents are measured by means ± SE (n = 3). The vertical bars represent standard error of the mean. Means marked with different letters are significantly different (p < 0.05).

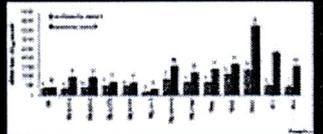


Figure 3 Comparative DPPH[•] RSA of the samples in different extracting solvents are measured by means ± SE (n = 3). The vertical bars represent standard error of the mean. Means marked with different letters are significantly different (p < 0.05).

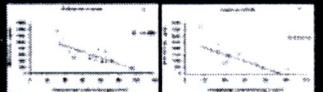
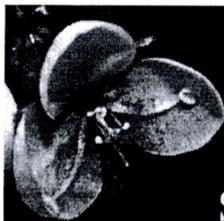


Figure 4 Correlation between TPC and DPPH[•] RSA of the plants in methanolic extract (A) and aqueous extract (B) of the plants.

Total phenolic content (TPC) and DPPH[•] radical scavenging activities (DPPH[•] RSA) between methanolic and aqueous extract from aerial vegetative part of *Murdannia bracteata*, *M. gigantea*, *M. macrocarpa* and *M. simplex* are evaluated by Folin-Ciocalteu assay and DPPH[•] assay. TPC of all species is established to correspond to DPPH[•] radical scavenging capacity. *Murdannia gigantea* generally exhibits the highest TPC and the strongest DPPH[•] scavenging capacity, followed by *M. simplex* and *M. macrocarpa*, respectively. Extracting solvents, variation of the species and environmental factors may be influenced on the difference.

Table 1 Details of the samples

ID	Species	SN	Source	Ex. Sol. concn.	Concentration
M1	<i>M. bracteata</i>	10	Forest, Chiang Mai Province	Methanolic	100 mg/L
M2	<i>M. bracteata</i>	20	Forest, Chiang Mai Province	Methanolic	100 mg/L
M3	<i>M. bracteata</i>	30	Forest, Chiang Mai Province	Methanolic	100 mg/L
M4	<i>M. bracteata</i>	40	Forest, Chiang Mai Province	Methanolic	100 mg/L
M5	<i>M. bracteata</i>	50	Forest, Chiang Mai Province	Methanolic	100 mg/L
M6	<i>M. bracteata</i>	60	Forest, Chiang Mai Province	Methanolic	100 mg/L
M7	<i>M. bracteata</i>	70	Forest, Chiang Mai Province	Methanolic	100 mg/L
M8	<i>M. bracteata</i>	80	Forest, Chiang Mai Province	Methanolic	100 mg/L
M9	<i>M. bracteata</i>	90	Forest, Chiang Mai Province	Methanolic	100 mg/L
M10	<i>M. bracteata</i>	100	Forest, Chiang Mai Province	Methanolic	100 mg/L
M11	<i>M. bracteata</i>	110	Forest, Chiang Mai Province	Methanolic	100 mg/L
M12	<i>M. bracteata</i>	120	Forest, Chiang Mai Province	Methanolic	100 mg/L
M13	<i>M. bracteata</i>	130	Forest, Chiang Mai Province	Methanolic	100 mg/L
M14	<i>M. bracteata</i>	140	Forest, Chiang Mai Province	Methanolic	100 mg/L
M15	<i>M. bracteata</i>	150	Forest, Chiang Mai Province	Methanolic	100 mg/L
M16	<i>M. bracteata</i>	160	Forest, Chiang Mai Province	Methanolic	100 mg/L
M17	<i>M. bracteata</i>	170	Forest, Chiang Mai Province	Methanolic	100 mg/L
M18	<i>M. bracteata</i>	180	Forest, Chiang Mai Province	Methanolic	100 mg/L
M19	<i>M. bracteata</i>	190	Forest, Chiang Mai Province	Methanolic	100 mg/L
M20	<i>M. bracteata</i>	200	Forest, Chiang Mai Province	Methanolic	100 mg/L
M21	<i>M. bracteata</i>	210	Forest, Chiang Mai Province	Methanolic	100 mg/L
M22	<i>M. bracteata</i>	220	Forest, Chiang Mai Province	Methanolic	100 mg/L
M23	<i>M. bracteata</i>	230	Forest, Chiang Mai Province	Methanolic	100 mg/L
M24	<i>M. bracteata</i>	240	Forest, Chiang Mai Province	Methanolic	100 mg/L
M25	<i>M. bracteata</i>	250	Forest, Chiang Mai Province	Methanolic	100 mg/L
M26	<i>M. bracteata</i>	260	Forest, Chiang Mai Province	Methanolic	100 mg/L
M27	<i>M. bracteata</i>	270	Forest, Chiang Mai Province	Methanolic	100 mg/L
M28	<i>M. bracteata</i>	280	Forest, Chiang Mai Province	Methanolic	100 mg/L
M29	<i>M. bracteata</i>	290	Forest, Chiang Mai Province	Methanolic	100 mg/L
M30	<i>M. bracteata</i>	300	Forest, Chiang Mai Province	Methanolic	100 mg/L
M31	<i>M. bracteata</i>	310	Forest, Chiang Mai Province	Methanolic	100 mg/L
M32	<i>M. bracteata</i>	320	Forest, Chiang Mai Province	Methanolic	100 mg/L
M33	<i>M. bracteata</i>	330	Forest, Chiang Mai Province	Methanolic	100 mg/L
M34	<i>M. bracteata</i>	340	Forest, Chiang Mai Province	Methanolic	100 mg/L
M35	<i>M. bracteata</i>	350	Forest, Chiang Mai Province	Methanolic	100 mg/L
M36	<i>M. bracteata</i>	360	Forest, Chiang Mai Province	Methanolic	100 mg/L
M37	<i>M. bracteata</i>	370	Forest, Chiang Mai Province	Methanolic	100 mg/L
M38	<i>M. bracteata</i>	380	Forest, Chiang Mai Province	Methanolic	100 mg/L
M39	<i>M. bracteata</i>	390	Forest, Chiang Mai Province	Methanolic	100 mg/L
M40	<i>M. bracteata</i>	400	Forest, Chiang Mai Province	Methanolic	100 mg/L
M41	<i>M. bracteata</i>	410	Forest, Chiang Mai Province	Methanolic	100 mg/L
M42	<i>M. bracteata</i>	420	Forest, Chiang Mai Province	Methanolic	100 mg/L
M43	<i>M. bracteata</i>	430	Forest, Chiang Mai Province	Methanolic	100 mg/L
M44	<i>M. bracteata</i>	440	Forest, Chiang Mai Province	Methanolic	100 mg/L
M45	<i>M. bracteata</i>	450	Forest, Chiang Mai Province	Methanolic	100 mg/L
M46	<i>M. bracteata</i>	460	Forest, Chiang Mai Province	Methanolic	100 mg/L
M47	<i>M. bracteata</i>	470	Forest, Chiang Mai Province	Methanolic	100 mg/L
M48	<i>M. bracteata</i>	480	Forest, Chiang Mai Province	Methanolic	100 mg/L
M49	<i>M. bracteata</i>	490	Forest, Chiang Mai Province	Methanolic	100 mg/L
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M51	<i>M. bracteata</i>	510	Forest, Chiang Mai Province	Methanolic	100 mg/L
M52	<i>M. bracteata</i>	520	Forest, Chiang Mai Province	Methanolic	100 mg/L
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M57	<i>M. bracteata</i>	570	Forest, Chiang Mai Province	Methanolic	100 mg/L
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M66	<i>M. bracteata</i>	660	Forest, Chiang Mai Province	Methanolic	100 mg/L
M67	<i>M. bracteata</i>	670	Forest, Chiang Mai Province	Methanolic	100 mg/L
M68	<i>M. bracteata</i>	680	Forest, Chiang Mai Province	Methanolic	100 mg/L
M69	<i>M. bracteata</i>	690	Forest, Chiang Mai Province	Methanolic	100 mg/L
M70	<i>M. bracteata</i>	700	Forest, Chiang Mai Province	Methanolic	100 mg/L
M71	<i>M. bracteata</i>	710	Forest, Chiang Mai Province	Methanolic	100 mg/L
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M96	<i>M. bracteata</i>	960	Forest, Chiang Mai Province	Methanolic	100 mg/L
M97	<i>M. bracteata</i>	970	Forest, Chiang Mai Province	Methanolic	100 mg/L
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M128	<i>M. bracteata</i>	1280	Forest, Chiang Mai Province	Methanolic	100 mg/L
M129	<i>M. bracteata</i>	1290	Forest, Chiang Mai Province	Methanolic	100 mg/L
M130	<i>M. bracteata</i>	1300	Forest, Chiang Mai Province	Methanolic	100 mg/L
M131	<i>M. bracteata</i>	1310	Forest, Chiang Mai Province	Methanolic	100 mg/L
M132	<i>M. bracteata</i>	1320	Forest, Chiang Mai Province	Methanolic	100 mg/L
M133	<i>M. bracteata</i>	1330	Forest, Chiang Mai Province	Methanolic	100 mg/L
M134	<i>M. bracteata</i>	1340	Forest, Chiang Mai Province	Methanolic	100 mg/L
M135	<i>M. bracteata</i>	1350	Forest, Chiang Mai Province	Methanolic	100 mg/L
M136	<i>M. bracteata</i>	1360	Forest, Chiang Mai Province	Methanolic	100 mg/L
M137	<i>M. bracteata</i>	1370	Forest, Chiang Mai Province	Methanolic	100 mg/L
M138	<i>M. bracteata</i>	1380	Forest, Chiang Mai Province	Methanolic	100 mg/L
M139	<i>M. bracteata</i>	1390	Forest, Chiang Mai Province	Methanolic	100 mg/L
M140	<i>M. bracteata</i>	1400	Forest, Chiang Mai Province	Methanolic	100 mg/L
M141	<i>M. bracteata</i>	1410	Forest, Chiang Mai Province	Methanolic	100 mg/L
M142	<i>M. bracteata</i>	1420	Forest, Chiang Mai Province	Methanolic	100 mg/L
M143	<i>M. bracteata</i>	1430	Forest, Chiang Mai Province	Methanolic	100 mg/L
M144					



Total Phenolic Content and DPPH[•] Radical Scavenging Activity in the Genus *Murdannia* (Family Commelinaceae)

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Figure 1 *Murdannia bracteata* (A), *M. discreta* (B), *M. edulis* (C), *M. gigantea* (D), *M. japonica* (E), *M. loriformis* (F), *M. macrocarpa* (G), *M. nudiflora* (H), *M. nudiflora* (I), *M. nudiflora* (J), *M. nudiflora* (K), *M. nudiflora* (L). A: white flowers, B: purple flowers, C: purple flowers, D: purple flowers, E: purple flowers, F: purple flowers, G: purple flowers, H: purple flowers, I: purple flowers, J: purple flowers, K: purple flowers, L: purple flowers.

Abstract

Methanolic and aqueous extracts of aerial vegetative parts of nine *Murdannia* species (23 samples) *M. bracteata*, *M. discreta*, *M. edulis*, *M. japonica*, *M. gigantea*, *M. loriformis*, *M. macrocarpa*, *M. nudiflora* and *M. simplex* (Family Commelinaceae), all from Thailand, are evaluated by Folin-Ciocalteu assay and DPPH assay. Samples from different locations display significantly different results as well as morphological differences. Methanolic extracts generally show higher values for both total phenolic content (TPC) and DPPH radical scavenging activity (DPPH RSA). *Murdannia gigantea* exhibits the highest TPC, whereas *M. edulis* has the strongest DPPH scavenging capacity. TPC of all species is demonstrated to correspond to DPPH radical scavenging capacity (correlation between IC₅₀ values: R² = 0.4607 in methanolic extract, R² = 0.4869 in aqueous extract). Extracting solvents, variation among the species and environmental factors may be influenced on the observed differences.

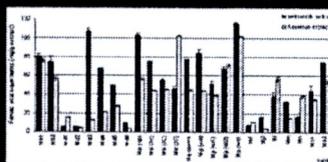


Figure 2 Comparative TPC of the samples in different extracting solvents are measured by means \pm SD (n = 4). The vertical bars represent standard error of the mean.

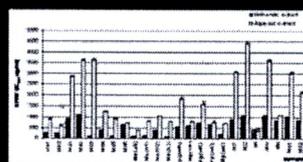


Figure 3 Comparative DPPH RSA of the samples in different extracting solvents are measured by means \pm SD (n = 4). The vertical bars represent standard error of the mean.

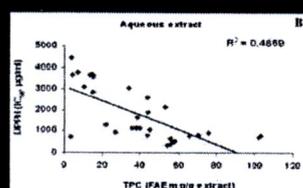
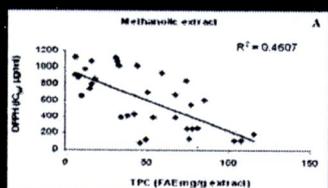


Figure 4 Correlation between TPC and DPPH RSA in methanolic extract (A) and aqueous extract (B) of the plants

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ตำแหน่ง ผู้ช่วยวิจัย ในโครงการการศึกษาพฤกษศาสตร์พื้นบ้านในโครงการอนุรักษ์พันธุกรรมพืชอันเนื่องมาจากพระราชดำริสมเด็จพระเทพรัตนราชสุดาฯ สยามบรมราชกุมารี

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