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Research article

Effect of harvesting age and drying condition on andrographolide content, antioxidant capacity, and antibacterial activity in *Andrographis paniculata* (Burm.f.) Nees

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Abstract: Quality of dried *Andrographis paniculata* (Burm.f.) Nees materials is important to determine its effectiveness in traditional medicine. The present study aimed to investigate an effect of harvesting age and drying condition on andrographolide content and its consequences on antioxidant and antibacterial activities. The plants were cultivated and harvested at 90, 100, 115, and 127 days after sowing (DAS) prior to drying under the sun or using hot air oven at 50, 65, and 80 ℃. The results indicated that drying condition significantly influenced andrographolide content, antioxidant capacities, and antibacterial activity of *A. paniculata*, whereas the harvesting age had no significant impact on those parameters. The andrographolide contents ranged from 0.74–4.11% (w/w) dry weight. The highest andrographolide contents were obtained at 90 DAS/65 ℃, 127 DAS/65 ℃, and 127 DAS/65 ℃. *A. paniculata* extracts were found to exhibit antibacterial activity against gram-positive bacterial strains (*B. cereus*, *M. luteus*, *S. epidermidis*, and *S. aureus*), which the highest antibacterial activity was observed at 90 DAS/65℃. The used of hot air oven at 65 ℃ effectively preserved andrographolide content and antibacterial activity of *A. paniculata*. In contrast, drying at 50 ℃ was the preferable drying condition for antioxidant capacity. The antioxidant activities of *A. paniculata* extracts ranged from 3.43–26.73 and 1.93–17.28 mg Trolox/g dry weight for DPPH• scavenging activity and FRAP reducing power activity, respectively. Overall, *A. paniculata* is suggested to dry

using hot air oven at 65 ℃ to maintain high levels of andrograhpolide and antibacterial activity. Drying using hot air oven at 50 ℃ is advised, if antioxidant activity is the main focus. Even though the harvesting age is not a key parameter, *A. paniculata* is suggested to harvest at 50% flowering stage onward for a better total herbage and andrographolide yield.

Keywords: *Andrographis paniculata* (Burm.f.) Nees; andrographolide; antimicrobial activity; antioxidant activity; plant maturity, drying; medicinal plant preparation

Abbreviations: DAS: days after sowing; DMRT: Duncan's Multiple Range Test; DMS: Department of Medicinal Sciences; DMSO: Dimethyl sulfoxide; MIC: minimal inhibitory concentration

1. Introduction

Andrographis paniculata (Burm.f.) Nees or known as "fah talai jone" in Thailand, is a medicinal plant that belongs to the Acanthaceae family. It is widely cultivated in subtropical and tropical regions and traditionally used to treat conditions such as common cold, upper respiratory infection, AIDs, and arthritis [1,2]. In Thailand, *A. paniculata* is in the National List of Essential Medicines to treat non-infectious diarrhea, pharyngotonsillitis, and common cold [3]. This plant species contains large quantity of bitter diterpenoid lactones, of which andrographolide is identified as a major bioactive compound [4]. Andrographolide is abundantly distributed in aerial parts of the plant, and its highest amount is found in leaves [5]. It has been reported to pose antimicrobial, anti-inflammatory, antioxidant, anticancer, and antiviral activities [1,4,6,7]. In the late 2020, the Thai government has approved the use of *A. paniculata* medicine to treat early/mind symptoms and reduce severity of Covid-19 [8]. *A. paniculata* and its bioactive compounds were reported to inhibit the SAR-CoV-2 entry and/or replication based on in vitro test systems [9].

A major problem hampering the use of *A. paniculata* in traditional medicine is a variation in andrographolide content of dried plant materials. In an effort to standardize the quality of the bioactive compounds from dried plant materials, the Thai Department of Medicinal Sciences requires that dried *A. paniculata* materials should contain more than 1% w/w of andrographolide [10]. The variation of andrographolide content is partly influenced by plant maturity (harvesting age) and drying conditions. Plant maturity is an important factor that is said to influence the levels of bioactive compounds in medicinal plants. Several studies found that andrographolide content in *A. paniculata* leaves declined as the plant maturity stage progressed [5,11,12]. An optimal harvesting age of *A. paniculata* are reported to be in the range of 120–130 days after sowing [5,12,13]. Besides plant maturity stage, drying is an important step in herbal preparation as it has a great influence on bioactive compounds in *A. paniculata* [14,15]. In the study of Gulati et al. [14], shade drying was found to maintain the highest amount of andrographolide followed by drying in hot air oven, sunlight, and microwave, respectively. The drying temperature also reported to affect the quality of dried *A. paniculata* materials and yields of bioactive compounds. For instance, plant materials dried in hot air oven at 60 ℃ yielded a higher amount of total lactones than at 40 ℃ and 50 ℃ [16].

For a sustainable use of *A. paniculata* as medicinal plant, it is important to pay attention to the impact that the quality of dried medicinal plant materials has especially on the amount of major bioactive compound, which will ultimately influence the efficacy of *A. paniculata*. Therefore, the

present study aimed to assess the influence of harvesting age and drying condition on andrographolide content and its consequences on biological activities focusing on antioxidant and antibacterial activities. Findings and outcomes of the study are to ultimately inform a proper guidance for the preparation of dried *A. paniculata* plant materials.

2. Materials and methods

2.1. Materials

Andrographolide (≥95%), acetic acid, 2,2-Di(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6 Tris(2-pyridyl-s-triazine (TPTZ), ferric chloride hexahydrate, hydrochloric acid (HCl), resazurin, sodium acetate trihydrate, were purchased from Sigma-Aldrich (MO, USA). Dichloromethane (AR grade), methanol (HPLC grade) and water (HPLC grade) were obtained from RCI Labscan (Bangkok, Thailand). Mueller-hinton broth and mueller-hinton agar were purchased from HiMedia Laboratories Pvt. Ltd (Mumbai, India). Dimethyl sulfoxide (DMSO) was obtained from LOBA Chemie (Mumbai, India). Methanol (industrial grade) was from Chemipan Corporation Co., LTD. (Rayong, Thailand).

Bacterial strains: *Bacillus cereus* (TISTR 1527), *Staphylococcus aureus* (TISTR 2329), and *Escherichia coli* (TISTR 074) were obtained from the Faculty of Natural Resources and Agro-Industry, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus, Thailand. *Micrococcus luteus* (ATCC 4698) and *Staphylococcus epidermidis* (ATCC 12228) were kindly provided from the Faculty of Public Health, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus, Thailand.

2.2. Field experiments

A. paniculata seeds were collected from field in Thailand and were authenticated by Asst. Prof. Dr. Hathairat Chokthaweepanich. The seeds were obtained from same region as the field experiment, therefore the plant growth and bioactive compounds should be similar in genetic and chemotypes as reported by Wijarat et al. [17] that the distribution of *A. paniculata* among each region likely belonged to the same variety and relatively undifferentiated across a large geographic range. Field experiments were conducted from June to November in 2020, at Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus, Thailand. The temperature and the relative humidity during that periods were in the range of 14–42 ℃ (average at 27 ℃) and 43–118% (average at 98%), respectively. The soil is classified as Phon Phisai series [Loamy-skeletal over clayey, kaolinitic, isohyperthermic, Typic (Oxyaquic Plinthic) Paleustults]. The field plots consisted of 36 plots with a plot size of 1.00 x 5.00 m and a space of 0.80 m in between plots. For field preparation, the soils were mixed with 1 kg/m^2 of chicken manure and rice husk. Prior sowing, all seeds were soaked in the water at 40 ℃ for 60 min and sown into the field plots (approximately 3.12 g of seeds/plot). Weed control was carried out manually and using machines throughout the growing periods and no pests or disease occurred during planting. Watering sources were mainly from rainfalls and occasionally from sprinkler system distributed around the fields. The aerial parts of the plants (i.e. stem, leave and flower (at flowering stage)) were randomly harvested at 90, 100, 115, and 127 days after sowing (DAS) with 9 plots per harvesting age.

2.3. Plant sample preparation and extraction

The fresh plant samples were weighted and washed prior to drying. The pooled plant samples for each harvesting age were randomly divided into 4 groups including drying under sunlight (widely used method for small scale farmers), drying with hot air oven at 50, 65, and 80 ℃. All samples were dried until the moisture contents of about 5.15 ± 2.08%, which was recorded by a moisture analyzer (Sartorius MA45, Germany). Dried plant samples were ground using high speed grinder, sifted through 80 mesh sieve and kept at room temperature in hermetically sealed plastic bags for further analysis.

The extraction procedures were based on Thai Pharmacopeia 2020 protocol described by Department of Medicinal Sciences (DMS) of Thailand with some modifications [10]. The extraction time and ratio of plant sample to extracted solvent were optimized (data not shown). The plant powders were extracted with methanol:dichloromethane (1:1 v/v) in the ratio of 1:25 (w/v) by continuously shaking for 30 min at 37 ℃. Each sample were extracted 3 times and the pooled extracts were filtered with Whatman No. 1 and dried under reduced pressure at 50 ℃ using rotary evaporator (Hei-VAP Expert, Heidolph, Germany). Aliquots of dried crude extract dissolving in methanol were prepared and kept at −20 ℃ until HPLC and antioxidant activity analysis. While, the rest of the dried crude extracts were dissolved in DMSO and kept at −20 ℃ until antibacterial activity analysis. The extractions were carried out in triplicates to help determine the level of variability in the samples used.

2.4. HPLC analysis of andrographolide

Andrographolide contents were determined using HPLC-DAD (1200 series Agilent, Germany) connected with a guard column XBD-C18 (4.6 x 12.5 mm, 5 µM) and a ZORBAX Eclipse C18 column (4.6 x 150 mm, 5 µM) (Agilent, Germany). The HPLC methods were based on Thai Pharmacopeia 2020 protocol with some modifications [10]. The samples were diluted with the mobile phases to get a concentration about 2 mg/mL and filtered with 0.45 µM nylon membrane filter prior to injection. Aliquots of 20 µL were injected and analyzed using isocratic conditions (35% water and 65% methanol) with a flow rate of 1 mL/min, run time of 10 min, at ambient column temperature, and reading at 224 nm. The amount of andrographolide present in samples was quantified based on calibration curves obtained from andrographolide standard compound. The experiments were carried out in triplicates.

2.5. Antioxidant activity analysis

2.5.1. DPPH free radical scavenging activity assay

The free radical scavenging activity of *A. paniculata* was performed based on methods described by Gasemzadeh et al. [18] with some modifications. In short, 0.5 mL of 0.1 mM DPPH$^{\bullet}$ in methanol were mixed with 0.5 mL of plant sample solution in methanol or Trolox solution. The mixtures were incubated in the dark for 30 min at room temperature and the absorbance was immediately measured at 517 nm using Synergy HT (BioTek instrument, USA). Blank incubation was carried out with methanol. The experiments were carried out in quadruplicates. The free radical scavenging activity was estimated based on the calibration curve of the DPPH free radical scavenging activity of Trolox and expressed as milligram of Trolox equivalent per gram of dried weight (mg Trolox/g DW). The DPPH free radical scavenging activity (%) was calculated using the following equation (Eq. 1):

$$\text{DPPH free radical scavenging activity (\%)} = (A_{blank} - A_{inhibitor})/A_{blank} \times 100 \quad (1)$$

where A_{blank} is the absorbance of the blank and $A_{inhibitor}$ is the inhibitor reading at 517 nm.

2.5.2. FRAP reducing antioxidant power assay

The reducing antioxidant power of *A. paniculata* was performed based on methods described by Gasemzadeh et al. [18] with some modifications. FRAP reagent was freshly prepared by mixing 10 volume of 300 mM acetate buffer pH 3.6 (adjusted pH with 0.5 M acetic acid) with 1 volume of 20 mM $FeCl_3$ and 1 volume of 10 mM TPTZ solution in 40 mM HCl. FRAP reagent (1.5 mL) was mixed with 50 µL of plant sample solution in methanol or Trolox solution. The mixtures were incubated in the dark for 4 min at room temperature and the absorbance was immediately measured at 593 nm using Synergy HT (BioTek instrument, USA). Blank incubation was carried out with ultrapure water. The experiments were carried out in quadruplicates. The FRAP value was estimated based on the calibration curve of the reducing antioxidant power of Trolox and expressed as milligram of Trolox equivalent per gram of dried weight (mg Trolox/g DW). The FRAP value was calculate by subtract the sample absorbance with the blank absorbance.

2.6. Antibacterial activity analysis

Bacterial strains were grown aerobically in Mueller-Hinton broth at 35 ℃ for 16–18 h until the initial cell cultures reached 10^8 CFU/mL approximately. The turbidity of microbial cell suspension was adjusted to 0.5 McFarland scale before use.

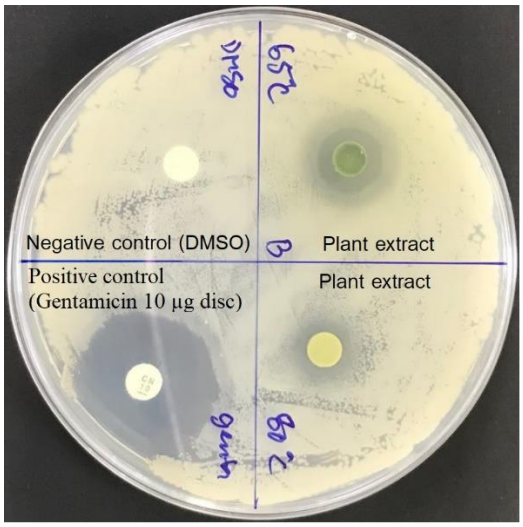

Figure 1. Plate design for the antibacterial zone inhibition experiment.

The agar disc-diffusion method was carried out to evaluate the antibacterial activity of *A. paniculata* extract according to Prajuabjinda et al. [19] with some modifications on incubation temperature of 35 ℃ and incubation time of 24 h. Disc-diffusion method determines presence or absence of bacterial growth inhibition. Briefly, 100 µL of bacterial inoculum at the cell density of 2 x 10^8 CFU/mL were inoculated onto mueller-hinton agar plates and left at room temperature for 15 min.

Once the bacterial cells dry, sterile filter paper discs (6 mm in diameter of the inhibition zone) were placed on the top of mueller-hinton agar plates. Subsequently, 20 µL of the extract (50 mg/mL) were pipetted into the sterile filter paper discs and left at room temperature for 30 min prior incubation at 35 ℃ for 24 h. The inhibition of bacteria growth was determined by measuring the clear zone around filter paper discs as depicted in Figure 1. Gentamicin (10 µg disc), an antibiotic drug uses to treat various types of bacterial infections, was used as positive control and DMSO was used as negative control or solvent control.

2.7. Determination of the minimal inhibitory concentrations

The Minimal inhibitory concentration (MIC) was carried out by the modified resazurin microdilution methods described by Elshikh et al. (2016) with slight modifications. Briefly, the extract solutions (0.02–25.00 mg/mL) were prepared by diluting the 50 mg/mL extract stock in DMSO with Mueller-Hinton broth. In 96 wells plate, 50 µL of the extract was mixed with 50 µL of bacterial inoculum (approximately 10^8 CFU/mL). The mixtures were incubated at 35 ℃ for 18 h before adding 10 µL of resazurin (0.015% w/v). After incubation at 35 ℃ for 2–4 h, the lowest concentration that had a blue color of resazurin was observed and noted as the MIC value. The MIC was also carried out with andrographolide standard compound at concentration ranging from 0.0075–1 mg/mL. The negative control was carried out without extract and bacterial inoculum.

2.8. Statistical analysis

Statistical analysis was carried out using a one-way ANOVA to determine any significant differences followed by Duncan's Multiple Range Test (DMRT) using SPSS version 28.0.1.0 (15) (IBM, USA). The significant level was set at $P \leq 0.05$.

3. Results and discussion

3.1. Effect of harvesting age and drying condition on andrographolide content

A. paniculata samples were harvest at four different harvesting ages of 90 DAS (vegetative stage), 100 DAS (immature flower bud stage), 115 DAS (less than 50% flowering stage) and 127 DAS (more than 50% flowering and seed-forming stage) as depicted in Figure 2. To determine quality of dried plant materials, andrographolide content is used as a marker where dried plant materials should contain at least 1% w/w of andrographolide as per the Thai Pharmacopeia 2020 protocol [10]. The HPLC chromatograms of andrographolide standard compound and *A. paniculata* extract are shown in Figure 3. At the established HPLC conditions, andrographolide was eluted at 2.63 min. The HPLC results revealed a significantly difference in the contents of andrographolide ranging from 0.74–4.11% (w/w) dry weight (Figure 4). Out of 16, 6 samples contained less than 1% w/w of andrographolide. The highest andrographolide contents were observed at 90 DAS/65 ℃, 127 DAS/65 ℃, and 127 DAS/80 ℃ with no statistically difference. Meanwhile, total andrographolide yields were estimated to be in the range of 3.80–61.14 g (Table 1) and the plant extracts harvested at 127 DAS/65 ℃ produced the highest total yield of andrographolide.

Table 1. Herbage, extraction, and andrographolide yields of *Andrographis paniculata* (Burm.f.) Nees at different harvesting age and drying condition.

Harvesting age (day after sowing)	Drying condition	Herbage yield (g fresh weight)	Herbage yield (g dry weight)	Herbage dry matter yield (%)[1]	Extraction yield (% dry weight)[2]	Total Andrographolide yield (g)[3]
90	Sunlight	2,460.0	534.0	21.71	23.39 ± 0.38	13.78 ± 3.89^{def}
	50 ℃	2,600.0	565.0	21.73	26.96 ± 1.50	4.19 ± 0.73^{f}
	65 ℃	2,600.0	564.5	21.71	17.62 ± 0.74	22.97 ± 2.22^{cd}
	80 ℃	2,600.3	543.5	20.9	15.68 ± 1.73	11.89 ± 2.32^{ef}
100	Sunlight	2,400.0	594.0	24.75	24.19 ± 2.34	6.29 ± 0.90^{f}
	50 ℃	2,760.0	698.5	25.31	20.05 ± 6.78	3.80 ± 0.75^{f}
	65 ℃	2,760.0	706.0	25.31	15.86 ± 1.48	22.96 ± 1.64^{cd}
	80 ℃	2,760.0	697.5	25.24	15.70 ± 0.44	19.59 ± 3.73^{de}
115	Sunlight	5,090.0	1,341.0	26.35	19.69 ± 1.06	6.00 ± 1.46^{f}
	50 ℃	4,879.0	907.0	18.59	23.70 ± 1.73	7.24 ± 0.66^{f}
	65 ℃	5,007.0	1,368.5	27.33	14.18 ± 1.21	32.19 ± 4.28^{c}
	80 ℃	4,980.0	1,441.0	28.94	15.97 ± 2.19	42.85 ± 11.18^{b}
127	Sunlight	5,250.0	1,341.0	34.86	22.78 ± 2.44	10.28 ± 5.08^{ef}
	50 ℃	4,400.0	1,464.5	33.28	20.35 ± 2.87	13.83 ± 11.43^{def}
	65 ℃	4,400.0	1,486.0	33.77	19.44 ± 2.06	61.14 ± 13.05^{a}
	80 ℃	4,450.0	1,446.0	32.49	17.22 ± 2.26	48.59 ± 2.34^{b}

[1]Herbage dry matter yield (%) was calculated as followed: (dry weight/fresh weight) x 100.

[2]Extraction yield (%) was calculated as followed: (dry crude extract weight/dry plant weight) x 100. Data were expressed as mean ± sd obtained from triplicated experiments.

[3]Total andrographolide yield was calculated as followed: (Andrographolide content (% dry weight) x Herbage yield (g dry weight))/100. Data were expressed as mean ± sd obtained from triplicated experiments. Different letters indicate statistically significant differences based on DMRT at $p \leq 0.05$.

Figure 2. Harvesting age of *Andrographis paniculata* (Burm.f.) Nees (a) 90 DAS, vegetative stage; (b) 100 DAS, immature flower bud stage; (c) 115 DAS, less than 50% flowering stage; (d) 127 DAS, more than 50% flowering and seed-forming stage.

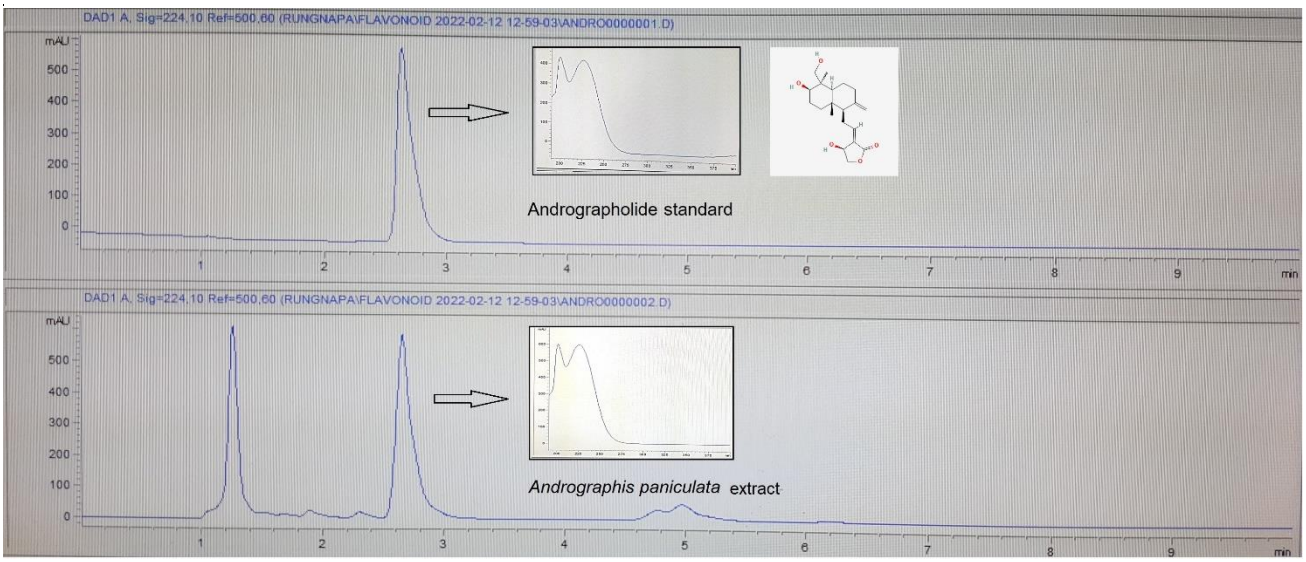

Figure 3. HPLC chromatograms of andrographolide standard compound and *Andrographis paniculata* (Burm.f.) Nees extract.

When looking at the harvesting age, there was no clearly defined trend in the content of andrographolide. But, the highest andrographolide contents (of about 4.11% (w/w) dry weight) were observed at vegetative (90 DAS) and at more than 50% flowering and seed-forming (127 DAS) stages (Figure 4). This finding corroborates with previous studies that reported the optimal harvesting age of *A. paniculata* to be in the range of 120–130 DAS [5,12,13,20]. Tajidin et al. [5] reported an increased in level of andrographolide content in leaves of Malaysian cultivar from vegetative to pre-flowering stages and a decreased in level of andrographolide after seed-forming stage. For Thai cultivar, it was reported that as plant maturity increase, there was an increasing trend in andrographolide content, which the highest andrographolide content was observed at 50% flowering, seed-forming, and matured seed stages at greenhouse and field experiment set up [12]. Bhan et al. [13] also reported an increase in andrographolide content in leaves of Indian cultivars correlated with the plant maturity. Additionally, andrographolide content was reported to be vary with changing seasons and light intensities [12,21,22]. Therefore, an optimal harvesting age of *A. paniculata* in relation to an appropriate maturity stage to obtain high andrographolide content is suggested rather than just simply considering the number of planting/harvesting days.

For drying conditions, the results obtained in the present study indicated that drying condition and temperature had a significant influence on andrographolide content. For example, drying at 65 ℃ was the most optimal temperature, whereas drying under sunlight and at 50 ℃ resulted in around 4-fold decrease in andrographolide content. These results are in an agreement with those in other studies. Saohin et al. [16] showed that hot air drying at 60 ℃ was the most suitable condition for leaves of *A. paniculata* comparing with drying at 40 ℃ and 50 ℃ in consideration of drying time, total lactones content and other powder characteristics for capsule preparation. Tummanichanont et al. [15] also reported that drying using tray dryer and heat pump dehumidified dryer at 60 ℃ resulted in the highest content of andrographolide comparing with drying at 40 and 50 ℃ for a relatively shorter drying time.

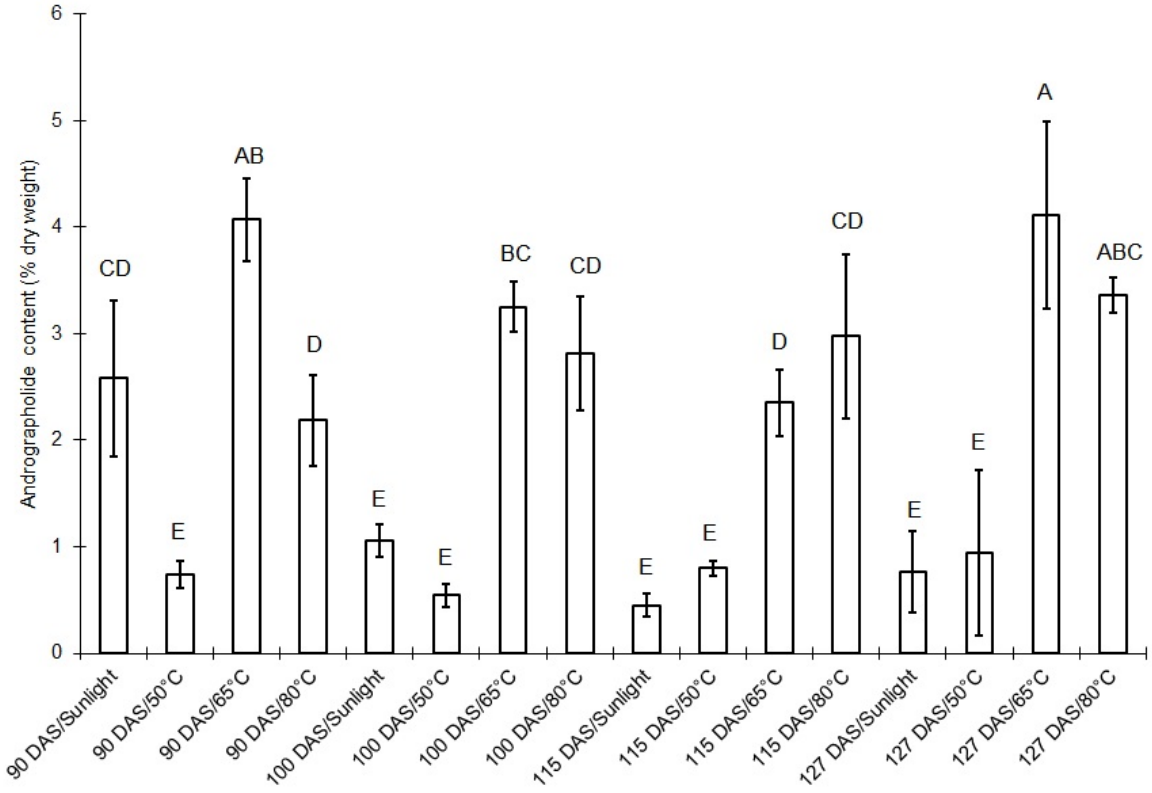

Figure 4. Andrographolide contents in *Andrographis paniculata* (Burm.f.) Nees extracts expressed as mean ± SD from triplicated experiments. Different letters indicate statistically significant differences based on DMRT at $p \leq 0.05$.

3.2. Effect of harvesting age and drying condition on antioxidant activity

Antioxidants are defined as a group of compounds that neutralized free radicals and reactive oxygen species, exerting beneficial health effect against oxidative stress, an onset of various chronic diseases such as cardiovascular diseases, cancer and inflammation [23]. In the present study, *A. paniculata* extracts exhibited the DPPH• scavenging activity and FRAP potential to reduce Fe (III) to Fe (II) in ranges of 3.43–26.73 and 1.93–17.28 mg Trolox/g DW, respectively (Figure 5). Based on these results, the antioxidant activity of *A. paniculata* was significantly influenced by drying condition not the harvesting age as the highest DPPH• scavenging activity and FRAP reducing power were observed in samples dried at 50 ℃ from all four plant maturity stages. Hence, andrographolide is unlikely to be a major bioactive compound responsible for antioxidant activity of *A. paniculata* as there was no correlation between andrographolide content (Figure 4) and antioxidant activities. This is supported by a study of Lin et al. [24] and Low et al. [25] reporting that antioxidant activities of *A. paniculata* was correlated with total flavonoid content in the extract. In contrast, Tummanichanont et al. [15] found that drying *A. paniculata* at 60 ℃ yielded higher DPPH• scavenging activity than drying at low temperatures of 40 ℃ and 50 ℃.

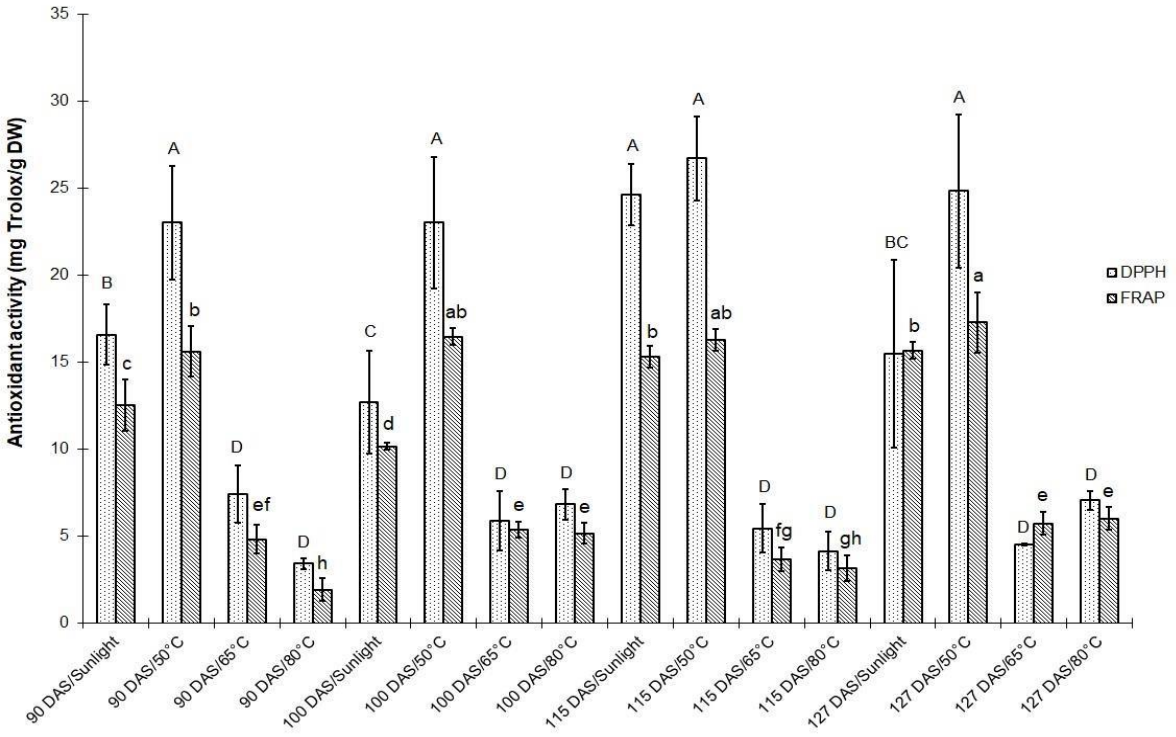

Figure 5. Antioxidant activities of *Andrographis paniculata* (Burm.f.) Nees extracts expressed as mean ± SD from quadruplicated experiments. Different letters indicate statistically significant differences based on DMRT at $p \leq 0.05$.

3.3. Effect of harvesting age and drying condition on antibacterial activity

The agar disc diffusion was the primary screening test to assess the antibacterial activity of *A. paniculata* extract against four strains of gram-positive bacteria (*B. cereus*, *M. luteus*, *S. epidermidis*, and *S. aureus*) and one strain of gram-negative bacteria (*E. coli*). The potential of the extracts to suppress the growth of these bacterial strains is illustrated in Table 2. Results revealed that *A. paniculata* extract (50 mg/mL) which was harvested at 90 DAS/65 ℃ exhibited the strongest inhibition activity against gram-positive bacterial strains. This is attributed to high inhibition zones of 14 ± 2.6, 11.3 ± 1.5, 10.0 ± 0.0, 9.0 ± 1.7 mm against *S. aureus*, *B. cereus*, *M. luteus*, and *S. epidermidis*, respectively. *A. paniculata* extracts at harvesting age of 100, 115 and 127 DAS and drying temperatures of 65 ℃ and 80 ℃ also displayed the antibacterial activities against *B. cereus*, *M. luteus*, *S. epidermidis*, and *S. aureus* with inhibition zone ranging from 7.0–11.0 mm. Noteworthy, gram-negative bacteria (*E. coli*) was resistant to all *A. paniculata* extracts at concentration of 50 mg/mL. The reason for the difference in susceptibility against gram-positive and gram-negative bacterial strains might be due to the presence of outer membrane in gram-negative bacteria and polarity nature of andrographolide [6,26].

The MIC determination was carried out with susceptible gram-positive bacterial strains, *B. cereus*, *M. luteus*, *S. epidermidis*, and *S. aureus* and the results are shown in Table 2. The results revealed that *A. paniculata* extracts at harvesting ages of 90, 100, 115, and 127 DAS and drying at 65 ℃ and 80 ℃ yielded the MIC values between 3.12–25.0 mg/mL. The 90 DAS/65 ℃ extracts exhibited the highest antibacterial activity with the MIC values of 3.12 mg/mL against *S. aureus*, *B. cereus*, and *M. luteus*

as well as 12.5 mg/mL against *S. epidermidis*. Andrographolide, a major bioactive compound in *A. paniculata* extracts, exhibited the MIC values of 0.5–1.0 mg/mL against four gram-positive bacterial strains. In the study of Banerjee et al. [26], andrographolide was also a potent inhibitor against gram-positive bacteria with the MIC values of 0.1–1 mg/mL, while the *S. aureus* was the most susceptible strain. The promising antibacterial activity potency of andrographolide is likely due to the inhibiting activity of this compound on intracellular DNA biosynthesis in bacteria [26]. Notably, the antibacterial activities of *A. paniculata* extracts against four gram-positive bacterial strains were well correlated with the content of andrographolide in the extracts (Figure 4), which suggests that this is partly attributed to the influence of this compound. Netshiluvhi and Eloff's study [27] also produced some good antibacterial activities of medicinal plants (as per MIC values of 0.42–2.5 mg/mL against bacteria that include *S. aureus*) of shade-dried leaf extracts from clone plant species that were grown under temperature regimes of 15 and 30 ℃ for a harvesting age of 182 days in the growth chambers.

Table 2. Antibacterial activity of *Andrographis paniculata* (Burm.f.) Nees extract against pathogenic bacteria.

Harvesting age (Days after sowing)	Drying condition[1]	Zones of inhibition (mm)[2]/ MIC[3] (mg/mL)				
		S. epidermidis ATCC 12228	*S. aureus* TISTR 2329	*M. luteus* ATCC 4698	*E. coli* TISTR 074	*B. cereus* TISTR 1527
90	sunlight	8.0 ± 0.0/12.5	9.7 ± 0.6/12.5	7.7 ± 1.2/25.0	0.0 ± 0.0[4]/NA[5]	9.7 ± 0.6/12.5
	50 ℃	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA
	65 ℃	9.0 ± 1.7/12.5	14.0 ± 2.6/3.12	10.0 ± 0.0/3.12	0.0 ± 0.0/NA	11.3 ± 1.5/3.12
	80 ℃	0.0 ± 0.0/25.00	7.0 ± 0.0/12.5	0.0 ± 0.0/12.5	0.0 ± 0.0/NA	9.0 ± 1.0/12.5
100	sunlight	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	7.0 ± 0.0/NA
	50 ℃	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA
	65 ℃	8.7 ± 2.1/12.5	11.0 ± 1.7/12.5	9.0 ± 1.7/6.25	0.0 ± 0.0/NA	8.7 ± 1.5/6.25
	80 ℃	8.3 ± 0.6/25.0	8.7 ± 0.6/12.5	9.0 ± 2.6/12.5	0.0 ± 0.0/NA	9.7 ± 2.1/12.5
115	sunlight	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA
	50 ℃	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	7.5±0.7/NA
	65 ℃	8.0 ± 1.4/25.0	8.7 ± 1.5/12.5	8.7 ± 1.5/12.5	0.0 ± 0.0/NA	8.0 ± 0.0/12.5
	80 ℃	0.0 ± 0.0/25.0	8.3 ± 1.2/12.5	8.0 ± 0.7/12.5	0.0 ± 0.0/NA	8.7 ± 1.2/12.5
127	sunlight	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA
	50 ℃	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA	0.0 ± 0.0/NA
	65 ℃	8.0 ± 1.7/25.0	7.5 ± 0.7/12.5	7.0 ± 0.0/12.5	0.0 ± 0.0/NA	7.3 ± 0.6/12.5
	80 ℃	7.7 ± 1.2/25.0	8.0 ± 1.4/12.5	7.5 ± 0.7/12.5	0.0 ± 0.0/NA	7.7 ± 0.6/12.5
Positive control for zone inhibition[6]		34 ± 1.7/NA	31.7 ± 2.9/NA	29.0 ± 1.0/NA	26.0 ± 0.0/NA	26.3 ± 0.6/NA
Positive control for MIC[7]		NA/1.0	NA/0.5	NA/1.0	NA/NA	NA/0.5

[1]Drying condition: sunlight drying and hot air oven drying at 50, 65, and 80 ℃.

[2]Zone inhibition was performed with 50 mg/mL *A. paniculata* extract in DMSO.

[3]MIC = Minimum Inhibition Concentration. Data were expressed as mean ± sd obtained from triplicated experiments.

[4]The value of 0.0 ± 0.0 implied that there was no inhibition.

[5]NA=Not Analyzed.

[6]Gentamicin (10 µg disc) was used as positive control for zone inhibition assay.

[7]Andrographolide at concentration ranged from 0.0.0075-1 mg/mL was used as positive control for MIC determination.

4. Conclusions

The findings did not show any clear trend with regards to the effect of harvesting age on the andrographolide content and herbal yield. However, the highest andrographolide content of slightly over 4% and herbal yield (total andrographolide yield) of 61.1 g were both observed at 127 harvesting days (more than 50% flowering and seed-forming stage) under the drying temperature of 65 ℃. These results suggest that the drying temperature of 65 ℃ is optimal for the achievements of high andrographolide content and herbal yield. It is therefore imperative to consider the right harvesting age in relation to appropriate maturity stage of the plant in order to obtain the desired andrographolide content and herbal yield. There was however a clear trend with regards to the effect of drying conditions (based sunlight and hot air oven) on andrographolide content, antioxidant, and antibacterial activity of *A. paniculata*. Overall, *A. paniculata* should be harvested based on plant maturity stage rather than number of plantation days. The optimal harvesting age of *A. paniculata* is suggested to be at 50% flowering stage and onward to obtain the highest total andrographolide yield. For drying condition, the hot air oven is more preferable than sunlight drying, while the optimal drying temperature should be at 65 ℃ in order to maintain high amount of andrographolide and antibacterial activity of dried *A. paniculata* materials. If antioxidant activity is a focus, hot air oven drying at 50 ℃ is preferable to sustain high antioxidant activity.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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