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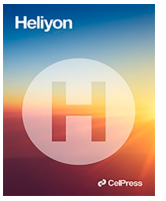

Research article

Exploring the excellence of commercial Brahmi products from Thai online markets: Unraveling phytochemical contents, antioxidant properties and DNA damage protection

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ABSTRACT

Brahmi (*Bacopa monnieri* (L.) Wettst.) is extensively used as a nutritional supplement in various commercial products as the plant contains abundant phytochemicals and has antioxidant properties. This study assessed the phytochemical contents, antioxidant properties, and DNA damage protection among seven Brahmi products sold through Thai online markets. Results showed that the P6 sample exhibited 3.5–7.5 fold higher bacoside contents than values observed in the other six product samples. The P6 sample also demonstrated the highest TTC, TFC, and TPC compared to the other brands. For antioxidant activity, the samples (P1–P7) displayed high capacity to scavenge DPPH free radicals with slightly significant differences ranging from 78.37 ± 0.25 to 87.21 ± 0.05 at *p*-value ≤0.01. The P6 sample showed strong protection against H_2O_2-induced oxidation of DNA strand breakage, indicating highly potent phytochemical compounds with effective free radical scavenging activity, and the ability to prevent DNA damage. The P6 sample showed promise as a valuable ingredient for the development of functional food products. However, further in vivo animal and clinical studies are required to explore the neuroprotective enhancement effects of Brahmi extracts.

1. Introduction

Bacopa monnieri (L.) Wettst., commonly known as Brahmi, is a medicinal herb that is native to India, China, and Thailand [1,2].

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Brahmi contains numerous bioactive compounds, with the main active constituents being the four steroidal saponin glycosides in bacoside A (as a mixture of bacoside A3, bacopaside II, bacopaside X, and bacopasaponin C) [3–5]. Brahmi has gained considerable attention for its traditional use in Ayurvedic medicine [6], particularly for enhancing memory [7], boosting brain function [8], and mitigating the effects of Alzheimer's disease [9,10]. These beneficial effects are attributed to various mechanisms including promoting antioxidant activity [11] and protecting against DNA damage [12,13]. Previous research showed that Brahmi treatment enhanced antioxidant enzyme activities such as superoxide dismutase, glutathione peroxidase, and catalase in a rat model [14,15], suggesting that Brahmi's therapeutic potential supported the body's natural defense mechanisms against oxidative stress.

Brahmi products have recently gained significant popularity as commercially marketed nutritional supplements in the global herbal industry [16]. These products are widely accessible in Thai markets, especially through online platforms, which showed increased demand during the Covid-19 outbreak. Brahmi products are offered by various manufacturers including household small and medium-sized enterprises (SMEs), and larger companies. Brahmi products are commercially produced as dried plants, dried plant powder, and dry extract powder and also commonly available as dried raw materials, capsules, tablets, and tea bags. However, the quality and content of these products are influenced by various factors including plant cultivars [17,18], plant growth stages [19], cultivation practices [20], environmental conditions [21], post-harvest procedures [22], and manufacturing processes [23,24]. These factors impact the bioactive compound contents that are responsible for the antioxidant and DNA damage protective properties of Brahmi products.

Despite their popularity, insufficient research has been conducted to evaluate the contained bioactive compounds, as well as the antioxidant and DNA damage protection potential of the different Brahmi products currently available in Thailand. Therefore, this study assessed the profiles of phytochemical contents, antioxidant properties, and DNA damage protective effects of commercial Brahmi products available online in Thailand, with specific focus on products made from dried plants and dried plant powder. This research will better inform consumers about the use of Brahmi products and also provide knowledge for the development of functional food products.

2. Materials and methods

2.1. Preparation of brahmi products' extracts

Seven commercial Brahmi products (P1–P7) from different manufacturers were purchased through Thai online markets (Table 1). Each sample was individually ground to a fine powder, and the chemical compounds were determined using the slightly modified methods of Bansal et al. [21] and Phrompittayarat et al. [23]. In this process, 50 mg of the fine powder was mixed with 1 mL of methanol in a 2 mL centrifuge tube and vigorously vortexed for 1 min. The mixture was then sonicated in an ultrasonic water bath for 15 min and subsequently centrifuged at 10,000 rpm for 5 min. The supernatant was collected, and the remaining residues were subjected to two additional extraction cycles. All supernatants from the three extraction cycles were pooled in a collection tube and then dried at 40 °C using a dry bath incubator. The dry residues were dissolved with 1 mL methanol by vigorously vortexing for 2 min and these extracted solutions were stored at −20 °C until further use.

2.2. Bacoside content quantification

Stock solutions of the four reference standards (bacoside A3, bacopaside II, and bacopaside X from Sigma-Aldrich, USA, and bacopasaponin C from ChromaDex Standards, USA) were individually prepared by dissolving in methanol. Subsequently, the reference standards for HPLC analysis were mixed with serial dilution ranging from 0 to 500 μg/mL for each reference standard.

The contents of the four bacoside A products in Brahmi were analyzed using the method described by Inthima and Sujipuli [25]. Before analysis, Brahmi product extracts were filtered through a 0.45 μm nylon syringe filter (Tianjin Fuji Science & Technology Co., Ltd., China). The filtered extracts were then injected into an HPLC system (1260 Infinity II LC System, Agilent Technologies, USA), equipped with a LiChroCART® 4-4 Purospher® STAR RP-18 endcapped (5 μm) guard column and a column (250 × 4.6 mm Merck, Germany). The mobile phase, comprising 65 % (v/v) acetonitrile and 35 % (v/v) aqueous phosphoric acid (0.2 % v/v, pH 3.0), was delivered at a flow rate of 1 mL/min. The presence of bacosides in the Brahmi products' extracts was detected at 205 nm using a diode array detector by comparing retention times with reference standards. The bacoside content in the Brahmi products' extracts was then calculated using a linearity equation that related peak areas to concentrations of reference standards (Fig. S1).

Table 1
Characteristics of commercial Brahmi products investigated in this study.

Brahmi product[a]	Characteristic	Color	Packaging	Manufacturer type
P1	Dried plant powder	Light brown	Zip lock foil bag	SME
P2	Dried plant	Green	Ziplock clear-plastic-bag	Household
P3	Dried plant	Dark brown	Sealed clear-plastic-bag	Household
P4	Dried plant powder	Light green	Tea bags in zip lock white-plastic-bag	Household
P5	Dried plant	Light brown	Zip lock foil bag	Household
P6	Dried plant extract powder	Light green	Capsules in black plastic bottle	Company
P7	Dried plant powder	Light green	Capsules in white plastic bottle	SME

[a] Brahmi products were purchased from Thai online markets.

2.3. Total phenolic content quantification

Total phenolic content (TPC) in the Brahmi products was evaluated using the Folin-Ciocalteu assay [26] as a mixture of gallic acid (Bio Basic, Canada) or Brahmi products' extracts (15 μL), deionized water (240 μL), and Folin-Ciocalteu reagent (15 μL). The mixtures were gently mixed and incubated for 5 min. Subsequently, 10 % w/v Na_2CO_3 (70 μL) was added, and the mixtures were continuously mixed and incubated at room temperature in the dark for 60 min. After incubation, the absorbances of the mixtures were measured at 750 nm using a microplate reader (BioTek Synergy H1, Agilent Technologies, USA). The TPC in the Brahmi products' extracts was then calculated using a linearity equation obtained from the standard calibration curve of absorbances against concentrations of gallic acid (ranging from 0 to 400 μg/mL, Fig. S2), and expressed in milligrams of gallic acid equivalent (GAE) per gram of dry Brahmi products' powder (mg GAE/g).

2.4. Total flavonoid content quantification

Total flavonoid content (TFC) in the Brahmi products was assessed by the modified $AlCl_3$ colorimetric assay [27]. Briefly, quercetin solution (Fisher Scientific, USA) or Brahmi products' extracts (30 μL) were mixed with 5 % w/v $NaNO_2$ (10 μL) and deionized water (120 μL). After vortexing and a 5-min incubation at room temperature (25 °C), 10 % w/v $AlCl_3$ (10 μL) was added, followed by another 6-min incubation at room temperature. Subsequently, 1 N NaOH (60 μL) and deionized water (70 μL) were added. Finally, the mixture was quantified for TFC at 510 nm using a microplate reader (BioTek Synergy H1, Agilent Technologies, USA). The standard calibration was plotted using quercetin at a serial concentration of 0–300 μg/mL (Fig. S2), with TFC expressed as milligrams of quercetin equivalent (QE) per gram of dry Brahmi products' powder (mg QE/g).

2.5. Total triterpenoid content quantification

Total triterpenoid content (TTC) in the Brahmi products was determined using a modified method based on Wei et al. [28]. In summary, ursolic acid (Sigma-Aldrich, USA) or Brahmi products' extracts (20 μL) were evaporated at 50 °C using a hot air oven until a completely dried powder was obtained. Then, a solution of 5 % w/v vanillin-acetic acid (10 μL) and sulfuric acid (18 μL) was added. These mixtures were thoroughly mixed and incubated at 70 °C for 30 min. After cooling, glacial acetic acid (72 μL) was added. The absorbances of the mixtures were measured at 573 nm using a microplate reader (BioTek Synergy H1, Agilent Technologies, USA). The TTC of Brahmi products' extracts was calculated based on a calibration curve of ursolic acid (Fig. S2) and expressed as milligrams of ursolic acid equivalent (UAE) per gram of dry Brahmi products' powder (mg UAE/g).

2.6. Analysis of DPPH radical scavenging activity

The scavenging activity of Brahmi products' extracts on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical inhibition was evaluated using a slightly modified method of Laywisadkul et al. [29]. In brief, Brahmi products' extracts (50 μL) were mixed with 0.1 mM DPPH (100 μL) and incubated at room temperature in the dark for 30 min. The absorbances of the mixtures were then measured at 517 nm by a microplate reader (BioTek Synergy H1, Agilent Technologies, USA). Methanol was used as the negative control, while standard compounds including bacoside A (mixture of bacoside A3, bacopaside II, bacopaside X, and bacopasaponin C), gallic acid, quercetin, and ursolic acid (at a final concentration of 250 μg/mL) were used as positive controls. The percentage inhibition of the DPPH radical was calculated using the following equation:

$$\%\ \text{inhibition} = \left[\left(A_{control} - A_{test\ sample}\right) / A_{control}\right] \times 100$$

2.7. Protective activity of brahmi products against H_2O_2-induced DNA damage

Escherichia coli (DH5α) carrying pGEM-T plasmid (Promega, USA) was cultured on Luria-Bertani (LB) liquid medium (Himedia™, India) supplemented with ampicillin at a final concentration of 50 μg/mL. The culture was then incubated at 37 °C with shaking at 150 rpm for 16–18 h. The pGEM-T plasmid was extracted using PureDireX Plasmid miniPREP Kit (Bio-Helix, Taiwan). Subsequently, the purified plasmid was quantified for concentration and assessed for quality using a NanoDrop™ Lite Spectrophotometer (Thermo Scientific, USA) and gel electrophoresis.

To evaluate the protective effect of Brahmi products against H_2O_2-induced DNA damage, a slightly modified method described by Anand et al. [13] was employed. In brief, pGEM-T plasmid (500 ng) was mixed with $FeSO_4$ (4 μM), H_2O_2 (10 mM), and Brahmi products' extracts (5 μL). The final volume was adjusted to 20 μL with nuclease-free water. The mixture was incubated at 37 °C for 1 h and then placed in an ice bath for 10 min to stop H_2O_2 activity. Plasmid without H_2O_2 treatment was used as the negative control, while plasmid treated with H_2O_2 but without Brahmi products' extracts served as the positive control. Standard compounds including bacoside A, gallic acid, quercetin, and ursolic acid (at a final concentration of 250 μg/mL) were used instead of Brahmi products' extracts for comparison.

To assess the presence of open-circular DNA (damaged DNA), 1 % agarose gel electrophoresis was performed. The DNA was stained with GelRed® (Biotium, USA) and then visualized and photographed by a gel documentation system (gelLITE, Cleaver Scientific, UK). The intensity of open-circular DNA was measured by ImageJ software [30] from the photos. The percentage of DNA damage was calculated as the following equation:

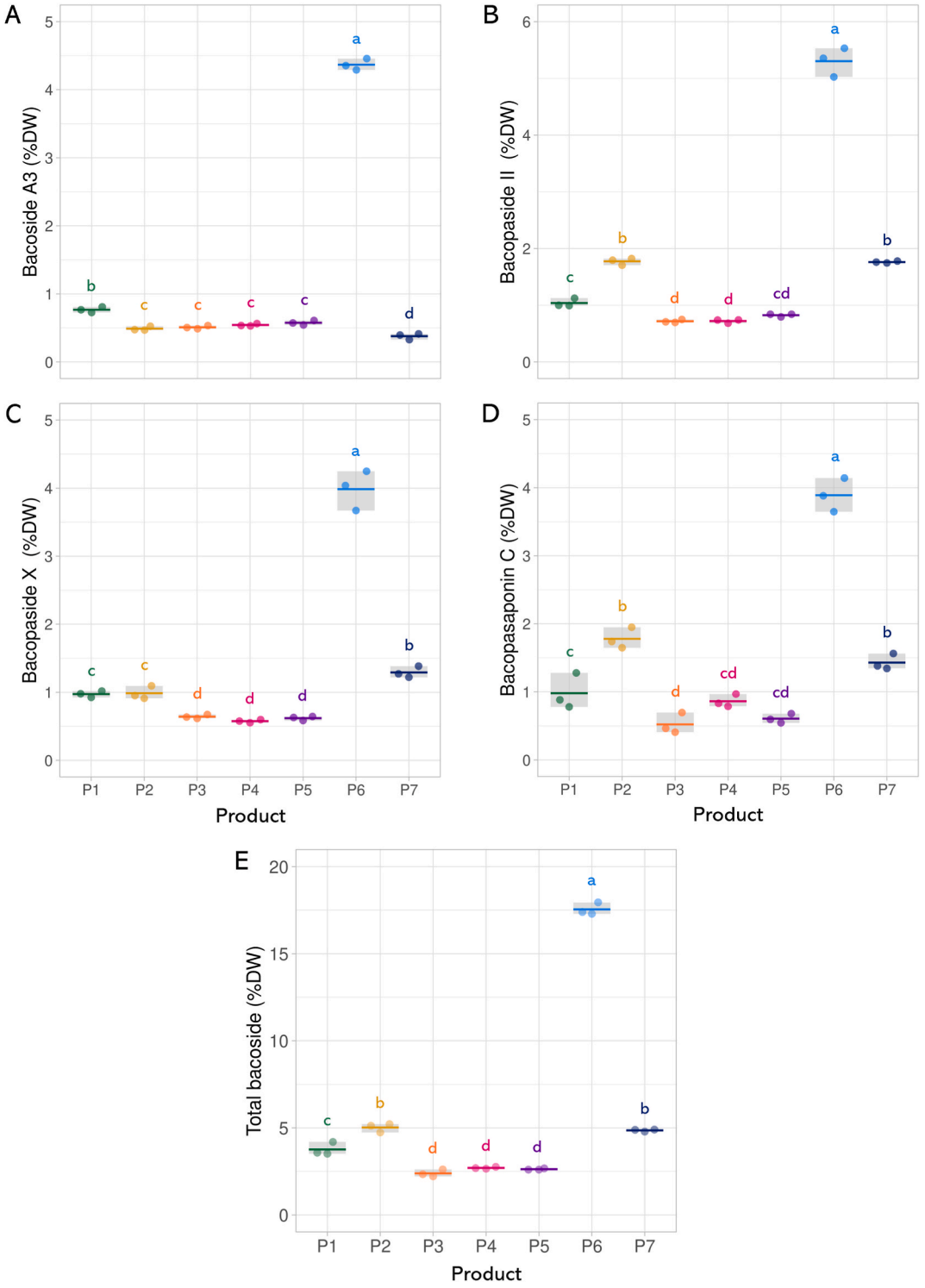

Fig. 1. Contents of bacoside A3 (A), bacopaside II (B), bacopaside X (C), bacopasaponin C (D), and total bacoside (E) in Brahmi products (P1–P7) presented as individual raw data (dots), mean values (horizontal lines, n = 3 samplings), and data ranges (grey bars). Different letters within the same figure indicate significant differences, analyzed by DMRT at $p \leq 0.01$.

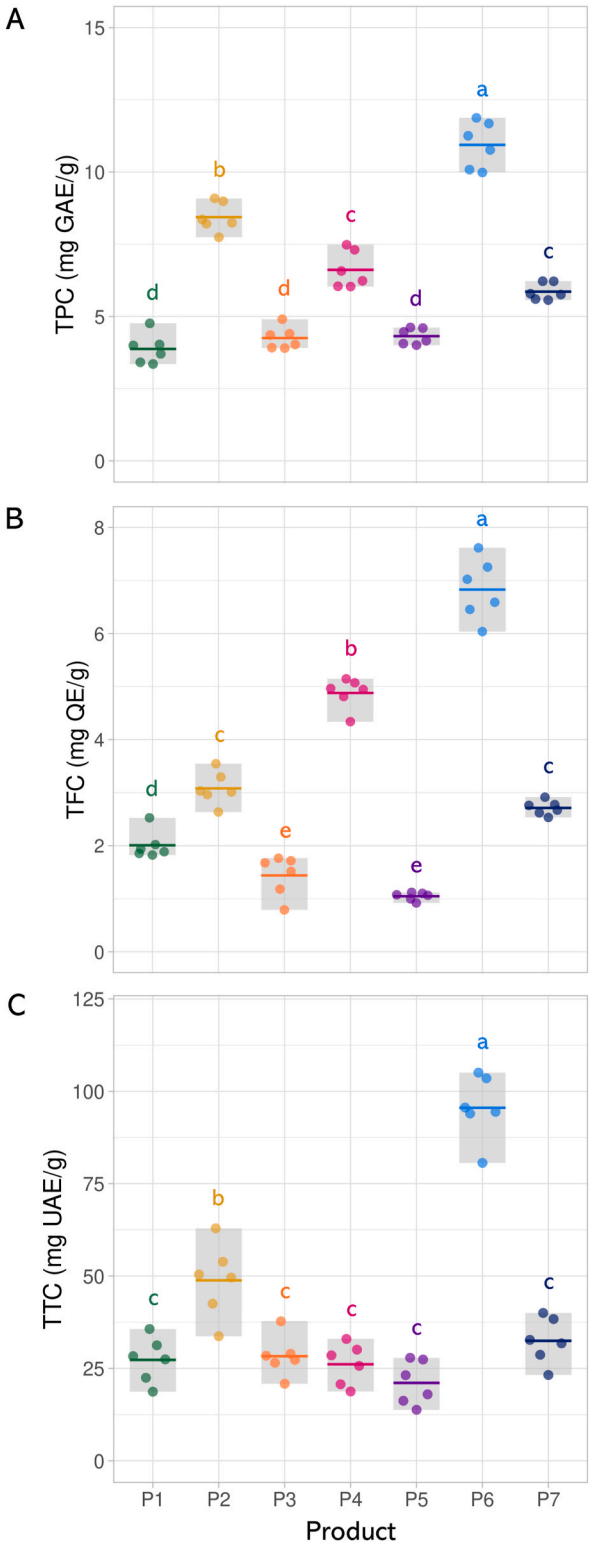

Fig. 2. Total phenolics (TPC, A), total flavonoids (TFC, B), and total triterpenoids (TTC, C) in Brahmi products (P1–P7) presented as individual raw data (dots), mean values (horizontal lines, n = 6 samplings), and data ranges (grey bars). Different letters within the same figure indicate significant differences, analyzed by DMRT at $p \leq 0.01$.

%DNA damage = (Band intensity of tested sample / Band intensity of positive control) × 100

To ensure accuracy, each treatment was carried out with five biological replicates, and visualized DNA bands on the gel were photographed twice as images for analysis.

2.8. Statistical analysis

The experiment was conducted using a complete randomized design (CRD) and performed with seven treatments (Brahmi products) with three to six sampling replicates from each product. Results were expressed as mean values ± standard error. Data analysis was carried out by one-way analysis of variance (ANOVA). Statistically significant differences in mean values among the samples were evaluated by Duncan's new multiple range test (DMRT) at p-value ≤0.01, with data visualized using PlotsOfData web application [31].

3. Results

3.1. Quantification of bacoside content in brahmi products

Bacoside contents among the seven commercial Brahmi products (abbreviated by P1–P7) were quantified using HPLC assay. As shown in Fig. 1A–E, the P6 sample had significantly highest bacoside A3 (4.37 ± 0.05 %DW), bacopaside II (5.30 ± 0.15 %DW), bacopaside X (3.99 ± 0.17 %DW), bacopasaponin C (3.89 ± 0.14 %DW), and total bacoside (17.55 ± 0.20 %DW) compared to the other six products (p-value ≤0.01), which showed slightly significant differences in bacoside A3 (ranging from 0.38 ± 0.03 to 0.77 ± 0.02 %DW, Fig. 1A), bacopaside II (ranging from 0.72 ± 0.02 to 1.77 ± 0.03 %DW, Fig. 1B), bacopaside X (ranging from 0.57 ± 0.01 to 1.29 ± 0.05 %DW, Fig. 1C), bacopasaponin C (ranging from 0.52 ± 0.09 to 1.78 ± 0.09 %DW, Fig. 1D), and total bacoside (ranging from 2.39 ± 0.12 to 5.03 ± 0.15 %DW, Fig. 1E). This indicated that the P6 sample contained the highest bacoside content as the main bioactive compound in Brahmi (at least 3.5 fold higher than the other six samples) and was the most useful product for consumers.

3.2. Quantification of phytochemical contents among the seven brahmi products

The TPC, TFC, and TTC across P1–P7 samples were quantified. Results showed that all the studied products contained significant amounts of phytochemicals with TPC, TFC, and TTC values ranging 3.88 ± 0.21–10.94 ± 0.33 mg GAE/g (Fig. 2A), 1.05 ± 0.03–6.83

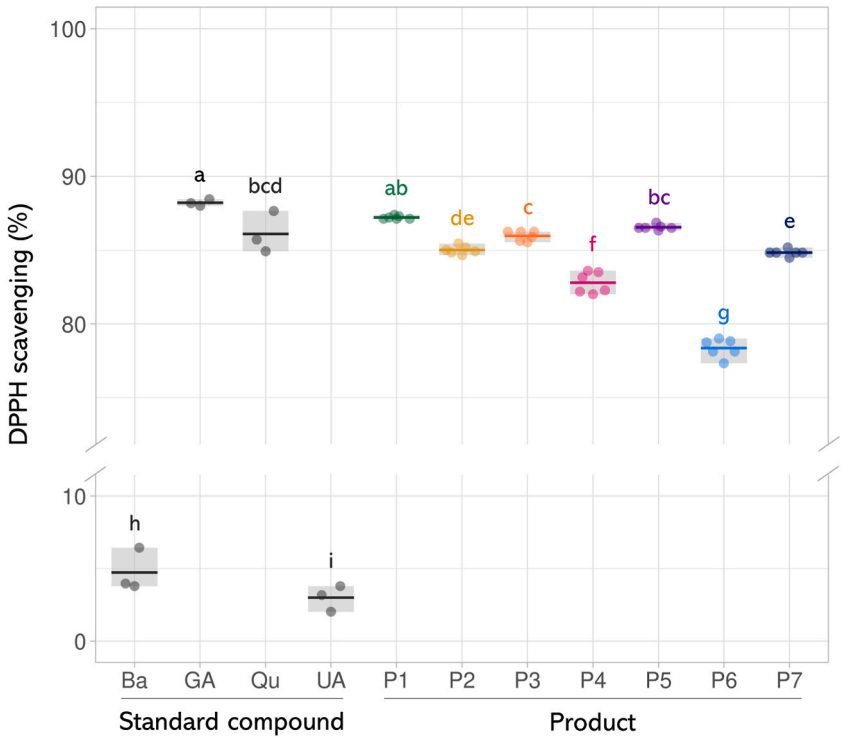

Fig. 3. Quantification of antioxidant activity of Brahmi products (P1–P7) compared with bacoside A (Ba), gallic acid (GA), quercetin (Qu), and ursolic acid (UA) standard compounds using DPPH scavenging assay presented as individual raw data (dots), mean values (horizontal lines, n = 3 and 6 samplings for standard compounds and Brahmi products, respectively), and data ranges (grey bars). Different letters within the same figure indicate significant differences, analyzed by DMRT at $p \leq 0.01$.

± 0.24 mg QE/g (Figs. 2B), and 21.07 ± 2.43–95.53 ± 3.56 mg UAE/g (Fig. 2C), respectively. The P6 sample contained significantly highest TTC, TFC, and TPC ($p \leq 0.01$).

3.3. DPPH radical scavenging activity of brahmi products

The free radical scavenging activities of all seven Brahmi products' extracts (P1– P7) were determined by the ability to scavenge DPPH free radicals and compared with chemical compounds as standard controls. Results showed that all the tested samples had high DPPH free radical scavenging ability, with slightly significant differences (ranging from 78.37 ± 0.25 to 87.21 ± 0.05 %) at *p*-value ≤0.01 compared to gallic acid (GA) and quercetin (Qu) standard compounds (Fig. 3). Antioxidant activity in all tested samples was significantly higher than for the bacoside A (Ba) and ursolic acid (UA) standard compounds (Fig. 3).

All the studied Brahmi products had high bacosides, phytochemical compounds, and free radical scavenging activity. The seven samples were further evaluated for their effect on preventing DNA damage by scavenging free radicals.

3.4. Protective activity of brahmi products against H_2O_2-induced DNA damage

The inhibition of H_2O_2-induced DNA damage was assessed by measuring the amount of open-circular (linear) pGEM-T plasmid DNA form (size greater than 3 kb indicated DNA-strand breakage) visualized on agarose gel electrophoresis. Results showed that all tested Brahmi products were capable of preventing H_2O_2-induced DNA strand break. This was observed by the analysis of DNA band intensity on agarose gel, which showed a decrease in the linear form and an increase in the original form of the pGEM-T plasmid DNA compared to the H_2O_2-treated plasmid without Brahmi products' extracts (positive control, Fig. 4). Of these, the extracted P6 sample exhibited the most effective DNA breakage prevention activity, resulting in 14 % DNA damage relative to the positive control (Fig. 4). This finding indicated that the P6 sample effectively protected against OH-induced oxidation of DNA strand breakage.

4. Discussion

Brahmi extracts contain numerous bioactive compounds such as phenolics [13], terpenoids and bacosides [3,4] and play an important role through significant antioxidant activity and protection against DNA damage. In this study, seven Brahmi products (P1–P7), available in Thai markets, were assessed for phytochemical contents, antioxidant profiles, and protection against DNA damage. The high bacoside A content in Brahmi extract has been well-recognized for its antioxidant activity [11] with an important role in neuro-protective disease inhibition, such as Alzheimer's disease [10]. Here, total bacoside content showed significant variation among the seven different Brahmi product brands ranging from 2.39 ± 0.12 to 5.03 ± 0.15 %DW. This result concurred with previous findings on the bacoside content of naturally grown bacopa plants which varied from 5.1 to 22.17 %DW [4], 1.82–3.65 %DW [18], and 2.55 %DW [32]. Extract P6 contained a higher bacoside mixture (17.55 ± 0.20 %DW) than the other samples because it was the only product in this study produced from dried plant extract powder.

Previous studies reported that antioxidant compounds from Brahmi products' extracts reduced oxidative stress against reactive

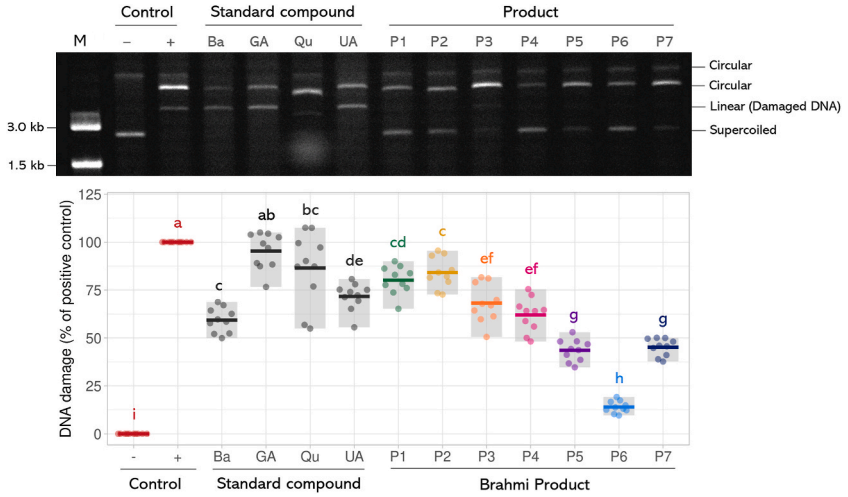

Fig. 4. Protective activities of Brahmi products (P1–P7) on pGEM-T plasmid DNA cleavage caused by H_2O_2 in comparison with bacoside A (Ba), gallic acid (GA), quercetin (Qu), and ursolic acid (UA) standard compounds. The negative control (−) consisted of the plasmid without H_2O_2 treatment, whereas the positive control (+) involved the plasmid treated with H_2O_2 but without Brahmi-product extracts or standard compounds. The P6 extract was diluted 5-fold before use to determine the protective activity. Data are presented as individual raw data (dots) from 10 gel photographs (5 DNA damage reaction), mean values (horizontal lines), and data ranges (grey bars). Different letters indicate significant differences, analyzed by DMRT at $p \leq 0.01$.

oxygen species (ROS) and inhibited or repaired damage to cells [33]. The total phenolic content (TPC) consistently demonstrated a positive correlation with antioxidant activity in plant extracts [34,35]. In this study, TPC varied significantly among the Brahmi product samples (ranging from 4.25 ± 0.16 to 10.94 ± 0.33 mg GAE/g product powder), while the P6 sample had significantly higher TPC (10.94 ± 0.33 mg GAE/g product powder) than the other six products. These findings were supported by observations that bacopa extract contained high TPC at 5.67–9.44 mg GAE/g plant [36], 54.8–70.3 μg GAE/mg extract [37], and 55.0 μg GAE/mg extract [13]. Reasons for the variations in antioxidant activities across disparate Brahmi product brands could be due to diverse accumulation of TPC, TFC and saponin contents [36] in raw plant materials from different genotypes of *Bacopa monnieri* [18,25] or plants harvested under different cultivation practices and environmental conditions [20,21]. Furthermore, the bioactivity and phytochemical content of herbal products can be significantly influenced by factors such as the drying process, packaging materials, and storage conditions. Specifically, drying fresh Brahmi plants using a cabinet dryer with a constant temperature of 50 °C retained higher levels of bacoside A compared to drying under sunlight or shade [24]. Moreover, drying the fresh Brahmi plant with a hot air cabinet dryer at 50 °C resulted in higher bacoside A and antioxidant activity compared to drying at 40 °C and 60 °C [22]. In our study, the visible difference in Brahmi product color (green and brown, as indicated in Table 1) was likely attributed to the drying process. Additionally, the choice of packing material also plays a role, with high-density polyethylene (HDPE) containers retaining the maximum andrographolide content of dry *Andrographis paniculata* over low-density polyethylene (LDPE) and polyethylene (PE) containers [38]. Storage temperature is also a critical factor; during the 28-day storage period, the content of bacopaside I and bacoside A3 in the crude extract remained unchanged at 5 °C but significantly decreased at 80 °C and gradually reduced at 40 °C and 60 °C [23]. Furthermore, considering that some bioactive compounds are light-sensitive [39], packaging that prevents exposure to light may aid in retaining these compounds in herbal products. However, it is essential to acknowledge that the tested products in our study arrived in different packaging and under unknown storage conditions, potentially introducing variations that could impact the phytochemical compounds in these products.

Based on a comparative analysis, all Brahmi product samples had high DPPH radical scavenging activity values ranging from 78.37 ± 0.26 to 87.21 ± 0.05 %. These high values were attributed to the presence of polyphenols with antioxidant capability to reduce free radical formation [34,35]. The bacopa extracts possibly decreased the free radicals because the contained phytochemical compounds (bacoside A, TPC, TFC, and TTP) reacted with the donating hydrogen atoms in the antioxidant principles [40]. Our findings suggested that Brahmi products showed highly potent antioxidant activity, which diminished free radical scavenging activities and could be considered as natural antioxidant sources with high potential for protecting against DNA damage generated from H_2O_2 treatment.

Hydrogen peroxide (H_2O_2) genotoxic agent is commonly used to induce oxidative DNA damage. The hydroxyl radical ($OH^{\bullet}$) interreacts with nitrogenous bases within the DNA molecule generating sugar and base radicals. This causes the breakdown of the sugar-phosphate backbone resulting in strand breaks [41]. Many previous studies reported that natural extracts with strong antioxidant properties showed high potential to prevent and repair oxidative DNA damage caused by reactive oxygen species (ROS) in living cells [35,42,43]. Antioxidants, including phenolics, flavonoids, carotenoids, and terpenoids, have been observed to interact with ROS, generating inert compounds that mitigate oxidative damage [44,45]. Cells possess a limited inherent capacity for antioxidants, underscoring the importance of supplementing with exogenous antioxidants, particularly those derived from natural herbs. In this study, all Brahmi products' extracts demonstrated the potential to prevent pGEM-T plasmid DNA damage. These extracts exhibited the ability to conserve a greater proportion of the original supercoiled DNA, while minimizing a reduced proportion of digested linear DNA in comparison to untreated Brahmi products' extracts.

The P6 sample exhibited the highest protective property of hydroxyl-radical-mediated-DNA-strand break, with similar observations from quercetin treatment, known as an antioxidant with the ability of DNA damage protection [46]. High amounts of phenolic compounds in the P6 sample with strong antioxidant properties were positively associated with DNA protection against oxidative stress [47]. These results agreed with previous findings that H_2O_2-induced plasmid DNA cleavage decreased in the presence of bacopa extracts [13,48], *Mentha arvensis* [49], *Abutilon indicum* [50], and *Terminalia catappa* [51]. One possible explanation is that bacopa extracts act as protective agents against H_2O_2-induced DNA damage by reducing the scavenging of highly reactive hydroxyl radicals [47]. Antioxidants provided protect against oxidative DNA damage in an *in vitro* experiment [50], while polyphenol compounds acted as antioxidants by effectively reducing ROS generation, thereby preventing DNA degradation [50]. Similarly, oxidatively induced DNA damage is mitigated by the contained antioxidant compounds of many medicinal plant extracts such as *Emblica officinalis* [52], *Rhaponticum carthamoides* [53], *Leonurus sibiricus* [54], and *Viburnum opulus* [55]. It is noteworthy that P5 and P7 exhibit substantial antioxidant activity (Fig. 3) and DNA protective activity (Fig. 4), even in the presence of a comparatively low phenolic content (Fig. 2). This observed effectiveness could potentially be attributed to the influence of other unexplored compounds not assessed in this study, such as carotenoids [56], phytosterols [57], and alkaloids [58]. Notably, these compounds have been previously identified in Brahmi [59–61].

This preliminary study showed that phytochemical compounds from Brahmi products' extracts had antioxidant properties and acted as cytoprotective agents. Nevertheless, the existing evidence is constrained, necessitating further research to definitively establish the potential advantages of Brahmi products in terms of DNA protection and recovery. In-depth in vivo animal and clinical studies on bacopa extracts are imperative to compare their neuroprotective enhancements with conventional therapeutic drug applications. Investigating the molecular mechanisms of action and exploring additional bioactive compounds in Brahmi are essential components of advancing our understanding.

5. Conclusions

Findings revealed variations in antioxidant properties and cytoprotective agents among seven commercial Brahmi products (P1–P7) available in the Thai market. The P6 product contained high phytochemicals and demonstrated significantly potent

antioxidant activity with hydroxyl radical scavenging effects that contributed to the protection of DNA damage under *in vitro* conditions. Results indicated that the P6 Brahmi product showed promise as a natural antioxidant for genetic stability maintenance and as a functional food product ingredient. However, current evidence remains limited and further research is required to ascertain the potential DNA-protective and DNA-recovering benefits of Brahmi products.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Junya Nopparat: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Kawee Sujipuli:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Kumrop Ratanasut:** Writing – review & editing, Investigation, Formal analysis. **Monthana Weerawatanakorn:** Writing – review & editing, Investigation, Formal analysis. **Surisak Prasarnpun:** Writing – review & editing, Investigation, Formal analysis. **Bussagon Thongbai:** Writing – review & editing, Investigation, Formal analysis. **Walailak Laothaworn:** Validation, Methodology, Investigation. **Phithak Inthima:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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.

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Appendix A. Supplementary data

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