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Original Research Article

GC-MS Analysis and Antioxidant Potential of the Stem and Leaf Extracts of *Dendrophthoe pentandra* (L.) Miq. Hemiparasite on *Shorea roxburghii* G. Don and *Cassia fistula* L.

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ABSTRACT

Dendrophthoe pentandra (L.) Miq. (Kafak mamung) is a hemiparasitic plant utilized for several ethnopharmacological and medicinal purposes. However, the hemiparasitic plant vary in correlation with the species from host plant, due to variation in the compound's profiles. This study was aimed to determine the compounds contained in leaf and stem extracts from various host plants. Soxhlet extraction was used to prepare crude extracts using ethanol and ethyl acetate as solvents. Phytochemical profiles of the stem and leaf were analyzed by GC-MS to determine the total phenolic content (TPC) and total flavonoid content (TFC), while antioxidant activities were examined using DPPH and ABTS assays. Folin-Ciocalteu colorimetry showed that ethanol extract of *D. pentandra* hemiparasite stem growing on *Shorea roxburghii* had the highest TPC (398.45± 2.49 mg GAE/g extract) and DPPH assays 88.518± 0.339%. Chemical analysis using GC-MS revealed the presence of 14 compounds including 9-octadecanamide (Z)-, hexadecanamide and octadecanamide. The phytochemical profiles of the compounds identified in the extracts of *D. pentandra* showed that this plant has a potential for future health promotion.

Keywords: Antioxidant, *Dendrophthoe pentandra*, Hemiparasite, Phytochemical profiles.

Introduction

Mistletoes (Kafak in Thai) are hemiparasitic plants that absorb water, nutrients and minerals from their hosts but have retained photosynthetic ability[1] The medicinal plant *Dendrophthoe pentandra* (L.) Miq. (Kafak mamung) is a Thai kafak species in the Loranthaceae Family commonly found growing on many different species of the host plant. Different species of kafak can grow on the same host plant or one species of kafak can grow on multiple host trees.[2] The kafak is named depending on its host plant such as Kafak mamung (kafak that grows on *Mangifera indica*). Kafaks are hemiparasitic plants and their bioactivities are related to their host plant. Chemical compositions of kafak extracts vary depending on the extraction techniques, solvent and species of host tree.[3] In Nepal, the plant is used as an alternative fodder [4] while in Indonesia, *D. pentandra* is sold in stores as a herbal tea and used to treat diabetes, hypertension and cancer.[5-6] The Akha people in China and Thailand use kafak as a traditional medicine for rheumatoid arthritis[7]. Previous phytochemical investigations of kafak reported catechin, procyanidin B-1, procyanidin B-3, bridelionoside A, quercetin, quercitrin and rutin. [8-10]. Here, GC-MS was used to determine the total phenolic and flavonoids contents and antioxidant activities of *D. pentandra* stem and leaf growing on *Shorea roxburghii* and *Cassia fistula* using ethanol and ethyl acetate solvents.

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Materials and Methods

Plant materials and extract preparation

The whole stem and leaf parts of *D. pentandra* were collected in May 2022 from Mahasarakham University, Thailand. The plant was identified by assistant professor Dr. Pasakorn Bunchalee. Voucher specimens (MSUT-7712-7713) were deposited in the herbarium at the Department of Biology, Faculty of Science, Mahasarakham University. The plant parts were dried in a hot air oven at 60°C. Stems and leaves were separated before extract preparation and then homogenized as fine powders. Then, 10 grams of plant powder were dissolved in 300 mL of ethanol and ethyl acetate solvents. The plant extracts were prepared by Soxhlet extraction for 4 h. The extracts were evaporated in a water bath at 60°C and preserved by freeze drying until use.

Determination of total phenolic contents

Total phenolic content was analyzed by the Folin-Ciocalteu colorimetric method modified by Amin *et al.*[11] Gallic acid was used as the standard (concentrations of 12.5-100 mg/mL). The plant extracts were dissolved in methanol and 0.5 mL of extracts (1 mg/mL) was mixed with 2.5 mL of Folin-Ciocalteu solution and left at room temperature for 5 min. Then, 2 mL of Na_2CO_3 solution was added. The solutions were mixed well and the volumes were adjusted to 5 mL by adding distilled water and then left at room temperature for 2 h. The solutions were measured for absorbance at 760 nm. Finally, the total phenolic content of the extracts was calculated using the gallic acid standard curve (mg of gallic acid equivalent (GAE)/g extract).

Determination of total flavonoid contents

Total flavonoid content was determined following the modified method of Pothitirat *et al.*[12] Quercetin was used as the standard (concentrations of 15.625- 250 µg/mL). Samples of 500 µL (1 mg/mL) were added with 1,500 µL of 95% ethanol and incubated at room temperature for 6 min. Then, 100 µL of 10% aluminium chloride was added and the solutions were mixed. After 5 min, 100 µL of 1 M potassium acetate was added to the solutions and mixed. The volume was adjusted to 5,000 µL with distilled water and left at room temperature for 30 min in

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the dark. The samples were measured as three replicates for absorbance at 415 nm. Total flavonoid contents was measured by comparison with the standard curves (mg quercetin/g extract).

DPPH Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay followed the method of Likhitwitayawuid *et al*,[13] and 100 µM DPPH was prepared in methanol. The extracts were prepared in methanol at a concentration of 1 mg/mL. Then, 180 µL of DPPH solution and 20 µL of extract solution were pipetted into a microplate.The solutions were incubated in a dark room at room temperature for 20 min. and measured for absorbance by a microplate reader at 517 nm. The absorbance of extract samples (As) was measured, while methanol was used as the blank (Ac), with ascorbic acid as the standard. The Percentage inhibition of free radicals was calculated by the following equation;

Percentage radical scavenging = $[(Ac – As)/Ac] \times 100$.

ABTS assay

The ABTS assay was carried out by following the modified method of Payet *et al*.[14] The ABTS·+ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate and stored in the dark at room temperature for 12-16 h before use. The ABTS solution was diluted with ethanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. Extracts were measured as three replicates. Methanol was used as the blank (Ac) and ascorbic acid was used as the standard. Percentage inhibition was calculated by the following equation;

Percentage radical scavenging = $[(Ac – As)/Ac] \times 100$.

Gas chromatography mass spectrometry **(***GC-MS***)**

The extracts were analyzed using GC-MS adapted from Suphrom *et al*.[15] The initial oven temperature was held at 70°C for 3 min. then programmed to increase at 5°C/min to 280°C and finally held for 10 min. The temperature of the transfer line heater was set at 280°C with the mass scanning range was set from 50-550 amu in full scan. Chemical compounds in the extracts were identified by computer matching of the retention times and mass spectral data with standards in the National Institute of Standards and Technology (NIST).

Statistical analysis

Total phenolic content and antioxidant activity were carried out in three replicates (n= 3), and the results are expressed as mean ± SD (standard deviation). Pearson's correlation coefficient was calculated using Microsoft excel 2021.

Results and Discussions

D. pentandra leaf and stem are shown in Figure 1. The percentage yield (% yield) showed that the ethanol extracts from the leaf and stem had the highest percentage yield. (Table 1). The total phenolic contents of *D. pentandra* extracts ranged from 24.583 to 398.450 mg GAE/g extract. (Table 2).

Figure 1: Leaf and stem of *Dendrophthoe pentandra* (L.) Miq.

Table 1: Percentage yield of *D. pentandra* extracts.

		Percentage yield	
Plants	**Part**	**Ethyl acetate**	**Ethanol**
DP hemiparasite on	leaf	3.397	9.502
Shorea roxburghii	stem	1.338	5.807
Shorea roxburghii	leaf	9.139	18.063
	stem	4.413	12.957
DP hemiparasite on	leaf	3.018	7.141
Cassia fistula	stem	1.761	8.05
Cassia fistula	leaf	8.759	13.135
	stem	1.739	4.784

The stem extracted with ethanol contained the highest total phenolic content of 398.450 ± 2.49 mg GAE/g extracts. The leaf extracted with ethanol contained the highest total phenolic content of 251.264 ± 1.00f mg GAE/g extract. Gallic acid; $y = 0.0335x + 0.0014$, $R^2 = 0.9288$**.** The ethanol solvent gave higher total phenolic contents compared to ethyl acetate.

Total flavonoid contents

Total flavonoid content of *D. pentandra* extracts are 14.760 to 225.912 mg QE/g extract (Table 2). The stem extracted with ethyl acetate contained the highest total flavonoid content of 73.965 ± 1.06 mg QE/g extract. The leaf extracted with ethyl acetate contained the highest the total flavonoid content of 225.912 ± 1.07 mg QE/g extracts. Quercetin; $y= 0.0117x$, $R^2= 0.9966$). The ethyl acetate solvent gave higher total flavonoids contents compared to ethanol.

Antioxidant activity

DPPH and ABTS assays of the test samples were evaluated at a concentration of 100 µg/mL. Compounds showing more than 50% inhibition were further analyzed for their IC_{50} values.

The DPPH results indicated that the ethanolic stems of *S. roxburghii* extracts had the highest antioxidant activity with IC_{50} of 0.425± 0.005 mg/mL. ABTS assay, results showed that the ethanolic stems of *D. pentandra* hemiparasite on *C. fistula* extracts had the highest antioxidant activity at IC_{50} of 0.062± 0.001 mg/mL (Table 3).

Correlation between total phenolic content and total flavonoid content

The Pearson's correlation coefficient (r) between total phenolic content and total flavonoid content was negative (-0.5794)

Correlation between total phenolic content and antioxidant activities

The correlation of total phenolic content, with DPPH and ABTS activities is shown in Figure 2(A) and (B), respectively. The correlation coefficient was higher between total phenolic content and ABTS activity than that of total phenolic content and DPPH activity (r = 0.7667). Osman *et al,*[16] suggested that there is a correlation between the total phenolic content and antioxidant activity of plant extracts. However, no correlation was found between the flavonoid content and the antioxidant activities of the extracts.

Correlation between DPPH and ABTS activities

The correlation of antioxidant activities (DPPH and ABTS), the two assays were supported by each other with a strong positive correlation (r = 0.7315).

GC-MS analysis of the crude extracts

The extracts were subjected to GC-MS analysis to identify the phytochemical constituents based on the peak area and retention time. Ethanol extracts of *D. pentandra* revealed the presence of 14 compounds, with 10 identified from leaf extracts and 7 from stem extracts (Table 4).

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Table 2: Total phenolic and flavonoid contents

Plants	Part	Solvent	Total phenolics (mg GAE/g extract)	Total flavonoids (mg QE/g extract)
DP	leaf	ethyl acetate	181.168 ± 1.07[i]	225.912 ± 1.07[a]
hemiparasite on		ethanol	251.264 ± 1.00[f]	110.598 ± 1.65[d]
Shorea roxburghii	stem	ethyl acetate	139.780 ± 3.00[j]	73.965 ± 1.06[f]
		ethanol	398.450 ± 2.49[a]	36.087 ± 3.43[j]
	leaf	ethyl acetate	234.428 ± 1.84[g]	122.100 ± 1.70[c]
Shorea roxburghii		ethanol	132.635 ± 1.35[k]	108.154 ± 1.36[de]
	stem	ethyl acetate	305.102 ± 1.72[d]	44.987 ± 4.20[i]
		ethanol	320.530 ± 3.66[c]	14.760 ± 0.47[l]
DP	leaf	ethyl acetate	117.066 ± 2.37[l]	120.798 ± 1.33[c]
hemiparasite on		ethanol	109.570 ± 3.76[m]	110.596 ± 1.26[d]
Cassia fistula	stem	ethyl acetate	136.160 ± 1.04[jk]	54.701 ± 2.56[g]
		ethanol	209.496 ± 2.05[h]	22.521 ± 3.18[k]
	leaf	ethyl acetate	103.344 ± 4.72[n]	156.994 ± 1.54[b]
Cassia fistula		ethanol	91.831 ± 0.400[o]	105.874 ± 1.63[e]
	stem	ethyl acetate	336.182 ± 3.92[b]	49.584 ± 3.33[h]
		ethanol	283.047 ± 1.12[e]	24.583 ± 0.43[k]

Table 3: Antioxidant activity (DPPH and ABTS activity)

Plant	Part	Solvent	DPPH (1 mg/mL)		ABTS (1 mg/mL)	
			% inhibition	IC_{50} (mg/mL)	% inhibition	IC_{50} (mg/mL)
DP hemiparasite on	leaf	ethyl acetate	73.810 ± 0.627[d]	0.613 ± 0.007[cd]	27.862 ± 0.365[i]	-
Shorea roxburghii		ethanol	85.192 ± 0.104[bc]	0.468 ± 0.008[cd]	45.649 ± 1.219[g]	-
	stem	ethyl acetate	51.607 ± 0.818[g]	0.824 ± 0.024[h]	39.184 ± 0.170[h]	-
		ethanol	88.518 ± 0.339[a]	0.448 ± 0.010[c]	76.493 ± 1.068[b]	0.078 ± 0.001[b]
	leaf	ethyl acetate	27.678 ± 0.818[j]	-	53.205 ± 3.111[f]	0.246 ± 0.004[e]
Shorea roxburghii		ethanol	33.933 ± 0.991[i]	-	44.587 ± 1.979[g]	-
	stem	ethyl acetate	59.107 ± 0.714[f]	0.670 ± 0.007[g]	61.960 ± 1.808[e]	0.098 ± 0.001[d]
		ethanol	84.830 ± 3.850[bc]	0.425 ± 0.005[b]	71.174 ± 2.358[c]	0.080 ± 0.002[b]
DP hemiparasite on	leaf	ethyl acetate	51.860 ± 0.403[g]	0.848 ± 0.026[i]	7.922 ± 1.565[k]	-
Cassia fistula		ethanol	28.477 ± 0.275[j]	-	8.972 ± 1.261[k]	-
	stem	ethyl acetate	63.781 ± 0.744[e]	0.754 ± 0.008[f]	21.569 ± 0.505[j]	-
		ethanol	84.532 ± 0.311[bc]	0.485 ± 0.010[d]	89.843 ± 0.248[a]	0.062 ± 0.001[a]
	leaf	ethyl acetate	39.813 ± 2.635[h]	-	2.876 ± 0.639[l]	-
Cassia fistula		ethanol	8.753 ± 0.452[k]	-	3.667 ± 2.447[l]	-
	stem	ethyl acetate	86.744 ± 1.452[ab]	0.546 ± 0.010[e]	65.442 ± 3.484[d]	0.090 ± 0.004[c]
		ethanol	82.854 ± 0.632[c]	0.426 ± 0.004[b]	66.437 ± 1.396[d]	0.088 ± 0.001[c]
Ascorbic acid				0.073 ± 0.001[a]		0.061 ± 0.001[a]

The major group of compounds in the leaf and stem extracts was long chain- fatty acids such as 9-octadecanamide (Z) and octadecanamide (Figure 3). 9-octadecanamide was the most abundant saturated fatty acid and has anti-inflammatory and antibacterial activities.[17] The bioactive compounds were identified and their bioactive uses are tabulated in Table 5. Bioactive compounds are normally reported for their antimicrobial, anti-inflammatory, anticancer and antioxidant properties. The present study suggested that there are 14 compounds found in the leaf and stem ethanolic extracts such as, 9-octadecenamide, (Z)-and octadecanamide. 9-Octadecenamide, (Z)- is a fatty acid amide known as oleamide. Oleamide can bind to cannabinoid receptors and produce a variety of biological actions such as sleep induction, immunosuppression, and anti-inflammatory.[25] The stem ethanol extracts of *D. pentandra* showed the highest phenolic content and antioxidant activity compared to the leaf extracts. Also, Alharits *et al*[26] evaluated the antioxidant capacity of mistletoe leaf and flower extracts. They reported that when compared to the leaf extract, the methanolic flower extract had higher phenolics, flavonoids, and antioxidants. Yismairai *et al*[27] investigated the antioxidant activity of three mistletoes living on three different host plants (*Mangifera indica*, *Bauhinia pupurea* and *Stelechocarpus burahol*).

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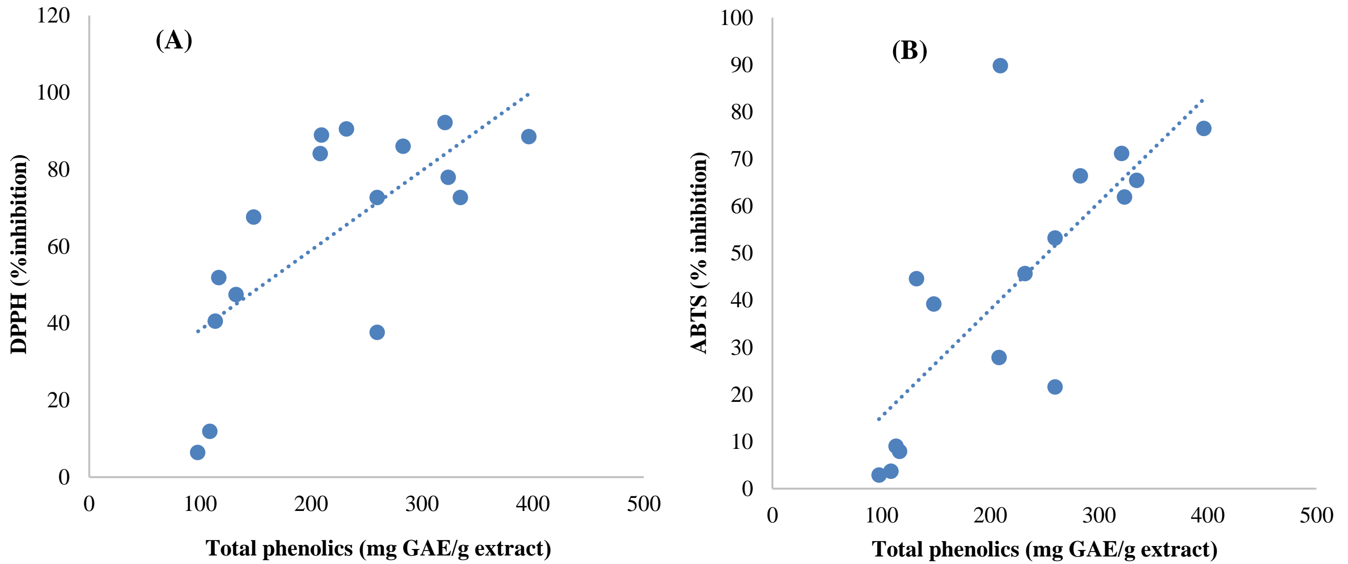

Figure 2: (A) Correlation between total phenolic content and DPPH activity of the crude extracts. Correlation coefficient, r = 0.7035. (B) Correlation between total phenolic content and ABTS activity of the crude extracts. Correlation coefficient, r = 0.7667.

Figure 3: A. Stem *Dendrophthoe pentandra* **B**. Leaf *Dendrophthoe pentandra*

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Table 4: Chemical compounds in the *Dendrophthoe pentandra* extracts identified by GC-MS.

Retention time (min.)	Compound	Peak Report TIC (Area %) Leaf	Stem
22.799	Heptadecane, 2,6,10,15-tetramethyl-		0.57
27.375	Heptadecane		0.92
28.184	Neophytadiene	1.31	
28.718	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.07	
31.416	Hexadecanoic acid, ethyl ester	0.84	
31.467	Docosanoic acid, ethyl ester		0.30
33.729	Phytol	1.68	
34.633	trans,trans-9,12-Octadecadienoic acid, propyl	0.14	
34.723	(E)-9-Octadecenoic acid ethyl ester	0.57	
35.177	Hexadecanamide		6.92
38.286	9-Octadecanamide, (Z)-	76.23	72.01
38.694	Octadecanamide	17.45	19.01
41.278	Diisooctyl phthalate	0.21	
45.406	Squalene	0.26	0.17
		100	100

Table 5: Bioactivity of phytochemical identified in the extracts of *Dendrophthoe pentandra*.

Compound name	Medicinal activity
Hexadecanamide	Analgesic, Anti-inflammatory[18]
Hexadecane	Antianginal, Ovulation inhibitor [19]
Hexadecanoic acid, ethyl ester	Antioxidant, Hemolytic, Hypocholesterolemic Flavor, Nematicide, Anti-androgenic[20]
Neophytadiene	Antipyretic, Analgesic, Anti-inflammatory, Antimicrobial, Antioxidant[21]
Octadecanamide	Antibacterial[22]
9-Octadecanamide, (Z)-	Antibacterial, Anti-inflammatory[17,25]
Phytol	Antimicrobial, Anti-inflammatory, Antioxidant, Cytotoxic, Antinociceptive [23]
Squalene	Antioxidant, Antibacterial, Antitumor, Cancer preventive, Immunostimulant, Chemopreventive[24]

The methanolic leaf extract from *S. burahol* had the highest activity. In the present study the ethanolic stem extract of *D. pentandra* hemiparasite growing on *Cassia fistula* had the highest antioxidant activity. Different plant parts, different host plants and types of extraction solvents are important factors that impact chemical compounds, phenolic contents and bioactivity of plant extracts.

Conclusion

The extracts of *D. pentandra* from various hosts showed significant antioxidant activity, whereas the ethanol extracts showed potential as antioxidant activity. Therefore, it is suggested that *D. pentandra* extracts vary in correlation with the species from host plant, due to variation in the phytochemical profiles. Extracts from this local plant can be used for health promotion and may also be suitable for development as supplementary food.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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