Journal of King Saud University – Science 32 (2020) 2762–2766

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

1-Deoxynojirimycin and polyphenolic composition and antioxidant activity of different native Thai silkworm (*Bombyx mori*) larvae

Samappito Wannee [a], Butkhup Luchai [b,*]

[a] Department of Food Technology, Faculty of Technology, Mahasarakham University, Mahasarakham 44000, Thailand
[b] Natural Antioxidant Innovation Research Unit (NAIRU), Department of Biotechnology, Faculty of Technology, Mahasarakham University, Mahasarakham 44000, Thailand

ARTICLE INFO

Article history:
Received 28 March 2019
Revised 1 May 2020
Accepted 25 June 2020
Available online 30 June 2020

Keywords:
Bombyx mori
Silk worm
Alkaloids
Polyphenols
Antioxidant activity

ABSTRACT

1-Deoxynojirimycin (DNJ) and polyphenolic compound are extensively found at different levels in many silkworm races. The present investigation comprises, estimation of DNJ, polyphenol, total phenolic (TPC) and total favonoid contents (TFC), antioxidant activities (DPPH, ABTS, and FRAP). The results showed that the fifth larval instars of Samrong and Nangsiw had high content of DNJ (96.42 mg/100 g dw and 65.23 mg/100 g dw, respectively). Nangsiw had the highest content of quercetin (82.94 mg/100 g dw) and (+)-catechin (81.88 mg/100 g dw). Nangsiw contained relatively highest ($p < 0.05$) TPC and TFC and exhibited highest scavenging free radical DPPH activity (58.96%), and highest ABTS radical scavenging capacity (12.35 mg TE/100 g dw) and highest ferric reducing antioxidant power (FRAP) (2.03 mg Fe (II)/g dw). This experiment suggests that Samrong and Nangsiw are a valuable source of natural alkaloid and phenolic antioxidants for commercial exploration.

1. Introduction

In recent years, phenolic compounds have been an increased interest because of their potential for possible health benefits and potent prevention of free radical-induced diseases. Enzymatic antioxidants include catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APOX) plays an important role in protecting oxidative cell injury (Mitra et al., 2019). Besides these, phenolic compounds could be also preventing cell damage from oxidative stress of free radicals (Mitra et al., 2019). Some glycosides, tannin, flavonoids, saponins, triterpenoids and alkaloids possess pharmacological properties like antioxidant, antimicrobial, antimalarial, anti-inflammatory, laxative, antidiabetic, hypoglycemic, antihelmintic, antithrombotic, cathartic, antifertility, anti-fungi and anticancer (Alara et al., 2017).

Mulberry (*Morus* sp.) leaves with high levels flavonoids and alkaloids are used to feed silkworms (*Bombyx mori*). Flavonoids and alkaloids are modified within the silkworm tissue. DNJ, an amino-sugar originally found in the mulberry leaves (Arfan et al., 2012). Yoon et al. (2019) reported that DNJ, a potent α-glucosidase inhibitor could be found in silkworm. In addition, Chen et al. (2014) found that DNJ composition changes during developmental stages have been also observed in silkworm (*Bombyx mori* L.), with 5th instar had the highest DNJ concentration and silkworm biomass. Silkworm pupae have long been used as food or food supplements with abundance in Chinese, Japanese and Korea (Tomotake et al., 2010). Ryu et al. (2013) found that the silkworm powder significantly suppressed blood glucose-lowering and thereby the larval powder displays more effective results on diabetic. In Thailand, although silkworms have been reared for a long time, the direct comparisons of DNJ and polyphenols content and antioxidant activity between the native Thai silkworm races have not previously been performed. Therefore, in the present work, we evaluated the DNJ and polyphenol contents in the 5th instar larvae of the most popular silkworm race of Northeastern Thailand. The selected silkworm race could be used for silkworm larval powder production or preparing DNJ-related products.

* Corresponding author at: Department of Biotechnology, Faculty of Technology, Mahasarakham University, Mahasarakham 44000, Thailand.
E-mail address: tak_biot2000@hotmail.com (B. Luchai).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

2. Materials and methods

2.1. Materials and reagents

Standard DNJ, phenolic compounds were of analytical grade purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other solvents were of highest grade available.

https://doi.org/10.1016/j.jksus.2020.06.012
1018-3647/

2.2. Proximate analysis

The proximate compositions including moisture, lipid and ash were analyzed according to the AOAC (AOAC, 1995) procedures.

2.3. Preparation of silkworm larvae crude extract

The fifth larval instars of 5 native Thai mulberry silkworm (*Bombyx mori*) races [Nangsiw, Nangtui, Nanglai, Nangluang, Samrong] were obtained from the Silk Innovation Center (Mahasarakham University, Thailand). Silkworm larvae were fed with fresh mulberry leaves in a controlled environment (24–26 °C, 70–80% RH) until the 3rd day of fifth instar (Fig. 1). The collected silkworm larvae samples were lyophilized and powdered. For the sample extraction and DNJ analysis was carried out according to the method by Yin et al. (2010).

For polyphenols and antioxidant activity analysis, silkworm powder (1 g) was extracted twice in 10 ml 60% aqueous methanol by ultrasonic for 1 h at room temperature. Extracted solution was centrifuged and filtered with the filter unit (Millipore, pore size: 0.45 μm). All samples were transferred to Eppendorf tubes and stored at −20 °C until analyzed.

2.4. HPLC analysis of DNJ and polyphenolic compounds

DNJ derivation: the DNJ content was carried out as described by Kim et al. (2003a,b) and Yin et al. (2010), the crude extract (35 μl of extract) or DNJ working solution in a 1.5-ml microtube was mixed with 169 μl of 0.4 M potassium borate buffer (pH 8.5) and 20 μl of 5 mM 9-fluorenylmethyl chloroformate (FMOC-Cl) dissolved in 50% (v/v) acetonitrile (CH_3CN). After keep in water bath (20 °C) for 20 min, 25 μl of 1 M glycine was added and mixed to quench the remaining FMOC-Cl. To stabilize the DNJ-FMOC formed, 66 μl of 0.1% (v/v) acetic acid was added. Finally, adjust the volume of 707 μl with distilled water and filtering through a 0.45 μm nylon syringe filter before analysed. DNJ determination: A Shimadzu HPLC system (Kyoto, Japan), equipped with a diode-array detector, was applied for DNJ content determination. DNJ-HCl was obtained from Sigma Chemicals (USA), and FMOC-Cl was from Fluca. The HPLC separation was achieved using an Apollo C_{18} (ø 4.6 mm × 250 mm, 5 μm) and mobile phase consisting of acetonitrile: 0.1% (v/v) aqueous acetic acid (11:16, v/v) at the flow rate of 0.6 ml/min and injection volume was 20 μl. Identification and quantification of DNJ using retention time and external standard, respectively.

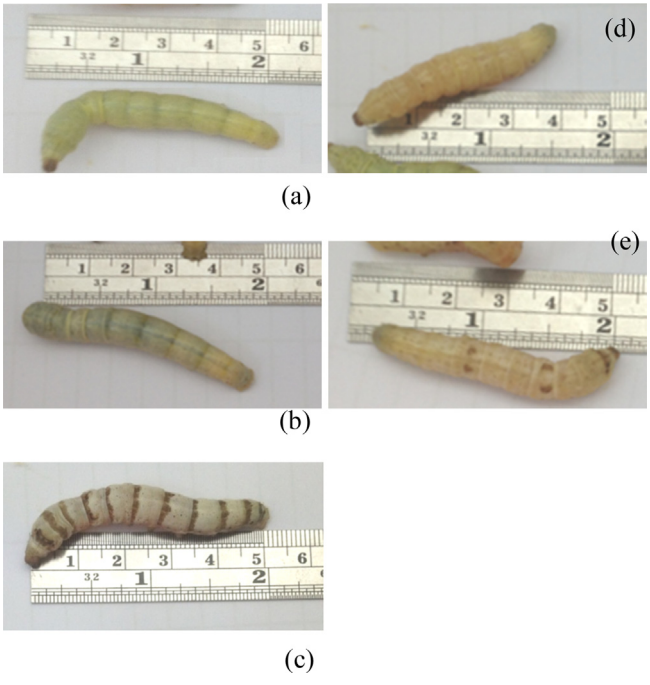

Fig. 1. Physical appearance of in the fifth-instar larvae of native Thai silkworm; (a): Nangsiw, (b): Nangtui, (c): Nanglai, (d): Nangluang, (e): Samrong.

Polyphenolic compounds determination: A Shimadzu HPLC system (Kyoto, Japan), equipped with a diode-array detector, was applied for polyphenols content. The HPLC separation was achieved using an Apollo C_{18} (ø 4.6 mm × 250 mm, 5 μm) and mobile phase consisting of acetonitrile/deionized water/phosphoric acid (2/97.8/0.2, v/v/v) (solvent A) and acetonitrile/deionized water/phosphoric acid (97.8/2/0.2, v/v/v) (solvent B) at a flow rate of 0.6 ml/min and injection volume was 20 μl. The detector and column temperature were set at 254 nm and 40 °C, respectively. The column was eluted with 20% solvent B, 50% solvent B at 30 min, 60% solvent B at 35 min, 20% solvent B at 40 min at isocratic elution until 55 min. Individual polyphenolic compound was quantified by external standard using purchased standards.

2.5. Measurements of total phenolic content (TPC)

The TPC in extracts was determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Briefly, 12.5 μl of sample and 12.5 μl distilled water were mixed with 12.5 μl Folin–Ciocalteu reagent (diluted tenfold). After 6 min, 125 μl of 7% Na_2CO_3 was added, followed by 100 μl of distilled water. After 90 min, absorbance was measured at 760 nm using a microplate reader (Synergy HT, BiotTek instruments, USA). TPC was calculated from the calibration curve of gallic acid and results expressed as mg GAE/100 g dw.

2.6. Measurements of total flavonoid content (TFC)

The determination of TFC of the samples was based on the method reported previously (Kim et al., 2003a,b). Sample (25 μl) was mixed with 5% $NaNO_2$ solution (7.5 μl) and distilled water (125 μl). After incubated for 5 min, 0.5mlof sample was mixed with 0.5 ml of 2% $AlCl_3$. Finally, 50 μl of 1 M NaOH and 27.5 μl of distilled water were added to the mixture 5 min later and the absorbance was measured at 510 nm. The TFC was estimated from a (+)-catechin standard curve and the results were expressed as mg CE/100 g dw.

2.7. Measurements of free radical scavenging activity

The extracts ability to scavenge DPPH radicals was measurement according to the method of Akowuah et al. (2005). Briefly, 100 μl of a 0.2 mM methanolic solution of DPPH was mixed thoroughly with 100 μl of extract. After left in the dark for 1 h, the absorbance was read using microplate reader (Synergy HT, BiotTek instruments, USA) at 520 nm. The capability of extracts was expressed as percentage quenching of DPPH radical using the following equation: Inhibition of DPPH (%) = $((A_0 - A_1)/A_0) \times 100$; Where A_0 was absorbance of control and A_1 was absorbance in the presence of sample.

2.8. Measurements of ferric reducing/antioxidant power (FRAP)

The reducing power of sample was determined according to the method of Benzie and Strain (1999). Briefly, 30 μl of sample was mixed with 270 μl of the FRAP reagent. After left for 30 min, the absorbance was read using microplate reader (Synergy HT, BiotTek instruments, USA) at 593 nm. The FRAP value of sample was

estimated against a standard curve using $FeSO_4 \cdot 7H_2O$ (6.25–400 μg/ml) and expressed as mg Fe (II)/g dw.

2.9. $ABTS^{\bullet+}$ radical scavenging assay

For the ABTS radical scavenging assay, the method of Seeram et al. (2006) was adopted. Briefly, 10 μl of sample was mixed thoroughly with 190 μl of ABTS radical cation solution. After left for 2 h, the absorbance was read using microplate reader (Synergy HT, BiotTek instruments, USA) at 595 nm. The ABTS radical scavenging value of sample was estimated against a standard curve using Trolox (6.25–400 μg/ml) and expressed as mg Trolox equivalent antioxidative capacity (TEAC)/g dw.

2.10. Statistical analysis

Experimental results were recorded as means ± SD of triplicate measurements. Linear regression analysis between some flavonoids and antioxidant activities was performed by SPSS v. 19.0. The data were analyzed by one way ANOVA and DMRT was performed to detect significant difference between means ($p < 0.05$).

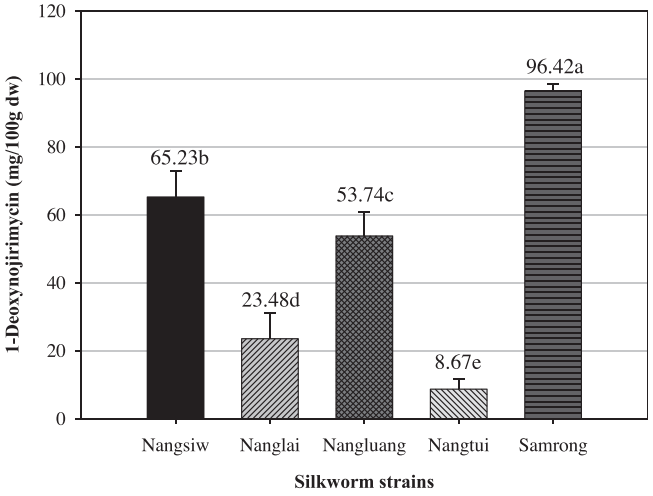

Fig. 2. The accumulation of DNJ in the fifth-instar larvae of native Thai silkworm. Data are the mean of three determinations. Vertical bars indicate standard deviation of total amounts of DNJ. Different letters (a,b,c,d,e) with each bar mean a statistical difference at $P < 0.05$ as measured by the DMRT.

3. Results and discussion

3.1. Proximate composition

Five silkworm races (Nangsiw, Nangtui, Nanglai, Nangluang, and Samrong) were reared by feeding fresh mulberry leaves at controlled environment (24–26 °C). Silkworm on 3rd day of 5th instar larval stage were collected randomly to analyze the weight, length and the percentage of ash and lipid on a dry matter basis. The proximate compositions of the silkworm larvae were presented in Table 1. The average weight and length varied from 1.66 to 2.04 g and from 4.75 to 5.70 cm, respectively. The average moisture content and ash of silkworm larvae varied from 5.17 to 6.71% and from 5.15 to 6.99 cm, respectively. The five races showed a rich source of lipid. Nanglai race had highest content of lipid (14.43%), while Samrong race had lowest content of lipid (11.66%).

3.2. DNJ content in silkworm races

There are many reports on the DNJ content in marine sponge (*Lendenfeldia chondrodes*) (Sakai and Kamiya, 2006), mulberry leaves (Yatsunami et al., 2008; Arfan et al., 2012) and silkworm (Yatsunami et al., 2011). However, the DNJ accumulation in different species of Thai silkworm larvae was also reported (Vichasilp et al., 2018). DNJ can be isolated from mulberry leaves (Arfan et al., 2012). The DNJ contents of 3rd day of 5th instar larval stage of silkworm was measured (Fig. 2). The results revealed that the DNJ content in the silkworm depended on the silkworm race. Among the races, the larvae of Samrong showed the highest content of DNJ (96.42 mg/100 g dw), followed by Nangsiw (65.23 mg/100 g dw), Nangluang (54.74 mg/100 g dw), Nanglai (23.48 mg/100 g dw) and Nangtui (8.67 mg/100 g dw), respectively. This finding is consistent with the previous report by Vichasilp et al. (2018) who analyzed DNJ in some silkworm larvae (36–111 mg/100 g dw). The best candidate among the silkworm species studied was Samrong. Samrong is one of the special native Thai silkworm varieties and the silkworm of 3rd day of 5th instar larval stage should be selected as the best harvest time for silkworm rich in DNJ. The presence of DNJ in silkworms is probably due the consumption of mulberry leaves (Arfan et al., 2012).

3.3. Polyphenolic composition of silkworm races

The distribution of polyphenolic compounds in the analysed samples is shown in Table 2. The results showed that the polyphenolic composition in the silkworm depended on the silkworm race. Quercetin, (+)-catechin, (−)-epicatechin and naringenin were the major polyphenolics presented in all the silkworm races. In most of the silkworm studied, rutin (quercetin 3-*O*-rhamnose glycoside), myricetin, *tran*-resveratrol, luteolin, and kaempferol were detected in traces. Among the races, the larvae of Nangsiw showed the highest content of (+)-catechin, (−)-epicatechin, and naringenin (Table 2). As shown in the Table 2, the most abundant polyphenolic compound was quercetin (44.35–82.94 mg/100 g dw). This flavonoid origin from mulberry leaves are modified within the silkworm tissue and accumulated in the larval body and silk fiber. Similar

Table 1
Proximate composition of native Thai silk larval powder of the fifth-instar.

Parameter	Silkworm strains [1)]				
	Nangsiw	Nangtui	Nanglai	Nangluang	Samrong
Weight (g)	1.66 ± 0.21	1.99 ± 0.31	2.04 ± 0.36	1.75 ± 0.31	1.80 ± 0.31
Length (cm)	5.15 ± 0.47	5.65 ± 0.47	5.70 ± 0.63	4.75 ± 0.49	4.60 ± 0.61
Moister content [2)]	6.71 ± 0.25	5.33 ± 0.22	5.17 ± 0.16	5.92 ± 0.18	2.91 ± 0.13
Ash [2)]	5.15 ± 0.01	6.99 ± 0.10	5.77 ± 0.12	6.86 ± 0.14	6.74 ± 0.26
Lipid [2)]	13.38 ± 0.39	13.95 ± 0.41	14.43 ± 1.38	13.75 ± 0.89	11.66 ± 1.40

[1)] Values are the means ± standard deviation.
[2)] The values are on a dry-matter basis (g/100 g dry weight).

Table 2
Phenolic composition (mg/100 g dw) of in the fifth-instar larvae of native Thai silkworm.

Phenolic compounds	Silkworm strains				
	Samrong	Nangluang	Nangtui	Nanglai	Nangsiw
(+)-Catechin	20.42 ± 1.42 d	76.80 ± 2.90b	28.13 ± 1.98c	15.69 ± 1.18 e	81.88 ± 2.70 a
(−)-Epicatechin	3.48 ± 0.45b	0.69 ± 0.04 d	2.74 ± 0.07 bc	2.04 ± 0.35c	50.08 ± 1.74 a
Rutin	3.50 ± 0.35 a	1.00 ± 0.01b	1.15 ± 0.02b	1.88 ± 0.27b	1.62 ± 0.01b
Quercetin	54.40 ± 2.77c	73.88 ± 2.20b	71.97 ± 3.01b	45.35 ± 3.31 d	82.94 ± 2.52 a
Myricetin	0.29 ± 0.00 a	0.63 ± 0.01 a	0.39 ± 0.00 a	0.27 ± 0.00 a	0.40 ± 0.00 a
trans-Resveratrol	1.70 ± 0.47b	1.35 ± 0.01	2.45 ± 0.06 a	1.46 ± 0.25b	2.12 ± 0.01 a
Luteolin	0.03 ± 0.00c	0.44 ± 0.04b	0.01 ± 0.00c	0.01 ± 0.00c	5.37 ± 0.06 a
Naringenin	8.07 ± 0.96b	2.17 ± 0.01c	12.75 ± 0.63 a	2.82 ± 0.45c	10.27 ± 0.17 a
Kaempferol	0.18 ± 0.01b	0.35 ± 0.01b	0.21 ± 0.01b	0.17 ± 0.01b	2.94 ± 0.05 a
$\Sigma_{polyphenols}$	126.05 ± 1.30 d	191.22 ± 2.05b	141.54 ± 1.16c	81.04 ± 1.42 e	278.65 ± 2.43 a

All analyses were the mean of triplicate measurements ± standard deviation.
Values in the same row with different lower-case letters are significantly different at $P < 0.05$ as measured by the DMRT.

Table 3
Total phenolic content, total flavonoid content and antioxidant activity of the fifth-instar larvae of native Thai silkworm.[1)]

Silkworm strains	Total phenolic (mg GAE/100 g dw) [2)]	Total flavonoids (mg CE/100 g dw) [2)]	FRAP (mg Fe(II)/g dw) [2)]	ABTS (mg TE/100 g dw) [2)]	DPPH (% Scavenging activity)
Samrong	83.38 ± 2.07b	143.53 ± 19.37 d	1.52 ± 0.04b	8.77 ± 1.72b	20.97 ± 1.25c
Nangluang	85.58 ± 5.46b	176.01 ± 18.58b	1.83 ± 0.01 ab	11.27 ± 1.57 a	38.37 ± 6.55b
Nangtui	84.34 ± 3.11b	168.87 ± 20.04c	1.65 ± 0.10b	10.93 ± 0.82 a	21.70 ± 3.53c
Nanglai	79.99 ± 5.19b	137.44 ± 26.13 e	1.38 ± 0.04c	1.71 ± 0.61c	11.95 ± 2.34 d
Nangsiw	106.01 ± 4.72 a	189.20 ± 18.99 a	2.03 ± 0.04 a	12.35 ± 0.75 a	58.96 ± 6.68 a

Values in the same column with different lower-case letters are significantly different at $P < 0.05$ as measured by the DMRT.
[1)] Values are the means ± standard deviation (n = 3).
[2)] Calculated by using dry weight of the methanolic extract.

results have been reported by Kurioka and Yamazaki (2002) in yellow green cocoon shell of Sasamayu silkworm, where quercetin, kaempferol, and glucosides of kaempferol were major components. Kurioka and Yamazaki (2002) demonstrated that the quercetin and it's glucosides were not found in mulberry leaves and they suggested that these metabolites could produce by the silkworm. Based on the above results, it was noticed that some of flavonoid glycosides in the silkworm was ingested from diets, indicating that silkworm larvae could synthesize some of flavonoid glycosides by itself.

3.4. Amount of total phenolics and total flavonoids

TPC varied widely in silkworm races and ranged from 79.99 to 106.01 mg GAE/100 g dw. This study showed that TPC in the selected Thai silkworm races as: Nangsiw > Nangluang > Nangtui > Samrong > Nanglai. TFC in the selected Thai silkworm races ranged from 137.44 to 189.20 mg CE/100 g dw. The highest ($p < 0.05$) TFC was presented in Nangsiw and the lowest was observed in Nanglai among the selected Thai silkworm races (Table 3). This study showed that TFC in the selected Thai silkworm races as: Nangsiw > Nangluang > Nangtui > Samrong > Nanglai.

3.5. Antioxidant capacity of silkworm larvae extracts

The analysed 3rd day of 5th instar larval stage of silkworm extracts revealed a wide range of antioxidant activities, with values ranging between 1.38 mg Fe(II)/g dw for Nanglai and 2.03 mg Fe (II)/g dw for Nangsiw with statistically significant differences ($p < 0.05$) between silkworm races (Table 3). Total antioxidant activity, measured by the $ABTS^{\bullet+}$ method, ranged from 1.71 to 12.35 mg TE/100 g dw, which Nangsiw exhibited the highest ($p < 0.05$) antioxidant capacity than those of the others. In addition, Nangsiw also exhibited the greatest DPPH free radical scavenging activity (58.96%). The result indicated that antioxidant capacity of 3rd day of 5th instar larval stage of silkworm extracts by DPPH assay had similar trend with $ABTS^{\bullet+}$ and FRAP assay. Thus, Nangsiw with high phenolic content exhibited highest antioxidant activity among the studied silkworm races. The antioxidant activity of DNJ and flavonoids in silkworms is the result of mulberry leaves (Arfan et al., 2012). These results suggest that DNJ is not playing a significant role as an antioxidant when compared to (+)-catechin, (−)-epicatechin, and naringenin present in Nangsiw. However, Samrong larvae powder with high DNJ might be used to relive the symptoms of diabetes.

Table 4
Correlations among some polyphenols, TPC, TFC and antioxidant activities of the fifth-instar larvae of native Thai silkworm.

	(+)-catechin	procyanidin B2	quercetin	$\Sigma_{polyphenols}$	TPC	TFC
$\Sigma_{polyphenols}$	0.774	0.936*	0.845			
TPC	0.750	0.847	0.758	0.963**		
TFC	0.892*	0.763	0.990**	0.842	0.786	
FRAP	0.942*	0.887*	0.953*	0.918*	0.868	0.969**
ABTS	0.696	0.812	0.909*	0.785	0.605	0.851
DPPH	0.932*	0.920*	0.861	0.944*	0.932*	0.893*

TPC: total phenolic content; TFC: total flavonoid content; FRAP: ferric reducing ability assay; ABTS: 2,2′-Azinobis(3-ethylbenzoline-6-sulphonate) radical cation decolorization assay; DPPH: radical scavenging activity; $\Sigma_{polyphenols}$: Summation of concentrations of individual polyphenols determined by HPLC.
Significance: *, $p \leq 0.05$ and **, $0.05 < p \leq 0.01$, as measured by the Pearson's correlation.

3.6. Relationship between phenolic composition and antioxidant activity

Univariate regression analysis between some polyphenols, TPC, TFC, and antioxidant activity are summarized in Table 4. (+)-Catechin content was significantly correlated ($p \leq 0.05$) with TFC, FRAP and DPPH. Quercetin content was highly correlated ($0.05 < p \leq 0.01$) with TFC whereas a less correlations ($p \leq 0.05$) with FRAP and ABTS was recorded. TPC was highly correlated ($0.05 < p \leq 0.01$) with $\Sigma_{polyphenols}$ and it had the positive correlations ($p \leq 0.05$) with DPPH but not with TFC, FRAP and ABTS in the silkworm larvae extracts. This may be due to the antioxidant activity (FRAP and ABTS assay) did not only depend on the amount of polyphenolic compounds but it also depended on chemical structure of phenolic compounds (Rice-Evans et al., 1996). Therefore, TPC may not always be a good indicator for antioxidant activities of the crude extracts. TFC was highly correlated ($0.05 < p \leq 0.01$) with FRAP and it had the positive correlations ($p \leq 0.05$) with DPPH.

4. Conclusion

In conclusion, we investigated DNJ and polyphenols distribution in 5 native Thai silkworm races in order to select appropriate silkworm race as sources of silkworm larval powder product with enriched DNJ and polyphenols content. Samrong had highest content of DNJ and this race could be applied commercially to prepare silkworm powder with high DNJ content. Nangsiw exhibited highest content of flavonoids (quercetin and (+)-catechin), TPC, TFC and exhibited highest antioxidant activity (DPPH, ABTS and FRAP assay). A significant positive correlations ($p \leq 0.05$) were obtained between some flavonoids (quercetin and (+)-catechin), TPC, TFC with antioxidant activity (FRAP and DPPH assay) revealed that polyphenolic compounds are responsible for antioxidant capacity of the investigated silkworm races.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was financially supported by Mahasarakham University, Thailand and the Thailand Research Fund, Thailand (TRF grant–TRG5480002). The useful suggestion and technical assistance from Mr. Suwat Promma is highly appreciated.

References

Alara, O.R., Abdurahman, N.H., Abdul Mudalip, S.K., Olalere, O.A., 2017. Effect of drying methods on free radicals scavenging activity of *Vernonia amygdalina* growing in Malaysia. J. King Saud Univ. – Sci. 31, 495–499.

Arfan, M., Khan, R., Rybarczyk, A., Amarowicz, R., 2012. Antioxidant activity of mulberry fruit extracts. Int. J. Mol. Sci. 13, 2472–2480.

Akowuah, G.A., Ismail, Z., Norhayati, I., Sadikun, A., 2005. The effects of different extraction solvents of varying polarities of polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity. Food Chem. 93, 311–317.

AOAC, 1995. Official Methods of Analysis (16th ed). Association of Official Analytical Chemists, Washington, DC.

Benzie, I.F.F., Strain, J.J., 1999. Ferric reducing / antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 299, 15–27.

Chen, H.-J., Shi, Y., Liu, J., Mu, L., Zou, Y.-X., Liao, S.-T., 2014. Changes of 1-deoxynojirimycin with hypoglycemic activity in silkworm (*Bombyx mori* L.) during different developmental stages. Med. Chem. 4, 630–634.

Kim, D., Chun, O., Kim, Y., Moon, H., Lee, C., 2003a. Quantification of phenolics and their antioxidant capacity in fresh plums. J. Agric. Food. Chem. 51, 6509–6515.

Kim, J.W., Kim, S.U., Lee, H.S., Kim, I., Ahn, M.Y., Ryu, K.S., 2003b. Determination of 1-deoxynojirimycin in *Morus alba* L. leaves by derivatization with 9-fluorenylmethyl chloroformate followed by reversed phase high-performance liquid chromatography. J. Chromatogr. A 1002, 93–99.

Kurioka, A., Yamazaki, M., 2002. Purification and identification of flavonoids from the yellow green cocoon shell (Sasamayu) of the silkworm, *Bombyx mori*. Biosci. Biotechnol. Biochem. 66, 1396–1399.

Mitra, S., Mobarak, S.H., Karmakar, A., Barik, A., 2019. Activities of antioxidant enzymes in three species of *Ludwigia* weeds on feeding by *Altica cyanea*. Journal of King Saud University –. Science 31, 1522–1527.

Rice-Evans, C.A., Miller, N.J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20, 933–956.

Ryu, K.-S., Lee, H.-S., Kim, K.-Y., Kim, M.-J., Sung, G.-B., Ji, S.-D., Kang, P.-D., 2013. 1-Deoxynojirimycin content and blood glucose-lowering effect of silkworm (*Bombyx mori*) extract powder. Int. J. Indust. Entomol. 27, 237–242.

Sakai, R., Kamiya, H., 2006. 1-Deoxynojirimycin derivatives from the marine sponge *Lendenfeldia chondrodes*. J. Antibiot. 59, 507–511.

Seeram, N.P., Henning, S.M., Lee, R., Niu, Y., Scheuller, H.S., Heber, D., 2006. Catechin and caffeine contents of green tea dietary supplements and correlation with antioxidant activity. J. Agric. Food. Chem. 54, 1599–1603.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. 16, 144–158.

Tomotake, H., Katagiri, M., Yamato, M., 2010. Silkworm pupae (*Bombyx mori*) are new sources of high quality protein and liquid. J. Nutr. Sci. Vitaminol. 56, 446–448.

Vichasilp, C., Jundapun, P., Wiwacharn, P., Wangtueai, S., 2018. Accumulation of 1-deoxynojirimycin (DNJ) in different species of silkworm larvae in Northeast of Thailand. Food and Applied Bioscience Journal 6, 133–139.

Yatsunami, K., Murata, K., Kamei, T., 2011. 1-Deoxynojirimycin content and alfa-Glucosidase inhibitory activity and heat stability of 1-deoxynojirimycin in silkworm powder. Food Nutrit. Sci. 2, 87–89.

Yatsunami, K., Ichida, M., Onodera, S., 2008. The relationship between 1-deoxynojirimycin content and alphaglucosidase inhibitory activity in leaves of 276 mulberry cultivars (*Morus* spp.) in Kyoto. J. Nat. Med. 62, 63–66.

Yoon, S., Nathan, A.K., Wong, M.C., Auh, J.-H., 2019. Comparative characterization of protein hydrolysates from three edible insects: mealworm larvae, adult crickets, and silkworm pupae. Foods 8, 1–16.