DOI: 10.15625/vap.2024.0106

MOLECULAR IDENTIFICATION OF TWO PROLIFERATIVE MODES OF *Cuscuta australis* R. Brown (Convolvulaceae) IN BRUNEI DARUSSALAM WITH DISTINCT PHENOLIC, FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY

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Abstract. Species of the genus Cuscuta, commonly known as dodders, have been traditionally incorporated in the herbal remedies of ancient Indian and Chinese medicinal practices. Despite their global distribution, the identification of individual species within this genus presents a significant challenge due to the absence of distinctive leaf morphology. This study aims to report the molecular identification, phenolic content, and antioxidant activities of two proliferative modes of dodders (fertile and sterile) observed in Brunei Darussalam. Employing a DNA barcoding approach, it was discovered that the sterile dodder exhibited a genetic divergence in DNA sequences (ITS and *trn*L-F) from the fertile *C. campestris*, but not from the fertile *C. australis*. This finding suggests that the sterile dodder prevalent in Brunei Darussalam is *C. australis*. Notably, the sterile *C. australis* demonstrated significantly elevated levels of total phenolics, flavonoids, and antioxidant capacities compared to its fertile counterparts. This study not only identifies the common sterile *Cuscuta* species in Brunei Darussalam but also highlights the intra-specific variation in the biochemical profiles of *C. australis*.

Keywords: parasitic plants, holoparasitic plants, genetic diversity, species boundary.

1. INTRODUCTION

Dodders are stem holoparasitic plants of the genus *Cuscuta* in the Convolvulaceae (Sarić-Krsmanović, 2020). The genus comprises 200 described species and inhabits all continents except Antarctica (Dawson, 1987). Many dodder species were commonly used in ancient Indian and Chinese medicinal remedies as liver and kidney tonics (Akbar, 2020).

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Taxonomic identification of dodders is problematic because many species are difficult to distinguish due to their uniformity in vegetative parts (García et al., 2014). The most common method for the identification and delimitation of *Cuscuta* species based on their fruit and flower morphology (Glofcheskie, 2023). Recently, DNA barcoding has provided an alternative approach to identifying species boundaries, phylogeny, character evolution and biogeography of flowering plants including *Cuscuta* species (García et al., 2014; Letsiou et al., 2024). Data of the internal transcribed spacers (ITS), which are noncoding sequences flanking the nuclear ribosomal RNA gene of 5.8S subunit of the ribosome, and chloroplast intergenic spacers (*trn*L-F), which are noncoding intergenic spacers of transfer RNA genes *trn*TUGU, *trn*LUAA and *trn*FGAA arranged in tandem, have been applied successfully to test species boundaries and define major world clades of dodders (Costea et al., 2008). Both ITS and *trn*L-F are suitable and powerful for resolving many plant taxonomic and phylogenetic issues (Besse, 2014).

Phenolics are the major bioactive secondary-compound group of *Cuscuta* species (Lee et al., 2011). For example, Azad and Mohamed (2023) reported that *C. reflexa* stems contains total phenolic content and total flavonoid content equal to 64.11 mg GAE/g and 41.08 mg QE/g dry weight, respectively. DPPH and ABTS radical scavenging was shown very potent with IC_{50} 295.12±1.33 µg/mL and 245.43±0.78 µg/mL, respectively. Phenolic compounds have one or more hydroxyl groups attached directly to an aromatic ring. An important property of phenolics, and its subgroup flavonoids, is antioxidant capacity which is defined as free radical-scavenging ability (Ghiselli et al., 2000). Soluble phenolic constituents exhibit stably in *Cuscuta* species irrespective of host plants and localities (Löffler et al., 1997). Therefore, profiles of phenolics are commonly used to authenticate the quality of dodder materials in herbal remedies (Ye et al., 2005).

The most updated plant checklist of Brunei Darussalam listed only one *Cuscuta* sp. in Brunei (Cood et al., 1996). However, Chak et al. (2010) found two proliferative modes, fertile and sterile, of *Cuscuta* species growing in Brunei Darussalam. Sterile *Cuscuta* sp. does not possess any visible floral structures but has a perennating stage, in which young shoots emerge and spread from senescent structures (Chak et al., 2010). Sterile *Cuscuta* sp. was taxonomically unclear at the species level due to the difficulty in applying the morphological approach for species identification. To overcome this constraint, in this study, we delimited species boundaries of dodders distributed in Brunei Darussalam using a DNA barcoding approach. We compared ITS and *trn*L-F DNA sequences of Brunei dodders with respective regions of *Cuscuta* DNA accessions delimited by the Stefanović et al. (2007) world phylogeny of *Cuscuta*. We also investigated total phenolics, flavonoids and antioxidant capacities of crude extracts of Brunei dodders. This was the first study to provide molecular evidence for species delimitation and preliminary profiles of bioactive compounds of dodders growing in Brunei Darussalam.

2. MATERIALS AND METHODS

2.1. Sample collection, DNA isolation, PCR amplification, sequencing and sequence alignment

Dodder distribution survey and sampling for DNA isolation were conducted since January, 2010. Samples were collected and silica dried for DNA analysis. Sampling sites were revisited and monitored from June, 2012 to December, 2014. Morphological species delimitation for fertile material was based on keys described by Spaulding (2013).

Total genomic DNA was extracted from silica-dried tissues using a DNeasy Plant Kit (Qiagen, Valencia, California, USA). The ITS region was amplified by polymerase chain reaction (PCR) using primers ITS4 and ITS5 (White et al., 1990). The *trn*L-F region was amplified by PCR using primers c and f. PCR products were purified using AMPure PCR purification kit (Agencourt, Beverly, Massachusetts, USA). DNA sequencing was completed by Macrogen (Seoul, Korea). Sequences were manually edited using Sequencer 3.0 (Gene Codes, Ann Arbor, Michigan, USA). DNA sequences were submitted to Genbank database and respective voucher specimens were deposited in Herbarium of Universiti Brunei Darussalam-UBDH (KT312995 and KT312996 Genbank accessions for UBDH-PC2 voucher specimen; KT312997 and KT312998 Genbank accessions for UBDH-PC3 voucher specimen).

ITS and *trn*L-F regions of Brunei dodders and Genbank accessions [*C. campestris* DNA accessions (EF194663 and EF194450) and *C. australis* DNA accessions (EF194667, EF194668, EF194669, EF194670, EF194457 and EF194458)] published by Stefanović et al. (2007) were aligned by Jalview®.

2.2. Determination of total phenolics, flavonoids and antioxidant capacity

Plant materials

Dodder samples for measurements of total phenolics and flavonoids and antioxidant capacity, were collected from two populations (fertile and sterile) in close vicinity (N 04 54.485' E 114 55.548' and N 04 54.714' E 114 55.494', respectively) in July, 2012. We inoculated these dodder samples on the host *Mikania micrantha* grown in pots at the plant house of Universiti Brunei Darussalam and monitored for proliferation for 2 years under uniform sunlight of 1,000 μmol quantum m^{-2} s^{-1}, temperature of 25-35 ^{o}C and relative humidity of 60-65%. In June, 2014, stems of comparable length and age from sterile and flowering dodders parasitizing *M. micrantha* were collected separately. Samples were then soaked in tap water and rinsed with double deionized H_2O before freeze drying at 4 ^{o}C for 2 weeks.

Sample extraction

Dried sample powder (0.05 g) was extracted with 20 mL solvents by shaking the mixture at room temperature for 2 h. The mixture was then centrifuged at 4,500 rpm for 20 min and the supernatant was separated for sequential assays. Extraction was carried out using different solvents namely absolute EtOH, 80% EtOH in water, absolute MeOH or 80% MeOH in water (Merck®, Darmstadt, Germany).

Determination of phenolics and flavonoids contents

Phenolic content was determined using Folin-Ciocalteu colorimetric method as described by Swain and Hillis (1959) and Azad and Mohamed (2023) with some

modifications. Briefly, 0.3 mL of sample extract was mixed with 2.25 mL of 10-fold diluted Folin-Ciocalteu reagent. After 5 min, 2.25 mL of 6% sodium carbonate was added to the mixture and allowed to stand at room temperature for 90 min. The absorbance of mixture was measured at 725 nm. A calibration curve was obtained using gallic acid (Sigma-Aldrich®, Buchs, Switzerland) as the standard. Results are expressed as gallic acid equivalent (GAE) [mg(GAE) g-1 (dry mass of sample)].

Flavonoid content was determined using aluminum colorimetric method as described by Zhishen et al. (1999) and Azad and Mohamed (2023) with some modifications. Briefly, 1.0 mL of sample extract was mixed with 0.2 mL of 10% aluminium chloride, 0.2 mL of 1 M potassium acetate, 3.0 mL of respective extracting solvent and 5.6 mL ddH_2O. After 30 min at room temperature, absorbance was measured at 414 nm. A calibration curve was obtained using quercetin (Sigma-Aldrich®, Buchs, Switzerland) as the standard. Results are expressed as quercetin (QC) equivalents [mg(QC) g^{-1}(dry mass of sample)].

Determination of antioxidant activity

Antioxidant capacity was determined by three assays viz: 2.2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing ability of plasma (FRAP). Calibration curves were obtained using Trolox (Sigma-Aldrich®, Buchs, Switzerland) as the standard. Results are expressed as Trolox equivalents (TE) [µmol(TE) g^{-1}(dry mass of sample)].

The ABTS assay was performed as described by Arnao et al. (2001) and Azad and Mohamed (2023) with some modifications. The working ABTS solution was prepared as described by Thaipong et al. (2006) and Azad and Mohamed (2023). For the ABTS assay, 0.15 mL of sample extract was mixed with 2.85 mL of working ABTS solution and kept at room temperature in dark. After 2 h, the absorbance was measured at 734.

The DPPH assay was performed according to Brand-Williams et al. (1995) with some modifications. The DPPH working solution was prepared as described by Thaipong et al. (2006). To perform DPPH assay, 0.15 mL of sample extract was mixed with 2.85 mL of working DPPH solution and kept at room temperature in dark. After 24 h, the absorbance was measured at 515 nm.

The FRAP assay was performed as described by Benzie and Strain (1999) with some modifications. Briefly, 0.15 mL of sample extract was mixed with 25 mL of 300 mM acetate buffer (3.1 g sodium acetate and 16 mL glacial acetic acid in 1,000 mL ddH_2O, pH = 3.6), 2.5 mL of 10 mM TPTZ solution in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$. The mixture was kept at room temperature in dark. After 30 min, the absorbance was measured at 593 nm.

2.3. Statistical analysis

Student t-tests for all statistical comparisons were conducted using R version 4.4.0 (R Core Team, 2024).

3. RESULTS

3.1. Occurrence and morphology of dodders in Brunei Darussalam

We recorded dodders at 19 sites in Brunei Darussalam during a survey conducted since January, 2010 (Table 1). Dodders were distributed mainly in disturbed habitats along roadsides, stream banks or nearby drainage canal sluices. We found that dodders were fertile (showing flowers and fruits) at 3 sites (Brunei-15, Brunei-16 and Brunei-29; see Table 1) and persistently sterile at the other 16 sites over 5 years of monitoring performed. Dodders were consistently either sterile (with flowers and fruits) or sterile characteristics at respective study sites from January, 2010 to December, 2014, each site was surveyed several times per year.

Table 1. *GPS coordinates and locations of sampling sites, DNA sequence lengths and proliferative modes of dodders in Brunei Darussalam.*

Sample code	GPS coordinate	Local name of the location	DNA length (nucleotide)		Proliferative mode
			ITS	trnL-F	
Brunei-1	N 04 57.237' E 114 54.234'	Rimba	612	484	Sterile
Brunei-3	N 04 55.057' E 114 55.305'	Tungku Highway	643	467	Sterile
Brunei-4	N 04 56.505' E 114 56.743'	Teguh Raya	640	484	Sterile
Brunei-8	N 05 01.681' E 115 02.990'	Kampong Sabuh	640	421	Sterile
Brunei-10	N 05 00.221' E 115 03.571'	Serasa Beach	627	483	Sterile
Brunei-11	N 04 59.315' E 115 01.262'	Sungai Buloh	640	428	Sterile
Brunei-12	N 04 57.148' E 114 58.166'	Tiong Hinc	641	459	Sterile
Brunei-15	N 04 54.714' E 114 55.494'	Menglait	639	484	Fertile
Brunei-16	N 04 54.714' E 114 55.494'	Menglait	650	470	Fertile
Brunei-17	N 04 54.485' E 114 55.548'	Sungai Kedayan	620	484	Sterile
Brunei-18	N 04 53.394' E 114 54.283'	Beribi	640	470	Sterile
Brunei-24	N 04 59.511' E 114 56.973'	Mukim Berakas	623	470	Sterile
Brunei-25	N 04 53.759' E 114 50.695'	Sungko Rong	625	484	Sterile
Brunei-27	N 04 49.106' E 114 50.431'	Kampong Pait	629	484	Sterile

Sample code	GPS coordinate	Local name of the location	DNA length (nucleotide)		Proliferative mode
			ITS	trnL-F	
Brunei-29	N 04 40.887' E 114 30.058'	Sungai Liang	602	431	Fertile
Brunei-30	N 04 36.112' E 114 19.848'	Seria	636	470	Sterile
Brunei-31	N 04 34.691' E 114 13.911'	Mumong	611	482	Sterile
Brunei-32	N 04 34.290' E 114 13.420'	Belait junction	638	488	Sterile
Brunei-33	N 04 35.623' E 114 30.735'	Luagan Lalak	623	481	Sterile

Floral morphology suggested that fertile dodders at two sites (Brunei-15 and Brunei-16) were *C. australis* while those at the other site (Brunei-29) were *C. campestris*. *Cuscuta australis* was characterized with flowers 5-merous, calyx equaling corolla tube, calyx lobes ovate-orbicular; stamens shorter than lobes; styles stout, subulate and shorter than ovary. *Cuscuta campestris* comprised with flowers 4 to 5-merous, calyx lobes not strongly overlapping at the base; calyx lobes obtuse; anthers 2-lobed; stigmas capitated. For this study, we assigned these fertile dodders as "Brunei fertile *C. australis*" and "Brunei fertile *C. campestris*".

3.2. Species identification of sterile dodder using ITS and *trn*L-F sequences

The genetic identity and distance of ITS and *trn*L-F sequences among Brunei dodders and Genbank accessions were shown in Table 2. We did not detect any sequence variation in ITS or *trn*L-F regions among Brunei sterile dodder, Brunei fertile *C. australis* and Genbank accessions of *C. australis*. However, Brunei sterile dodder and Brunei fertile *C. australis* exhibited sequence variation in ITS and *trn*L-F regions compared with Brunei fertile *C. campestris* and Genbank *C. campestris* accessions.

Table 2. *The percent sequence identity (Identity) and genetic distance (Distance) for ITS (without parenthesis) and trnL-F (in parenthesis) regions of Brunei dodders*

	Brunei *C. campestris*	Genbank *C. campestris*	Genbank *C. australis*	Brunei fertile *C. australis*	Brunei sterile *C. australis*	
Brunei *C. campestris*		100 (100)	99.3 (96.2)	99.3 (96.2)	99.3 (96.2)	Identity
Genbank *C. campestris*	0 (0)		99.1 (96.2)	99.1 (96.2)	99.1 (96.2	
Genbank *C. australis*	3 (15)	5 (15)		100 (100)	100 (100)	
Brunei fertile *C. australis*	3 (15)	5 (15)	0 (0)		100 (100)	
Brunei sterile *C. australis*	3 (15)	5 (15)	0 (0)	0 (0)		
	Distance					

3.3. Total phenolics, flavonoids and antioxidant capacity of dodder extracts

Table 3 shows total phenolics and flavonoids of stem extracts of Brunei sterile dodder and fertile *C. australis*. Sterile dodder had significantly higher total phenolics and flavonoids than fertile *C. australis* (18.8-49.8% and 51.4-75.8%, respectively, $P < 0.001$). The flavonoid/ phenolic content ratios were 68.6-85.2% in sterile dodder and 59.5-71.4% in fertile *C. australis*.

Table 3. *Total phenolics, flavonoids and antioxidant capacity of stem crude extracts of C. australis growing in Brunei Darussalam.*

Extraction method and sample source	Phenolics [mg(GAE) g^{-1}(dry mass)]	Flavonoids [mg(QE) g^{-1}(dry mass)]	Antioxidant capacity [µmol(TE) g^{-1}(dry mass)]		
			ABTS	DPPH	FRAP
Absolute ethanol					
Fertile *C. australis* (*n = 3*)	22.0 ± 1.5^{a}	15.7 ± 1.0^{a}	187 ± 10^{a}	104 ± 13^{a}	93 ± 11^{a}
Sterile *C. australis* (*n = 3*)	32.4 ± 0.9^{b}	27.6 ± 0.4^{b}	232 ± 10^{b}	168 ± 03^{b}	212 ± 02^{b}
80% ethanol					
Fertile *C. australis* (*n = 3*)	25.7 ± 1.0^{c}	15.3 ± 0.4^{c}	231 ± 08^{c}	115 ± 21^{c}	131 ± 07^{c}
Sterile *C. australis* (*n = 3*)	38.5 ± 2.4^{d}	26.4 ± 0.9^{d}	297 ± 24^{d}	224 ± 03^{d}	246 ± 27^{d}
Absolute methanol					
Fertile *C. australis* (*n = 3*)	30.2 ± 1.2^{e}	19.7 ± 0.2^{e}	249 ± 10^{e}	204 ± 23^{e}	150 ± 15^{e}
Sterile *C. australis* (*n = 3*)	41.0 ± 1.9^{f}	31.7 ± 0.9^{f}	297 ± 09^{f}	306 ± 15^{f}	263 ± 03^{f}
80% methanol					
Fertile *C. australis* (*n = 3*)	35.7 ± 1.3^{g}	21.8 ± 1.0^{g}	238 ± 10^{g}	256 ± 08^{g}	164 ± 12^{g}
Sterile *C. australis* (*n = 3*)	42.4 ± 0.8^{h}	33.0 ± 1.1^{h}	266 ± 07^{h}	279 ± 07^{h}	241 ± 08^{h}

Note: Data are expressed as mean±SD. Tests for differences between fertile and sterile C. australis were conducted using Student t-test at 95% confidence interval. Different superscript letters denote the statistically significant difference between fertile and sterile dodders. The comparison was made in the same regime of solution and assay. TE: Trolox equivalent; GAE: gallic acid equivalent; QE: quercetin equivalent.

Brunei sterile dodder also exhibited significantly higher antioxidant capacity than fertile *C. australis* in all assays (Table 3; $P < 0.001$). Antioxidant capacity of sterile dodder was the highest in absolute methanol extract assayed by DPPH (306 µmol TE g^{-1} dry mass), whereas fertile *C. australis* showed the highest antioxidant capacity in 80% methanol extract assayed by DPPH (256 µmol TE g^{-1} dry mass).

There was a significantly strong positive correlation between total phenolic and flavonoid contents (Table 4, $P < 0.01$). In addition, total phenolic and flavonoid contents also showed significantly strong positive correlations with antioxidant capacities of dodder extracts measured by ABTS, DPPH and FRAP assays ($P < 0.05$).

Table 4. *Coefficient (without parenthesis) and respective P values (in parenthesis) of Pearson correlation test (n = 8) of total phenolics, flavonoids and antioxidant capacities of dodder extracts measured by ABTS, DPPH and FRAP assays.*

	Phenolics	Flavonoids	ABTS	DPPH	FRAP
Phenolics	1	0.915	0.854	0.938	0.925
Flavonoids	(0.0014)	1	0.723	0.812	0.942
ABTS	(0.0070)	(0.0426)	1	0.786	0.885
DPPH	(0.0006)	(0.0144)	(0.0209)	1	0.791
FRAP	(0.0010)	(0.0005)	(0.0035)	(0.0195)	1

4. DISCUSSION AND CONLUSION

In this study we found that dodders in Brunei Darussalam exhibited sterile and sterile modes in their life cycles for at least 5 years since January, 2010 to December, 2014 as in agreement with report of Chak et al. (2010). Our data on genetic identity and distance among the sterile dodder, *C. australis* and *C. campestris* showed clearly that the sterile dodder is *C. australis*. Stefanović et al. (2007) reported that *C. australis*, *C. obtusiflora* and *C. campestris* are among Cuscuta species with the widest geographical distributions and ecological amplitudes and form a monophyletic group with several other species. *Cuscuta australis* is distributed in Asia, Australia and Europe, whereas *C. obtusiflora* has spanned the entire western hemisphere and *C. campestris* has spread worldwide (Dawson et al., 1994; Stefanović et al., 2007). Stefanović et al. (2007) used ITS and *trn*L-F sequence data to reliably delimit most species of dodder including "Clade B" where *C. australis* was resolved. Thus, we have confidence that results of our molecular identification coupled with the morphological characters of the fertile *C. australis* correctly indicate the species identity of the sterile dodder in Brunei Darussalam.

Vegetative parts of dodders are very simple in structure and exhibit great uniformity. Therefore, fruit, flower, especially calyx structures are useful characters for the identification and delimitation of *Cuscuta* species (Glofcheskie et al., 2023). However, this method cannot be applied to the sterile dodders due to the lack of floral characteristics. Here we illustrated the successful application of a molecular DNA barcoding approach to overcome the lack of floral organs of the sterile dodder in Brunei Darussalam.

Our findings showed that profiles of total phenolics, flavonoids and antioxidant capacities of *C. australis* in Brunei Darussalam exhibited significant intra-specific variation according to different proliferative modes (fertile or sterile). Sterile *C. australis* had significantly higher total phenolics, flavonoids and antioxidant capacities. We also detected strong positive correlations between antioxidant capacities with total phenolics or flavonoids. Antioxidant activity of dodders is mainly attributed to phenolic compounds (Craft et al., 2012). Anjum et al. (2013) also demonstrated a strong correlation between total phenolics and antioxidant activity of the methanol extracts from *Cuscuta* sp. stem. Ye et al. (2002) suggested that the medicinal values of *Cuscuta* seeds can be evaluated according to flavonoid content.

Stems of *C. australis* investigated in this study showed higher total phenolic contents (35.7 and 42.4 mg GAE g^{-1} dry mass for fertile and sterile dodders, respectively) compared to *C. australis* collected in Thailand (33.21 mg GAE g^{-1} dry mass; see Phomkaivon and Areekul (2009) using a similar extracting solvent (80% MeOH) and determination assay (Folin-Ciocalteu). Total phenolic content of aqueous and methanol extracts from *C. chinensis* (9.1 and 6.69 mg GAE g^{-1} dry mass, respectively) reported by Wong et al. (2006) was much lower than our results. However, Azad and Mohamed (2023) reported that the total phenolic content of *C. reflexa* stem (64.11 GAE g^{-1} dry mass) was much higher than *C. australis* investigated in our study. Currently, many methods are used to evaluate the antioxidant capacity of plant samples but no single test can be satisfactory due to the complex matrix of antioxidant activity in plant sources (Grigelmo-Miguel et al. 2010). The common approach is to use many assays for screening antioxidant properties of plants (Aruoma, 2003; Grigelmo-Miguel et al., 2010). In this investigation, we used three common assays (ABTS, DPPH and FRAP) to measure total antioxidant capacities of dodder extracts. Regardless of the different assays used, Brunei sterile *C. australis* showed significantly higher antioxidant capacity than fertile plants. Our findings suggested that stems of *C. australis* in Brunei Darussalam have medicinal potential (e.g. radical-scavenging capacity related to antioxidants) and the sterile *C. australis* stocks have a much higher potential to be used in herbal remedies than the fertile stocks.

During this 5-year investigation, we found that the sterile *C. australis* distributed more widely in Brunei Darussalam than the fertile *C. australis* and *C. campestris*. The distribution of dodders may relate to contents of phenolics and flavonoids in tissues. This is reasonable because an accumulation of phenolic compounds in plant tissues can be observed under stress conditions (Michalak, 2006). Thus, high contents of phenolics and flavonoids may facilitate dodders to overcome stress induced by defense system of different host species to successfully parasite and infest hosts (Glatzel and Geils, 2009). However, we do not have enough evidence to illustrate the involvement of phenolics and flavonoids in the invasive capacity of Brunei dodders.

In conclusion, the DNA barcoding molecular approach using sequences of ITS and *trn*L-F in this study were successful in identifying the consistently sterile dodder populations in Brunei Darussalam as *C. australis*. This study also investigated, for the first time, profiles of phenolics, flavonoids and antioxidant capacities of Brunei sterile and fertile *C. australis*. The relationships of proliferative modes, distribution and bioactive compound profiles of *C. australis* in Brunei Darussalam should be compared with similar populations in other regions to exactly understand the reasons behind the existence of persistently sterile *Cuscuta* populations over a number of life cycles in specific locations.

ACKNOWLEDGEMENTS

This work was supported by funding provided by the Brunei Research Council (UBD-Science and Technology Research Grant No. 8) and Graduate Research Scholarship Programme, Universiti Brunei Darussalam. Logistic and laboratory facilities provided by the Faculty of Science and Institute for Biodiversity and Environmental Research (IBER),

Universiti Brunei Darussalam are gratefully acknowledged. We thank to W.H. Chak, R. Jabaily and A.L. Bieber for field work and assistance with molecular assays.

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SỬ DỤNG PHƯƠNG PHÁP PHÂN TỬ XÁC ĐỊNH DẠNG HỮU DỤC VÀ BẤT DỤC CỦA TƠ HỒNG (*Cuscuta australis* R. Brown, Convolvulaceae) CÓ HÀM LƯỢNG TỔNG PHENOLIC, FLAVONOID VÀ KHẢ NĂNG OXY HÓA KHÁC NHAU

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Tóm tắt: Các loài thuộc chi *Cuscuta*, thường được biết đến với tên gọi dây tơ hồng, thường được sử dụng trong các phương thuốc thảo dược của y học cổ truyền Ấn Độ và Trung Quốc. Mặc dù phân bố rộng rãi trên toàn cầu, việc xác định các loài trong chi này gặp nhiều thách thức do chúng không thể hiện các đặc điểm hình thái lá đặc trưng. Nghiên cứu này đã sử dụng phương pháp phân tử để phân định loài, đánh giá hàm lượng phenolic, flavoid và hoạt tính chống oxy hóa của hai dạng dây tơ hồng có đặc điểm tăng sinh khác nhau (dạng hữu dục có hoa và bất dục không có hoa) được quan sát tại Brunei Darussalam. Sử dụng phương pháp mã vạch DNA, chúng tôi phát hiện rằng dây tơ hồng bất dục có sự khác biệt di truyền trong trình tự DNA (ITS và *trn*L-F) so với *C. campestris* hữu dục, nhưng không khác so với *C. australis* hữu dục. Kết quả này cho thấy, dây tơ hồng bất dục phát triển phổ biến tại Brunei Darussalam là *C. australis*. Đáng chú ý, *C. australis* bất dục này có tổng hàm lượng phenolic, flavonoid và khả năng chống oxy hóa cao hơn đáng kể so với dạng hữu dục tương ứng.

Keywords: Cây ký sinh, thực vật dị ký sinh, đa dạng gene, ranh giới loài.

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