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Microscopic Characteristics, Chemical Composition and Antioxidant Activity of Stem and Leaf Extracts of *Scurrula atropurpurea* **(Blume) Danser. Hemiparasite on** *Erythrophleum succirubrum* **Gagnep.**

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ARTICLE INFO ABSTRACT

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Scurrula atropurpurea (Blume) Dans. is a hemiparasitic plant that parasitizes a variety of host plants. It is used traditionally for treating various ailments. The chemical composition and antioxidant properties of this plant, particularly when attached to *Erythrophleum succirubrum* are not well documented. This study aimed to provide a pharmacognostic description, and evaluate the chemical composition and antioxidant activity of the stem and leaf extracts of *S. atropurpurea* and *E. succirubrum*. The extracts were obtained by Soxhlet extraction using dichloromethane, ethyl acetate, and ethanol, successively. The pharmacognostic features of *S. atropurpurea* was examined microscopically. The total phenolic and flavonoid contents were evaluated using standard methods. The antioxidant activity was assessed using DPPH and ABTS radical scavenging assays. The compounds in the extracts were identified by GC-MS analysis. Results showed that the ethanol extract of *S. atropurpurea* stem had the highest total phenolic content (387.365±4.643 mg GAE/g extract), while the dichloromethane extract of *E. succirubrum* leaf had the highest total flavonoid content $(170.311\pm2.20 \text{ mg }$ OE/g extract). The ethanol extract of *E. succirubrum* stem had the highest antioxidant activity ($IC_{50} = 0.290 \pm 0.029$ mg/mL) in the DPPH assay, while the ethanol stem extract of *S. atropurpurea* had the highest antioxidant activity ($IC_{50} = 0.509 \pm 0.041$ mg/mL) in the ABTS assay. GC-MS analysis identified 13 compounds in the extracts of *S. atropurpurea* with the major constituents being 9-octadecanamide (Z)-, tetradecanamide, octadecanamide, and hexadecanamide. *S. atropurpurea* extracts exhibited significant antioxidant activity, underscoring the plant's potential therapeutic value and supporting its traditional use in folk medicine.

Keywords: Scurrula atropurpurea, Anatomical features, Gas chromatography-Mass spectrometry, Antioxidant activity.

Introduction

Scurrula atropurpurea, commonly known in Thai as 'Gar fak kwan taw,' is a hemiparasitic plant belonging to the family Loranthacea that grows in tropical and subtropical regions. In Thailand, the genus *Scurrula* includes seven species with similar morphological characteristics and individual species identification can be challenging. ¹ A hemiparasitic plant commonly attaches to a variety of host plants including tea plants)Camellia sinensis (L.) Kuntze(, mango (*Mangifera indica* L.), guava (*Psidium guajava* L.), and various *Ficus* L. species as well as ornamental plants in both urban and rural landscapes. The selection of host plants varies depending on geographical location and specific ecological conditions. *S. atropurpurea* has cultural significance in some regions, and is linked to traditional practices and local folklore.

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Economically, its association with tea cultivation makes it relevant in agricultural contexts, especially in areas where tea is a major crop, and understanding its effects on tea plants is crucial for developing sustainable agricultural practices. ² Phytochemicals identified in the whole plant (stems and leaves) of *S. atropurpurea* include various flavonoids and polyphenolic compounds such as kaempferol, quercetin, and casticin which are known for their potential health benefits. Quercetin and kaempferol support cardiovascular health by improving endothelial function and reducing inflammation, 3 lowering blood pressure⁴, providing antioxidative benefits,⁵ and treating diabetoporosis. ⁶ No chemical compounds have been specifically reported from the stem because existing data primarily focused on the whole plant including both leaves and stems or chemicals derived from leaf extracts. Aditiyarini et *al*. 2 examined the leaf extract of *S. atropurpurea* through GC-MS analysis and identified 12 compounds categorized into fatty acid esters and sugars, indicating a complex phytochemical composition. In traditional medicine, various parts of the plant including the leaves, stems, and roots have been utilized for their potential medicinal properties. The leaves, rich in essential oils, flavonoids, tannins, and other phytochemicals are commonly used in herbal teas, extracts, and poultices. The stems contain a variety of bioactive compounds such as alkaloids, flavonoids, and terpenoids contributing to their medicinal use. Each part of the plant offers unique health benefits due to its specific composition of phytochemicals. Understanding the medicinal properties of different plant parts enhances the development of herbal remedies and contributes to the field of pharmacognosy, which studies the medicinal properties of natural products.⁷ The quality control of herbal raw materials involves several procedures including the examination of macroscopic and microscopic characteristics, qualitative evaluation of chemical

compounds, and chromatographic analysis. Microscopic examination plays a crucial role in research involving medicinal plants. Plant species can be accurately identified by examining cellular structures such as trichomes, stomata, vascular bundles, and glandular hairs. These data provide valuable information about the distribution and localization of phytochemicals within plant tissues that is essential for understanding the biosynthesis, storage, and accumulation of bioactive compounds.⁸ Microscopic characteristics refer to the structural features of plant tissues that are observed and analyzed using a microscope. These characteristics are crucial for the identification, classification, and understanding of the functional roles of different plant species. Some plants have specialized structures for producing and storing secondary metabolites, such as essential oils or resin, which protect the plant from herbivores and pathogens.⁹ The microscopic examination of medicinal plants offers valuable information for botanical identification, quality control, phytochemical and pharmacological studies, and antioxidant screening.

Two species of mistletoe, *Dendrophthoe pentandra* and *S. atropurpurea* were collected from Mahasarakham University. Previous research primarily focused on comparing *D. pentandra* with mistletoes on different host plants.¹⁰ This study examined *S. atropurpurea* that can grow on the host plant *E. succirubrum* which is known to be toxic¹¹ and explored the phenolic content and antioxidant properties of both *S. atropurpurea* and *E. succirubrum*. The microscopic characteristics and the impact of ethanol, dichloromethane, and ethyl acetate solvents on the chemical composition of the leaf and stem extracts were also investigated.

Materials and Methods

Collection and identification of plant materials

The entire stem and leaf sections of *S. atropurpurea* were collected in May 2024 from Mahasarakham University (GPS coordinates: Lat 16.24655964320057, Long 103.25110704647679), Thailand. The plant was identified by Assistant Professor Dr. Pasakorn Bunchalee, and voucher specimens (MSUT-8662) were deposited in the herbarium of the Department of Biology, Faculty of Science, Mahasarakham University.

Preparation of extracts

The plant parts were dried in a hot air oven at 60°C and then ground into fine powder. Fifty grams of the powdered stem and leaf material were extracted using 400 mL each of dichloromethane, ethyl acetate, and ethanol. The extraction was carried out using a Soxhlet apparatus for 6 hours, followed by evaporation in a water bath at 60°C. The resulting extracts were stored at -4°C for future use.

Microscopic characterization

Transverse sections of the fresh leaves and stems of *S. atropurpurea* were prepared. Each section was placed on a glass slide and stained with specific reagents. Separate slides were prepared for safranin O, ferric chloride hexahydrate (FeCl₃.6H₂O) solution, and iodine staining. The slides were examined at 10x and 40x magnification using a compound microscope (ZEISS Primostar 3, manufactured by Carl Zeiss, USA). For light microscopy, the epidermis was mounted on a glass slide and examined. Three to five samples were prepared from each surface for quantitative analysis.

Determination of total phenolic content

Total phenolic content was determined using a modified Folin-Ciocalteu colorimetric method based on Amin *et al*. ¹²Gallic acid, at concentrations ranging from 12.5 to 100 mg/mL, was used as the standard. The plant extracts were dissolved in methanol, and 0.5 mL of each extract (1 mg/mL) was combined with 2.5 mL of Folin-Ciocalteu reagent and left to react at room temperature for 5 minutes. Thereafter, $2 \text{ mL of Na}_2\text{CO}_3$ solution was added. The mixture was thoroughly mixed, adjusted to 5 mL with distilled water, and incubated at room temperature for 2 hours. Absorbance was recorded at 760 nm using a UV-Visible Spectrophotometer (Thermo Scientific GENESYS 10S Series) manufactured by Thermo Fisher Scientific, USA. The total

phenolic content was determined using the gallic acid standard curve, expressed as mg of gallic acid equivalent (GAE) per gram of extract.

Determination of total flavonoid content

Total flavonoid content was assessed using a modified version of the method described by Pothitirat and Gritsanapan. ¹³ Quercetin, in concentrations ranging from 15.625 to 250 µg/mL, served as the standard. To each sample (500 µL at 1 mg/mL), 1,500 µL of 95% ethanol was added and incubated at room temperature for 6 minutes, then 100 µL of 10% aluminum chloride was added and the mixture was allowed to stand. After 5 minutes, 100 µL of 1 M potassium acetate was added, and the volume was adjusted to $5,000 \mu L$ with distilled water. The mixture was then kept in the dark at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm in triplicate. The total flavonoid content was calculated against the quercetin standard curve and expressed as mg of quercetin equivalent per gram of extract.

Determination of antioxidant activity

DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed according to the method described by Likhitwitayawuid and Sritularak.¹⁴ A 100 µM DPPH solution was prepared in methanol. Solutions of varying concentrations (1000, 500, 250, 125, and 62.5 μg/mL) were made for each extract and ascorbic acid. In a microplate, 180 µL of the DPPH solution was combined with 20 µL of the extract solution. The mixture was incubated in the dark at room temperature for 20 minutes, and the absorbance was measured at 517 nm using a microplate reader. The absorbance of the extract samples (As) was compared to methanol (blank, Ac), with ascorbic acid used as the standard. The percentage of radical scavenging activity was calculated using the following equation:

DPPH radical scavenging activity $(\%) = [(Ac - As)/Ac] \times 100$ (Eqn. 1)

ABTS assay

The ABTS assay was conducted following a modified version of the method described by Payet *et al*.¹⁵ The ABTS⁺ cation radical was produced by reacting 7 mM ABTS in water with 2.45 mM potassium persulfate. The mixture was left in the dark at room temperature for 12-16 hours before use. The resulting ABTS solution was diluted with ethanol to achieve an absorbance of 0.700 ± 0.02 at 734 nm. Solutions at varying concentrations (1000, 500, 250, 125, and 62.5 μg/mL) were prepared for each crude extract and ascorbic acid. Triplicate measurements were taken, using methanol as the blank and ascorbic acid as the standard. The percentage inhibition was calculated using the following equation:

Percentage radical scavenging = $[(Ac - As)/Ac] \times 100$. (Eqn. 2)

Gas chromatography-mass spectrometry (GC-MS)

The leaf and stem extracts of *S. atropurpurea* were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett Packard (Agilent Technologies, Palo Alto, CA, USA) model 6890 gas chromatograph equipped with a mass selective detector (MS). The GC separation utilized a fused silica capillary Hewlett Packard HP-5 column (5% phenyl methyl siloxane) measuring 30 m x 0.25 mm i.d. with a film thickness of 0.25 µm based on the method of Suphrom *et al*. ¹⁶ Helium was employed as the carrier gas at a flow rate of 1 mL/min. The initial oven temperature was set at 70°C for 3 minutes, then increased at a rate of 5° C/min to 280°C, and held at this final temperature for 10 minutes. The transfer line heater was maintained at 280°C. The mass spectrometer scanned within a range of 50-550 amu in full scan mode. Chemical compounds in the extracts were identified by comparing their retention times and mass spectra with standards from the National Institute of Standards and Technology (NIST).

Statistical analysis

All experiments were conducted in triplicate, with the results presented as mean \pm standard error of mean (SEM). Statistical comparisons were made using Duncan's test and ANOVA, with significance defined at $p <$ 0.05. Data analysis was performed using Excel 2019 and IBM SPSS Statistics 29**.**

Results and Discussion

Extraction yields

Crude extracts of *S. atropurpurea* leaf and stem were obtained by Soxhlet extraction using ethanol, dichloromethane, and ethyl acetate as solvents (Figure 1). The percentage yields of the extracts were determined based on their dry weights. The extraction yields varied significantly with the polarity of the solvents used. The results showed that the ethanol extracts from both leaves and stems had the highest yields. Solvent polarity significantly influenced the percentage yield of the extracts (Table 1). Ethanol, being the most polar solvent, resulted in the highest extraction yield, followed by ethyl acetate and dichloromethane. This result indicated that more polar solvents extracted a broader range of phytochemicals from *S. atropurpurea*. Different solvents have varying polarities, which affect their ability to extract different types of phytochemicals.

Figure 1: Leaf and stem of *S. atropurpurea*.

Microscopic characteristics of Scurrula atropurpurea

The Microscopic profiles of *S. atropurpurea* stems and leaves are shown in Figures 2 and 3. The stem bark serves as a storage site for tannins and is covered by a cuticle (Figures 2B and 2N). The cork cambium comprised 3–4 layers that run parallel to the bark (Figures 2B and 2N). The chlorenchyma in the outer cortex contains starch

granules. The vascular tissue showed phloem arranged in an approximately circular pattern around the xylem, with phloem fiber caps extending into the outer cortex (Figure 2H). The xylem consists of vessel elements characterized by alternating elliptic bordered pits, as well as spiral and scalariform thickenings (Figures 2O, 2P, and 2Q).

Table 1: Percentage yield of plant extracts

Fibers and parenchyma cells are interspersed among the vessel elements, with the parenchyma cells containing starch grains (Figure 2K). The pith contains parenchyma cells that store starch grains and rectangular prisms of calcium oxalate (Figures 2C, 2E, 2F, 2I, and 2L). Additionally, a single oil body was present within a cluster of cells (Figures 2C, 2F, 2I, 2L, 2M, 2N, and 2O). Juvenile leaves were covered with branched stellate hairs (Figure 2R), and the abaxial epidermis of the leaf contained parasitic stomata (Figures 2S, 2T, and 2U). Microscopic observations of the stem and leaf powder of this species revealed the following: The secondary cell walls of vessel cells exhibited alternating elliptic bordered pits with spiral and scalariform thickenings (Figures 3A, 3B, and 3C). Fiber cells (Figure 3D), parasitic stomata (Figure 3E), stellate hairs (Figures 3F and 3G), rectangular prisms of calcium oxalate (Figure 3H), and tannins (Figure 3I) were also readily identifiable. Previous microscopic studies on Loranthaceae revealed that structures such as trichomes (hair-like structures), lenticels, texture of the cell wall and the waxy layer (such as a cuticle) were among the most taxonomically significant characteristics for this plant family. 17

Plant secondary metabolites were detected in cells of the whole plant body but the site of biosynthesis was restricted to an organ and transported to different regions through vascular tissues to the site of storage, depending on the polarity of the metabolite. Hydrophilic compounds including alkaloids and tannins are typically stored in vacuoles or idioblasts while lipophilic compounds such as terpenebased essential oils are stored in thylakoid membranes, cuticles, resin ducts, and trichomes. The storage locations for these compounds include various tissues and structures such as leaves, shoots, roots, flowers, and specialized accumulation sites such as glandular trichomes, periderms, and phellem.¹⁸

Total phenolic content

The total phenolic content of the stem and leaf extracts ranged from 14.076 to 387.365 mg GAE/g extract (Table 2). the total phenolic content was estimated from the equation of the Gallic acid calibration curve, $Y = 0.0342x - 0.0772$; $R^2 = 0.9993$. The ethanol extracts of both the stem and leaves of *S. atropurpurea* contained the highest total phenolic content of 387.365 \pm 4.643 mg GAE/g extract, and 154.820 \pm 4.895 mg GAE/g extract, respectively.

Figure 2: Microscopic profiles of *S. atropurpurea* fresh stems and leaves. **A-L.** Transverse section of stem. **A-C.** Unstained tissues. **D-F.** Tissues stained with iodine solution. **G-I.** Tissues stained with ferric chloride hexahydrate (FeCl3.6H2O) solution. **J-L.** Tissues stained with safranin O. **M-Q.** Longitudinal section of stem and stained with safranin O. **R.** Branched stellate hair on leaves. **S-U.** Epidermis of lower leaf showing parasitic stomata. **S.** Unstained tissues. **T.** Tissue stained with iodine solution. **U.** Tissue stained with ferric chloride hexahydrate. Abbreviations: *b* bark, *cc* cork cambium, *ch* chkorenchyma, *cu* cuticle, *ic* inner cortex, *le* lenticel, *oc* outer cortex, *pa* paracytic stoma, *pf* phloem fibers, *ph* phloem, *pi* pitted vessel, *rp* rectangular prism, *sc* scaraliform vessel, *sp* spiral vessel, *xf* xylem fiber, *xy* xylem.

Total flavonoid content

The total flavonoid content was estimated from the equation of the quercetin calibration curve, $Y = 0.0121x - 0.0409$; $R^2 = 0.9982$. The total flavonoid content of the stem and leaf extracts ranged from 19.662 \pm 2.27 to 170.311 \pm 2.20 mg QE/g extract (Table 2). The dichloromethane extracts of the leaves of both *S. atropurpurea* and *E.*

succirubrum had higher flavonoid contents compared to the other solvents extracts. The total flavonoid contents of the dichloromethane extracts were 130.313 ± 3.12 mg QE/g extract, and 170.311 ± 2.20 mg QE/g extract for the leaves of *S. atropurpurea* and *E. succirubrum*, respectively.

Figure 3: Microscopic profiles of *S. atropurpurea* stem and leaf powder. **A.** Alternated elliptic bordered pits vessel thickening. **B.** Spiral vessel thickening. **C.** Scalariform vessel thickening. **D.** Group of fiber cells. **E.** Parasitic stomata. **F & G.** Stellate hair. **H.** Rectangular prism of calcium oxalate. **I.** Group of cells accumulate tannin.

Antioxidant activity

The DPPH and ABTS assays were conducted to assess the antioxidant activity of the test samples. Samples exhibiting over 50% inhibition were further analyzed to determine their IC₅₀ values. The results of the DPPH assay indicated that the ethanol extract of the stem of *Erythrophleum succirubrum* had the highest antioxidant activity with IC₅₀ of 0.290 ± 0.029 mg/mL. For the ABTS assay, results showed that the ethanol stem extracts of *S. atropurpurea* had the highest antioxidant activity with IC₅₀ of 0.509 ± 0.041 mg/mL (Table 3).

Correlation between total phenolic content and antioxidant activity The correlation between total phenolic content and DPPH and ABTS radical scavenging activities is shown in Figure 4.

Table 2: Total phenolic and flavonoid contents of plant extracts.

Values are expressed as mean \pm SD of triplicate measurements, (n = 3). Different letters in the same column indicate significant differences at p < 0.05.

Table 3: Antioxidant activity of *Scurrula atropurpurea* and *Erythrophleum succirubrum* extracts

Values are expressed as mean \pm SD of triplicate measurements, (n = 3). Different letters in the same column indicate significant differences at p < 0.05.

The results showed a correlation coefficient, $r = 0.891$ for DPPH, and r $= 0.895$ for ABTS. The Pearson correlation coefficient (r) indicated a strong correlation between antioxidant activity and total phenolic content. Phenolic compounds are considered as strong antioxidants due to their capacity to donate hydrogen atoms to free radicals.¹⁹ The ethanolic extract from the stem of *S. atropurpurea* demonstrated greater phenolic and antioxidant activity than the leaf extract, indicating that the stem extract had a higher phenolic content. This result concurred with the findings from the work of Mustarichie et al,²⁰ who investigated the antioxidant activity of *S. atropurpurea*. Results showed that ethanol, as a highly polar solvent, had the highest phenolic content and antioxidant activity compared to the ethyl acetate and hexane fractions. The results of the present study correlated with that of a previous study that investigated the antioxidant activity of *Dendrophthoe pentandra* (L.) Miq. hemiparasite on *Shorea roxburghii* G. Don and *Cassia fistula* L, where the ethanol stem and leaf extracts were found to have the highest activity, demonstrating that solvent

polarity significantly influenced the chemical composition and antioxidant activity of plant extracts.¹⁰

Correlation between DPPH and ABTS radical scavenging activities There was strong and positive correlation ($r = 0.895$) between the DPPH and ABTS radical scavenging activities.

Compounds identified from GC-MS analysis

This study identified 14 compounds in the ethanol extracts of *S. atropurpurea* leaves and stems, with (Z)-9-octadecenamide and octadecanamide being the major constituents, followed by neophytadiene and hexadecenoic acid ethyl ester. Two of these compounds are known to have antioxidant properties. Previous research by Ohashi *et al*., ²¹ and Aditiyarini *et al*. 2 concurred with the GC-MS results of the present study, with (Z)-9-octadecanoic acid identified as the main compound in both studies.

Figure 4: (A) Correlation between total phenolic content and DPPH activity of the crude extracts. Correlation coefficient, r = 0.895 **(B)** Correlation between total phenolic content and ABTS activity of the crude extracts. Correlation coefficient, $r = 0.891$

Retention	Compound name	Peak Report TIC (Area %)	
time (min)		Leaf	Stem
24.999	3-O-Methyl-d-glucose	1.77	
28.171	Neophytadiene	0.72	$\overline{}$
29.084	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.22	$\overline{}$
31.374	Hexadecanoic acid, ethyl ester	3.23	0.13
33.741	Phytol	1.15	
34.617	2-Chloroethyl linoleat	0.27	
34.633	Ethyl 9,12-hexadecadienoate		0.06
34.697	(E)-9-Octadecenoic acid ethyl ester	2.11	0.21
34.817	9-Octadecenoic acid, ethyl ester	0.52	$\overline{}$
35.150	Octadecanamide	4.66	12.66
35.177	Hexadecanamide	$\overline{}$	5.45
38.281	9-Octadecanamide, (Z)-	73.86	81.49
38.656	Tetradecanamide	10.56	
45.396	Squalene	0.92	$\overline{}$
		100	100

Table 4: Compounds identified from the GC-MS in *Scurrula atropurpurea* stem and leaf extracts

.**Conclusion**

This study presents a pharmacognostic description of the microscopic diagnostic features of *S. atropurpurea*. It also showcased the plant's antioxidant properties, and identified key compounds in the leaf and stem extracts through GC-MS analysis. Further exploration of *S. atropurpurea* bioactive constituents using advanced techniques such as LC-MS/MS will yield a more comprehensive chemical profile and may uncover additional compounds with broader medicinal value. Data obtained in this study present a sound basis for further research into developing alternative herbal medicines for the prevention and treatment of many human diseases.

Conflicts of interest

The authors declare no conflicts of interest.

Author's 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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