Wiley
Journal of Food Quality
Volume 2025, Article ID 1971279, 30 pages
https://doi.org/10.1155/jfq/1971279

WILEY

Research Article

Development and Comparative Evaluation of Two Polyherbal Nutraceuticals, BobyGuards A and B: Phytochemical Composition, Antioxidant Potential, and Nutritional Value

Borelle Mafogang,[1] **Stephane Zingue**,[2] **Charles Taptue Kotue**,[1] **Michelle Djouhou**,[1] **Franck Armand Fozing Fomo**,[3] **Roger Ponka**,[4] **and Elie Fokou**[1]

[1]*Department of Biochemistry, University of Yaoundé I, Yaoundé, Cameroon*
[2]*Department of Pharmacotoxicology and Pharmacokinetics, University of Yaoundé I, Yaoundé, Cameroon*
[3]*Department of Chemistry, University of Douala, Douala, Cameroon*
[4]*Department of Agriculture, Livestock and Derived Products, University of Maroua, Maroua, Cameroon*

Correspondence should be addressed to Roger Ponka; rponka@yahoo.fr

Received 11 November 2024; Revised 3 June 2025; Accepted 18 June 2025

Academic Editor: Sunil Pareek

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Research in nutrigenomics highlights the ability of nutrients to modulate oxidative stress and reduce free radical production, playing a key role in chronic disease prevention and management, particularly cancer. Nutraceuticals, especially polyherbal blends, offer enhanced bioactivity due to synergistic interactions among phytochemical constituents. This study aimed to evaluate the phytochemical composition, antioxidant activities, and nutritional value of two polyherbal nutraceutical formulations, BobyGuard A (BGA) and BobyGuard B (BGB). Formulations were prepared using *Curcuma longa, Piper nigrum, Dioscorea bulbifera, Spirulina platensis, Adansonia digitata,* and *Brassica oleracea*. Standardization included assessments of phytochemical profiles, organoleptic, and physicochemical characteristics. Proximate composition, vitamin C, and mineral contents were measured, and LC–MS/MS was employed for the tentative identification of bioactive compounds. Antioxidant capacity was assessed using DPPH, OH, FRAP, and TAC assays. Both formulations shared similar qualitative phytochemical profiles, with phenols and flavonoids consistently detected. Quantitatively, BGA showed significantly higher ($p < 0.05$) levels of vitamin C, manganese, copper, sulfur, tannins, water and alcohol extractives, and total antioxidant capacity. Conversely, BGB exhibited higher protein, crude fiber, potassium, total phenols (6580.36 ± 238.10 mg GAE/100 g DM), flavonoids (1107.27 ± 9.74 mg QuE/100 g DM), carotenoids (403.52 ± 5.35 mg/100 g DM), and stronger antioxidant activities (OH: 86.03 ± 0.71%; DPPH: 91.09 ± 0.45%). LC–MS/MS identified 50 compounds in BGA and 53 in BGB, including curcumin, piperine, catechin, and pheophytin b. BGB's superior antioxidant activity and richer phytochemical profile suggest stronger potential to mitigate oxidative stress and related cellular damage. In contrast, BGA's elevated mineral content, particularly copper and manganese, may enhance enzymatic functions involved in metabolic and redox homeostasis. These findings highlight the distinct yet complementary therapeutic roles of both formulations, supporting their use in chronic disease prevention and management strategies.

Keywords: antioxidant activity; BobyGuard A; BobyGuard B; nutrient profile; phytochemical composition; polyherbal nutraceuticals

1. Introduction

The global rise in chronic diseases has underscored the urgent need for effective preventive and management strategies that extend beyond conventional medicine. Oxidative stress has been identified as a significant contributing factor in the onset and progression of several chronic conditions, including cancer, cardiovascular diseases, and

neurodegenerative disorders [1, 2]. In this context, dietary interventions have gained increasing attention, especially those emphasizing foods rich in antioxidants and essential nutrients that may help mitigate oxidative damage. Bioactive compounds derived from plants known as phytochemicals such as alkaloids (e.g., piperine), carotenoids (e.g., phytoene), and polyphenols (e.g., curcumin), along with micronutrients like vitamin C, zinc, selenium, and manganese, have shown potential in supporting the prevention and management of chronic diseases through their antioxidant, anti-inflammatory, and immunomodulatory properties [3].

In recent years, there has been growing interest in nutraceutical foods or food products that offer health and medical benefits beyond basic nutrition [4] due to their abundance in phytochemicals and nutrients [5, 6]. Among these, polyherbal formulations, which combine multiple medicinal foods and plants, are gaining significant attention for their potential synergistic effects in promoting health and preventing disease [7, 8]. These formulations are particularly attractive because they offer a holistic approach to health management, drawing on the diverse phytochemical properties of different plants. The World Health Organization (WHO) has also recognized the critical role of medicinal herbs and foods in public healthcare, particularly in developing nations [9].

A key aspect of polyherbal formulations is standardization, which ensures the consistency and quality of the product [10]. However, challenges arise due to variations in plant sources, growing conditions, and extraction methods, which necessitate stringent quality control measures. Developing a polyherbal nutraceutical involves carefully selecting appropriate plant extracts, determining optimal proportions, ensuring consistent quality, and evaluating toxicity [11]. Combining various herbs in specific ratios can produce a desirable therapeutic effect, as the phytochemical constituents of individual plants may not be sufficient on their own to achieve the desired benefits [12]. Some plants, including *Curcuma longa* (turmeric), *Piper nigrum* (black pepper), *Dioscorea bulbifera* (air potato), *Spirulina platensis* (spirulina), *Brassica oleracea* var. capitata f. alba (white cabbage), *Afrostyrax lepidophyllus* (country onion), and *Adansonia digitata* (baobab), are used for the prevention and management of chronic diseases due to their rich content of bioactive phytochemicals, antioxidants, and essential nutrients [13–40]. These botanicals have demonstrated diverse health benefits supported by both traditional knowledge and scientific research. Thus, the aim of this study is to evaluate the nutritional composition, antioxidant capacity, and phytochemical profile of two new polyherbal nutraceutical formulations, BobyGuard A (BGA) and BobyGuard B (BGB) developed using these plants.

2. Materials and Methods

2.1. Reagents. Quercetin dihydrate, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), gallic acid, 2,6-di-tert-butyl-p-cresol or butylated hydroxytoluene (BHT), Folin–Ciocalteu's phenol reagent, L-ascorbic acid, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), aluminum chloride ($AlCl_3$), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), potassium persulfate, iron (II) sulfate, glacial acetic acid, hydrochloric acid (HCl), sulfuric acid (H_2SO_4), and ammonium molybdate. All other reagents and chemicals used in this study were of analytical grade.

2.2. Raw Materials. The ingredients of BGA and BGB were selected based on their reported nutritional value, antioxidant potential, anticancer activity, and traditional uses (Table 1).

The different raw materials were collected from various localities in Cameroon and authenticated at the Cameroon National Herbarium (CNH) by comparison to deposited material by Mr Ngansop, a botanist (Table 2).

2.3. Preparation of BGA and BGB

2.3.1. Treatment of Raw Material

2.3.1.1. Spirulina (Spirulina platensis). Fresh spirulina was oven-dried at 40°C for 48 h following the protocol described by Moor et al. [41].

2.3.1.2. Baobab (Adansonia digitata). Baobab fruits were processed according to Maptouom et al. [42], with slight modifications. The fruits were dehusked, and the pulp was separated from the seeds by pounding in a traditional mortar. The obtained pulp was sieved using a No. 40 mesh to remove fibrous materials and then stored in opaque plastic bags to protect against light and moisture.

2.3.1.3. White cabbage (Brassica oleracea var. Capitata f. alba). Fresh cabbage leaves were chopped and blended with deionized water in a 1:1.5 (w/v) ratio. The mixture was microwaved at 390 W for 3 min and then filtered through the Whatman No. 4 filter paper. The filtrate was oven-dried at 45°C for 72 h to yield a powdered cabbage extract [43].

2.3.1.4. Turmeric (Curcuma longa). Turmeric rhizomes were washed, sliced, and oven-dried at 45°C for 48 h before being pulverized. Extraction was performed following the method of Sogi et al. [44], with modifications. The powder was mixed with 82% ethanol in a 1:5 (w/v) ratio and stirred at 60°C for 30 min using a magnetic stirrer. This step was repeated until exhaustion. The solution was filtered through the Whatman No. 4 paper, and the filtrate was evaporated and oven-dried at 40°C for 72 h to obtain the final dry extract.

2.3.1.5. Air Potato (Dioscorea bulbifera). Bulbils of air potato were cleaned, chopped, and dried at 45°C for 48 h and then ground into powder. The powder was macerated in 70% ethanol (2:5 w/v) for 72 h at room temperature with occasional stirring. The mixture was filtered using the Whatman No. 4 paper, and the resulting filtrate was evaporated and oven-dried at 45°C until completely dry, following a modified protocol by Wang et al. [45].

Table 1: BobyGuard A and B's ingredients.

No.	Common name (local name)	Botanical name	Traditional uses	Bioactivities and mechanisms	References
1	Baobab	*Adansonia digitata*	Used traditionally to treat microbial infections, fever, hemoptysis, diarrhea, dysentery, cystitis, diuretic effects, and hepatic disorders	Exhibits hepatoprotective and nephroprotective effects; antidepressant activity; cardioprotective properties; antioxidant modulation and antiapoptotic effects via regulation of p53 and Bcl-2 in Ehrlich ascites carcinoma model. Rich in vitamin C which supports immune function and may facilitate cancer immunotherapy	[13–19]
2	White cabbage	*Brassica oleracea* var. capitata f alba	Traditionally applied as a poultice (cataplasm) for burns	Contains bioactive compounds such as 3,3′-diindolylmethane and sulforaphane (derived from indole-3-carbinol and isothiocyanate, respectively), which induce apoptosis, inhibit cell cycle progression, and enhance chemotherapeutic efficacy in breast cancer models	[20–22]
3	Country onion	*Afrostyrax lepidophyllus*	Used for sinusitis, abdominal, and navel pain	Demonstrates protective effects against oxidative liver enzyme damage; improves growth performance and nutrient utilization in animal models; exhibits cardioprotective effects against doxorubicin-induced toxicity	[23–27]
4	Black pepper	*Piper nigrum*	Traditionally used for malaria treatment	Possesses antioxidant, anticancer, anti-inflammatory, and antimicrobial activities; enhances curcumin bioavailability by inhibiting metabolic enzymes, thereby reducing breast tumor incidence and chemotherapy-induced toxicity	[28–30]
5	Turmeric	*Curcuma longa*	Used traditionally for liver diseases, jaundice, menstrual disorders, hematuria, and flatulence	Exhibits antioxidant, antineoplastic, anti-inflammatory, and antimicrobial properties; modulates multiple cancer-related signaling pathways including STAT3, JAK, MAPK, and EGFR, inhibiting metastasis	[31–33]
6	Air potato	*Dioscorea bulbifera*	Traditionally used for abscesses, boils, wound infections, memory enhancement, antiaging, and abdominal pain	Displays antitumor, anti-inflammatory, and antioxidant activities; analgesic effects linked to anti-inflammatory action; cytotoxic against breast cancer cell lines (MCF-7, MDA-MB-231), inhibiting cell migration	[34–37]
7	Spirulina	*Spirulina platensis*	Traditionally consumed as a nutritional supplement, with purported benefits in cancer, hypertension, obesity, and dyslipidemia management	Exhibits antioxidant and anticancer effects; ameliorates malnutrition; shown to alleviate chemotherapy-induced myelosuppression and enhance immune function in cancer patients	[38–40]

2.3.1.6. Country/Bush Onion (Afrostyrax lepidophyllus). Fresh bark was shade-dried at ambient temperature and pulverized. Extraction was conducted using a water–ethanol solution (30:70 v/v) in a 1:5 (w/v) ratio for 48 h, as per Moukette et al. [25], with adjustments. The extract was filtered through the Whatman No. 4 paper, and the residue was re-extracted using the same conditions. Combined filtrates were evaporated and oven-dried at 45°C for 48 h.

2.3.1.7. Black Pepper (Piper nigrum). Black pepper extraction followed the method described by Khan et al. [46], with adjustments. Ground black pepper was mixed with 95% ethanol (1:4 w/v) and stirred at 30°C using a magnetic stirrer for 2 h. The mixture was filtered through Whatman paper No. 4, and the extract was evaporated and oven-dried at 45°C for 48 h.

Baobab fruit pulp powder and all dried extracts were separately stored in conical tubes at −20°C, while spirulina was kept in an airtight container at ambient temperature.

Table 2: Entrants' characteristics and BobyGuard A and BobyGuard B's compositions.

Common name/local name	Botanical name	Identification number	Part used	Zone of collection	Extraction yield (%)	Proportion by weight in BGA (%, w/w)	Proportion by weight in BGB (%, w/w)
Baobab	*Adansonia digitata*	7815 SRF/Cam	Fruit pulp	Benoue, North region 9°10′38.52″N 13°26′18.24″E	NA	16	21
White cabbage	*Brassica oleracea* var. capitata f. alba	25,686 SRF/Cam	Leaves	Menoua, West region 5°27′59.99″N 10°06′60.00″E	2.20	7	10
Country/bush onion	*Afrostyrax lepidophyllus*	39,020 SRF/Cam	Bark	Bonano, East region 2°04′31.51″N 15°17′1.63″E	1.52	5	—
Black pepper	*Piper nigrum*	25,818 SRF/Cam	Seed	Penja, Littoral region 4°38′26.88″N 9°40′58.49″E	10.65	9	12
Turmeric	*Curcuma longa*	42,173 SRF/Cam	Rhizome	Santchou, West region 5°16′53.63″N 9°58′33.95″E	15.16	13	17
Air potato	*Dioscorea bulbifera*	29,684 SRF/Cam	Bulbils	Bamendjou, West region 5°23′21.85″N 10°19′52.34″E	13.61	20	—
Spirulina	*Spirulina platensis*	—	Whole algae	Nomayos, Centre region 3°47′24.39″N 11°26′25.55″E	NA	30	40

Abbreviation: NA = not applicable.

2.3.2. Extraction Yield. Extraction yield was *calculated* using the following formula:

$$\text{Extraction yield} = \frac{(\text{Dry extract weight})}{(\text{Raw material weight})} \times 100. \quad (1)$$

2.3.3. BGA and BGB Development. Formulations, BGA and BGB, were prepared by blending the processed ingredients in specific proportions based on the minimum effective doses reported in the literature (Table 2). The components were thoroughly mixed and homogenized. The polyherbal blends were then oven-dried at 45°C for 24 h, followed by a final blending step. The formulations were stored in air-tight containers at ambient temperature until further use.

2.4. Standardization of Entrants, BGA, and BGB

2.4.1. Organoleptic Characterization. Organoleptic properties, including color, odor, taste, texture, and flowability, of individual ingredients, BGA, and BGB were evaluated following the methodology of Siddiqui and Hakim [47]. Sensory assessments were carried out at the Laboratory for Food Science and Metabolism, University of Yaoundé I, Cameroon. A trained panel of 10 adult volunteers (aged ≥ 21 years) was recruited. Written informed consent was obtained from all participants, and the study adhered to the ethical standards outlined in the Declaration of Helsinki. A sensory profile test was conducted under standardized conditions: Samples were presented on white disposable plates under natural daylight. Between samples, panelists were instructed to cleanse their palates with tap water at room temperature and a piece of bread to minimize sensory carryover.

2.4.2. Phytochemical Screening. Phytochemical screening was conducted to identify the classes of secondary metabolites present in the ingredients, BGA, and BGB. Aqueous methanolic extracts (20:80 v/v) of the ingredients, BGA, and BGB were prepared by mixing 2 g of each product with 25 mL of solvent and stirring the mixture using a magnetic stirrer for 3 h. The solution was then filtered through Whatman paper No. 4, and the filtrate was used for analysis. The following tests were performed [48].

2.4.2.1. Alkaloids (Mayer's Test). A solution of 60 mL of mercuric (II) chloride (2.27% w/v) and 10 mL of potassium iodide (50% w/v) was prepared and diluted to 100 mL. The formation of a white-to-yellowish precipitate indicated the presence of alkaloids.

2.4.2.2. Saponins (Foam's Test). A 0.5-g sample was mixed with 10 mL of distilled water and agitated. Persistent foam formation upon heating in a water bath for 5 min indicated the presence of saponins.

2.4.2.3. Tannins (Ferric Chloride Test). A 6 mL sample solution was prepared in hot distilled water (0.17% w/v) and filtered. The filtrate was divided into three portions. Each portion was mixed with separate solutions: 0.9% sodium chloride (NaCl), a mixture of 0.9% NaCl and 1% gelatin, and ferric chloride. A precipitate in the second portion and a blue, blue-black, or greenish color indicated the presence of tannins.

2.4.2.4. Glycosides (Bontrager's Test). A 1 mL sample solution was mixed with 1 mL of 5% sulfuric acid and heated in a water bath. The solution was filtered, and the filtrate was mixed with 2 mL of chloroform and allowed to stand for 5 min. The lower chloroform layer was mixed with dilute ammonia (50% of its volume). A rose pink to red color in the ammoniacal layer indicated the presence of anthraquinone glycosides.

2.4.2.5. Flavonoids (Ammonia Test). A 5 mL diluted ammonia solution and concentrated sulfuric acid were added to 1 mL of the sample solution. The appearance of a yellow color indicated the presence of flavonoids.

2.4.2.6. Steroids (Salkowski's Test). A few drops of concentrated sulfuric acid were added to 5 mL of the sample solution. The mixture was shaken and allowed to stand. A red color in the lower layer indicated the presence of steroids.

2.4.2.7. Triterpenoids (Salkowski's Test). A few drops of concentrated sulfuric acid were added to 5 mL of the sample solution. The mixture was shaken and allowed to stand. A golden yellow layer at the base of the tube indicated the presence of triterpenoids.

2.4.2.8. Phenols (Lead acetate Test). A 5 mL sample solution was mixed with 3 mL of 10% lead acetate solution. The formation of a white precipitate indicated the presence of phenols.

2.4.2.9. Anthocyanins (Borntrager's Test). A 2 mL sample solution was mixed with 2 mL of 2N HCl and 2 mL of ammonia solution. The presence of anthocyanins was confirmed by the appearance of a pink solution that turned blue-violet upon the addition of ammonia.

2.4.2.10. Reducing Sugar (Fehling's Test). A 1 mL sample of Fehling's solution A and B was added to 1 mL of the sample solution, and the mixture was boiled in a water bath. The formation of a red precipitate indicated the presence of reducing sugars.

2.4.3. Physicochemical Characterization of BGA and BGB. The physicochemical assessment of BGA and BGB included determining features such as percentage loss on drying, solubility, total ash, pH, and extractives [49, 50].

2.4.3.1. Percentage of Loss on Drying. A precise weight of 3 g of BGA or BGB (M1) was accurately measured in an evaporating dish. The sample was dried at 105°C for 5 h and then reweighed (M2). The percentage loss on drying was calculated using the following formula:

$$\text{Percentage loss on drying} = \frac{(\text{M1} - \text{M2})}{M1} \times 100. \quad (2)$$

2.4.3.2. Total Ash Determination. BGA or BGB was placed in a silica dish and incinerated in a furnace at 550°C until the sample turned white. The total ash content was calculated using the following formula:

$$\text{Total ash} = \left(\frac{\text{Ash weight}}{\text{Initial weight of nutraceutical}}\right) \times 100. \quad (3)$$

2.4.3.3. pH Determination. Solutions of BGA or BGB (1% and 10%) in 2% ethanol were prepared and allowed to stand for 30 min before pH evaluation.

2.4.3.4. Solubility Test. In a dry test tube, 1 g of BGA or BGB was weighed, and 5 mL of the solvent (ethanol, water, or ethyl acetate) was added. The mixture was shaken thoroughly for about 30 min using a magnetic stirrer, and the solutions were observed for solubility in different solvents.

2.4.3.5. Determination of Alcohol and Water-Soluble Extractive. Approximately 3 g of BGA or BGB was mixed with 50 mL of 95% ethanol (for alcohol-soluble extractive) in a closed flask and stirred for 5 h using a magnetic stirrer. The extract was filtered without losing any solvent and dried at 105°C to a constant weight. This procedure was repeated using water to assess water-soluble extractive. The extractive content was calculated using the following formula:

$$\text{Alcohol | water soluble extractive} = \frac{\text{Dry extract weight}}{\text{Sample weight}} \times 100. \quad (4)$$

2.5. Macronutrient, Crude Fiber, and Vitamin C Contents of BGA and BGB

2.5.1. Macronutrient and Crude Fiber Contents of BGA and BGB. The macronutrient content (protein, lipid, and carbohydrate) and crude fiber content were determined following AOAC standard methods [51], with values expressed as percentages (g/100 g).

2.5.1.1. Lipid Content. Lipid content was measured using the Soxhlet method. The sample was wrapped in preweighed filter paper and extracted with hexane at 70°C for 8 h. The residue was dried at 105°C for 3 h, cooled, and weighed. Lipid percentage was calculated from weight differences. Analyses were done in triplicate.

2.5.1.2. Protein Content. Crude protein was determined by the Kjeldahl method. The sample was digested with sulfuric

acid and a $CuSO_4$–K_2SO_4 catalyst at 420°C. After digestion, the sample was distilled with NaOH and collected in boric acid containing Tashiro's indicator. Titration was done with N/100 H_2SO_4. Nitrogen was converted to protein using a factor of 6.25. Tests were done in triplicate.

2.5.1.3. Carbohydrate Content. Total sugars were estimated using the phenol–sulfuric acid method. The sample was boiled in water, filtered, and treated with zinc sulfate and potassium ferricyanide. After dilution, the extract was reacted with phenol and H_2SO_4. Absorbance was read at 490 nm using a glucose standard curve. Each sample was analyzed three times.

2.5.1.4. Crude Fiber Content. Crude fiber was assessed on a defatted sample (P1) using acid and alkaline digestion (H_2SO_4 and KOH), followed by acetone rinsing and drying at 105°C. The residue was weighed (P2), then incinerated at 550°C and reweighed (P3). Three trials were conducted for each sample:

$$\text{Crude fiber content} = \frac{\text{P2} - \text{P3}}{P1} \times 100. \quad (5)$$

2.5.1.5. Energy Content. The energy content was computed through calculation, knowing that 1 g of carbohydrate and 1 g of protein each provide 4 kcal, and 1 g of fat provides 9 kcal. The formula applied was as follows:

$$\begin{aligned}\text{Energy (kcal)} = {} & (\text{Total carbohydrate (g)} \times 4\,\text{kcal}) \\ & + \text{Total protein (g)} \times 4\,\text{kcal} \\ & + (\text{Total lipid (g)} \times 9\,\text{kcal}).\end{aligned} \quad (6)$$

2.5.2. Vitamin C Content of BGA and BGB. Vitamin C was determined by titration with DCPIP [52]. The sample was extracted in 90% acetic acid, filtered, and diluted. Five mL of the extract was titrated with DCPIP until persistent pink appeared. A blank and standard were also titrated. Three trials were conducted for each sample.

The vitamin C content was calculated using the following formula:

$$\text{Vitamin C} = \left(\frac{\text{Sample} - \text{Blanck}}{\text{Standard} - \text{Blank}}\right) \times \text{dilution factor}. \quad (7)$$

2.6. Heavy Metals and Other Mineral Characterizations of BGA and BGB. Heavy metal and mineral content determinations of BGA or BGB were conducted using energy-dispersive X-ray fluorescence (EDXRF) [53]. BGA or BGB was introduced into the apparatus (PANalytical Epsilon 3XLE). Samples underwent excitation for 300 s, and a multichannel spectrometer employing a solid Si-pin-diode X-ray detector with a 140-μm Be window was used to detect the characteristic X-rays. Quantitative analysis was conducted using the Omnian standardless software.

2.7. Phytochemical Quantification and Determination of Antioxidant and Antiradical Activities of BGA and BGB

2.7.1. Test With Ethanolic Extracts

2.7.1.1. Extract Preparation. A sample of BGA or BGB was extracted using 70% ethanol (1:10 w/v). The mixture was agitated using a magnetic stirrer for 2 h, after which it was filtered through Whatman paper No. 4. The filtrate was used for further analysis.

2.7.1.2. Total Phenol Content. The total phenol content was determined using the Folin–Ciocalteu reagent method [54]. The sample extract was mixed with 10% 2N Folin reagent and incubated for 5 min at room temperature. Then, 10% sodium carbonate was added, and the mixture was incubated for another 15 min. The absorbance was measured at 765 nm. A gallic acid standard curve (0.2 mg/mL) was used, and results were expressed as grams of gallic acid equivalent per 100 g of dry weight (g GAE/100 g).

2.7.1.3. Alkaloid Content. Extract solution (1 mg/mL) was mixed with $FeCl_3$ (2.5 mM in 0.5 M HCl) and 0 1,10-phenanthroline. After incubation for 30 min at 70°C, absorbance was measured at 500 nm. Alkaloid content was determined using a calibration curve and expressed as mg equivalent berberine/g dry extract [55].

2.7.1.4. Flavonoid Content. The extract was combined with distilled water and $NaNO_2$ (5%) (v/v/v). After 5 min of incubation, $AlCl_3$ (10%) was added and incubated for an additional 5 min. The reaction mixture was treated with 1 mM NaOH, and the volume of each tube was adjusted with distilled water before measuring absorbance at 510 nm. Flavonoid content was determined using a quercetin calibration curve and expressed as mg quercetin equivalents per g dry extract (mg quercetin eq/g dry extract) [56].

2.7.1.5. Flavonols. The extract (0.1 mg/mL) was combined with 2% $AlCl_3$ and 3 mL of sodium acetate solution (50 g/L), and the mixture was incubated for 2 h and 30 min at 20°C. Absorbance was read at 430 nm. Flavonol concentration was determined using a quercetin calibration curve and expressed as mg quercetin equivalents per g of dry extract (mg quercetin eq/g dry extract) [56].

2.7.1.6. DPPH Assay. The inhibitory activity of BGA or BGB was determined using the DPPH procedure [56]. A volume of 50 μL of the sample was introduced into a test tube, to which DPPH solution (0.05 mM) was added. The solution was incubated in darkness for 30 min, after which the absorbance was read at 517 nm. The percentage of DPPH radical remaining in the reactive milieu (% DPPH) was determined after incubation.

2.7.1.7. FRAP Assay. The FRAP assay measures the reduction of Fe^{3+} to Fe^{2+}, forming a colored complex with TPTZ. To the extract, freshly prepared FRAP reagent was added. After 5 min of incubation, absorbance was

measured at 593 nm. Results were expressed as mg iron sulfate equivalent antioxidant capacity per 100 g of sample [57].

2.7.1.8. Hydroxyl Radical Scavenging Activity Assay. Hydroxyl radical scavenging activity was assessed through the Fenton reaction [58]. To each extract, 60 µL of $FeCl_3$ (1 mM), 90 µL of phenanthroline (1 mM), 2.4 mL of phosphate buffer (0.2 M, pH 7.8), and 150 µL of 0.17 M H_2O_2 were successively added. The mixture was homogenized and incubated at room temperature for 5 min, after which the absorbance was measured at 560 nm against a blank. The negative control contained all reagents except the extract, while gallic acid was used as the standard.

2.7.1.9. Total Antioxidant Capacity (TAC). TAC was evaluated using the phosphomolybdenum method [59]. The extract sample diluted fourfold was mixed with phosphomolybdate reagent. The tubes were incubated at 95°C for 90 min and cooled, and absorbance was measured at 695 nm. TAC was expressed as mg ascorbic acid equivalents per gram of extract (mg AAE/g), with all tests performed in triplicate.

2.7.2. Test With Other Extracts

2.7.2.1. Carotenoids. Carotenoid concentration was determined using a standard method [60]. The sample was triturated with petroleum ether. The solution was transferred to a 20-mL flask and made up to the mark with petroleum ether. Absorbance was measured at 450 nm using a spectrophotometer, with a control vial containing only petroleum ether. Carotene content (expressed as β-carotene) in mg/100 g of the product (Qc) was calculated using the following formula:

$$\mathrm{Qc} = \frac{\varepsilon}{2650} \times 105, \tag{8}$$

where ε = specific extinction coefficient of the concentration solution in g/100 mL at its maximum absorption. $\varepsilon = ((\mathrm{OD})/\mathrm{C}) \times l$, where OD is the optical density read, l is the thickness of the cell, and C is the concentration in g of product/100 mL of petroleum ether.

2.7.2.2. Phytate. Phytate content in BGA or BGB was measured following the method by Unuofin et al. [61]. The sample was mixed with 2% HCl and shaken for 3 h using a magnetic stirrer. The mixture was filtered, and 25 mL of the filtrate was transferred to a 250-mL flask. A total of 5 mL of 0.3% ammonium thiocyanate solution (as an indicator) and 53.5 mL of distilled water were added to adjust the acidity. The solution was titrated with iron III chloride (0.00195 g of iron per mL) until a persistent brownish-yellow color appeared. Phytate content was determined using the following equation:

$$\text{Phytate content} = \text{Titrate} \times 0.00195 \times 1.19 \times 100. \tag{9}$$

2.7.2.3. Tannin. The sample was extracted 95% ethanol, stirred, and centrifuged. To the extract, ferric reagent and butanol–HCl were added and heated at 100°C for 1 h. After cooling, absorbance was measured at 550 nm, and tannin content was calculated as leucocyanidin equivalents. Condensed tannins (% dry matter) were calculated as leucocyanidin equivalents using the following formula:

$$\text{Tannin content} = \frac{\mathrm{Ab} \times 78.26 \times \text{Dilution factor}}{\text{Dry sample weight}}. \tag{10}$$

2.7.2.4. Oxalate. The sample was placed in an Erlenmeyer flask with 3M H_2SO_4 and stirred for 1 h. The mixture was filtered, and the filtrate was heated in a water bath at 80°C–90°C for 1 h and then maintained at 70°C. The hot sample was titrated with 0.05 M $KMnO_4$ until a pale pink color persisted. Oxalate content was calculated as follows:

$$\text{Oxalate content} = \frac{\left(\mathrm{KMnO_4}\,(0.05\ \mathrm{M}) \times 2.2\right)}{\text{Dry sample weight}} \times 100. \tag{11}$$

2.8. LCMS Analysis. Analyses were performed using a liquid chromatography system (Ultimate 3000, Thermo Scientific) equipped with a standard autosampler. The UHPLC column used was an Accucore C18 reversed phase (50 × 2.1 mm, 2.6 µm, 150 Å). Separation was carried out at 35°C with a gradient elution program at a flow rate of 0.4 mL/min. The mobile phases consisted of water (+0.1% formic acid) (A) and acetonitrile (+0.1% formic acid) (B). The injection volume in the UHPLC system was 5 µL. The following multistep linear gradient was applied: 5% B isocratic for 5 min, 5% B to 60% B for 7 min, 60% B isocratic for 5 min, 60% B to 95% B for 4 min, and 95% B isocratic for 2 min. The initial conditions were maintained for 1 min. Detection was performed using a diode array detector (DAD) (λ 190–600 nm). The UHPLC system was coupled to a Q-TOF mass spectrometer (Bruker Daltonics) equipped with an ESI interface operating in positive ion mode with a capillary voltage of +4.5 kV. The optimal values for the ESI–QTOF–MS parameters were as follows: drying gas temperature, 200°C; drying gas flow, 10 L/min; and nebulizing gas pressure, 2 bar. Detection was carried out within a mass range of 50–1300 m/z. MS/MS analyses were performed by direct injection, where the selected mass peaks were fragmented. The collision energy for MS/MS experiments was 40 eV. Nitrogen was used as the drying, nebulizing, and collision gas. The accurate mass data of the molecular ions were processed using Data Analysis 4.3 software (Bruker Daltonics), which provided a list of possible elemental formulas via the Generate Molecular Formula Editor.

2.9. Statistical Analysis. The findings are presented as the mean ± standard error of the mean. The statistical significance of variations at $p < 0.05$ between the experimental compounds was analyzed using the Student's t-test, and a Pearson correlation matrix was computed using R Studio version 4.2.

3. Results and Discussion

3.1. Extraction Yield, Sensory Properties, and Phytochemical Screening of Entrants, BGA, and BGB. The extraction yields of the entrants varied from 1.52% (*A. lepidophyllus*) to 15.16% (*C. longa*), as depicted in Table 2. This wide range underscores the differential solubility and abundance of phytoconstituents inherent in each botanical source. Extraction yield is a critical parameter as it directly correlates with the quantity of bioactive compounds retrievable from the plant matrix, impacting both the efficacy and reproducibility of downstream applications. Higher yields, as observed in *C. longa*, suggest a rich reservoir of phytochemicals, including alkaloids, glycosides, phenolics, saponins, and tannins compounds well-documented for their antioxidant, anti-inflammatory, antiglycation, and antimicrobial properties [62]. These functional attributes are foundational for the therapeutic potential of BG formulations.

To authenticate the collected samples, a pharmacognostical assessment was performed. The organoleptic characterization of the entrants and polyherbal nutraceuticals revealed that the color varied from creamy white to brown, passing through green, dark orange, orange green, brown, and dark brown (Figure 1 and Table 3), with predominantly characteristic odors. These sensory attributes serve not only as preliminary quality control markers but also as indirect indicators of phytochemical composition. For instance, the pungent, sweet–bitter taste profiles of BGA and BGB (Table 3) suggest the presence of alkaloids and flavonoids, which often impart such sensory notes [63].

Most of the products had a paste or coarse paste texture. BGA and BGB were characterized by a pungent, sweet–bitter taste (Table 3). The recognition and sensory evaluation of the distinct plants used in the preparation of BGA and BGB unveiled unique attributes concerning macrostructure, color, odor, texture, and taste. As a result, the authenticity of the collected raw material was verified, providing valuable information for accurately identifying the specific species employed in the formulations. This sensory verification is crucial for quality assurance, particularly for complex polyherbal products where batch-to-batch variability is a concern.

Table 4 depicts the classes of phytochemicals detected in the entrants, BGA, and BGB. Qualitative chemical tests indicated the presence of glycosides, tannins, triterpenes, saponins, flavonoids, alkaloids, phenols, steroids, and carbohydrates in both entrants and the polyherbal formulations. All the products contained phenols, and most did not contain saponins. BGA and BGB had almost identical phytochemical profiles (sugars, flavonoids, phenols, alkaloids, glycosides, and anthocyanins) except for triterpenes, which were detected in BGA but not in BGB. These compounds scavenge free radicals, reducing oxidative stress and its associated disorders (Figure 1), such as inflammation, cardiovascular diseases, and neurodegenerative conditions.

These phytochemicals collectively contribute to the antioxidant potential by scavenging reactive oxygen species (ROS) and mitigating oxidative stress—a pathological driver implicated in chronic inflammatory conditions, cardiovascular diseases, and neurodegeneration. The dietary relevance of such compounds is further emphasized by their capacity to modulate endogenous enzyme systems like paraoxonases, which regulate oxidative and inflammatory pathways [64]. Moreover, flavonoids, alkaloids, and saponins exert cytoprotective effects by influencing cell proliferation, differentiation, and apoptosis, thus offering promising avenues for the prevention and management of multifactorial diseases such as cancer and diabetes [65].

3.2. Physicochemical Properties of BGA and BGB. Preliminary physicochemical assessment, encompassing parameters like pH, moisture content, total ash, and alcohol/water-soluble extractives, provided insights into the characteristics of the polyherbal nutraceuticals (Table 5 reports them). The pH was found to be significantly higher ($p < 0.05$) in BGB at a 1% concentration. The pH ranged from 6.24 ± 0.01 to 5.64 ± 0.00 for BGA and from 6.17 ± 0.01 to 5.61 ± 0.00 for BGB in 1% and 10% solutions, respectively. The pH value of a formulation is a significant parameter, considering that drugs classified as weak acids are more efficiently absorbed from the stomach than from the upper intestine [66]. An exploration of the correlation between pH and microbial contamination suggested that a neutral or alkaline pH might foster higher levels of microbial contamination in herbal preparations. For both formulations, the pH was below 7, indicating their acidic nature.

The loss of weight on drying was significantly higher ($p < 0.05$) in BGA ($11.74 \pm 0.04\%$ w/w) than in BGB ($11.50 \pm 0.01\%$ w/w), while the total ash content was significantly greater ($p < 0.05$) in BGB. The moisture level in plant pharmaceuticals can fluctuate between 8% and 14%, and maintaining an appropriate moisture level is crucial to prevent hydrolysis of constituents, bacterial and fungal growth, and biochemical reactions. Pharmacopoeial monographs typically establish limits for moisture levels, particularly in medications with hygroscopic properties or where excessive moisture could cause product deterioration. Moisture content is intricately linked to product shelf-life; elevated moisture can catalyze hydrolytic degradation and foster microbial contamination, and thus, rigorous control of this parameter is essential for ensuring long-term stability [67]. Determining moisture content is crucial for calculating the levels of other food components, such as protein, carbohydrates, minerals, and phytonutrients, on a consistent basis (dry weight basis). The ash value is also a crucial parameter in the quality assessment of plant pharmaceuticals, with a high ash value indicating potential contamination, substitution, or adulteration during drug preparation or marketing [68].

Alcohol and water-soluble extractives were found to be significantly higher ($p < 0.05$) in BGA (35.58 ± 0.16 and 29.64 ± 0.13, respectively) than in BGB (35.58 ± 0.16 and 27.46 ± 0.11, respectively), and in both formulations, alcohol extractives were greater than water extractives. Both formulations were found to be soluble in ethanol and slightly soluble in ethyl acetate. Extractive values in various solvents provide insights into the solubility of different chemical

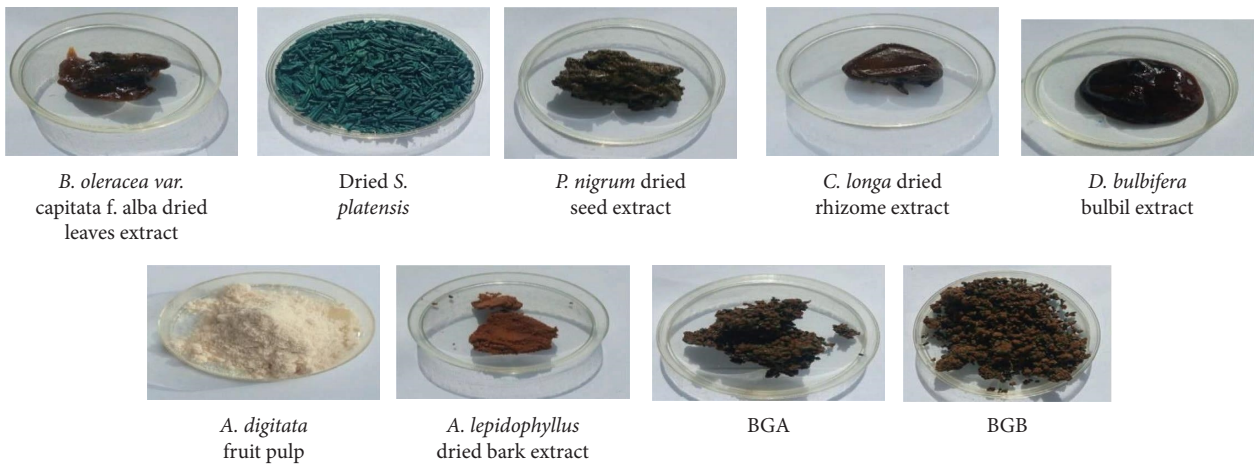


Figure 1: Presentation of the different entrants, BobyGuard A, and BobyGuard.

Table 3: Organoleptic properties of entrants, BobyGuard A, and BobyGuard B.

Herb	Odor	Color	Texture	Taste	Flow property
A. digitata	Characteristic	Creamy white	Coarse powder	Sweet sour	Not free flowing
S. platensis	Characteristic	Green	Coarse powder	Salty characteristic	Not free flowing
C. longa	Aromatic	Dark orange	Sticky paste	Pungent	Not free flowing
B. oleracea var. capitata f. alba	Characteristic	Brown	Paste	Sweet	Not free flowing
P. nigrum	Aromatic	Dark green	Sticky paste	Pungent	Not free flowing
D. bulbifera	Characteristic	Dark brown	Paste	Bitter	Not free flowing
A. lepidophyllus	Characteristic	Dark brown	Paste	Bitter	Not free flowing
BGA	Aromatic	Brown	Coarse paste	Pungent, sweet, bitter	Not free flowing
BGB	Aromatic	Orange green	Coarse paste	Pungent, sweet, bitter	Not free flowing

Table 4: Qualitative phytochemical screening of entrants, BobyGuard A, and BobyGuard B.

Herb/ extract	*D. bulbifera* (extract)	*C. longa* (extract)	*P. nigrum* (extract)	*S. platensis*	*A. digitata* fruit pulp	*A. lepidophyllus* (extract)	*B. oleracea* var. capitata f. alba (extract)	BGA	BGB
Phenol	+	+	+	+	+	+	+	+	+
Flavonoid	+	+		+	+	+	+	+	+
Alkaloid	+	+	+	−	+	+	+	+	+
Tannins	+	−	+	−	+	+	+	+	+
Steroids	−	+	−	−	+	−	+	+	+
Saponin	−	+	+	−	−	−	−	−	−
Glycoside	+	−	+	−	+	−	+	+	+
Triterpene	+	+	+	−	+	+	+	+	−
Anthocyanin	−	+	+	−	+	+	+	+	+
Sugars	+	+	−	+	+	+	+	+	+

Note: +: detected; −: not detected.

constituents, serving as an important test to assess drug quality. The difference in extractive efficiency between alcohol and water can be attributed to the solubility of different compounds in each solvent. Alcohol, particularly ethanol, is a more effective solvent for extracting a wider range of phytochemicals compared to water due to its polarity and ability to dissolve both polar and nonpolar compounds [69]. Variations in chemical constituents result in changes in extractive values, thereby affecting the drug's activity [70]. The observed solubility patterns with both formulations soluble in ethanol and slightly soluble in ethyl acetate offer practical implications for formulation development, particularly in the choice of excipients and delivery vehicles to maximize bioactive release. The variation in physicochemical properties between BGA and BGB reflects not only compositional differences but also potential divergence in pharmacokinetic behavior and clinical efficacy.

3.3. Nutrient Content of BGA and BGB. Table 6 presents the macronutrient and vitamin C contents of BGA and BGB. Both formulations contained protein, carbohydrates, crude fiber, lipids, and vitamin C, with all these nutrients, except for vitamin C, being significantly higher ($p < 0.05$) in BGB.

Table 5: Physicochemical characteristics of BobyGuard A and BobyGuard B.

Formulation	Loss on drying (%)	Ash (g/100 g)	Water-soluble extractive (%)	Alcohol-soluble extractive (%)	pH (1%)	pH (10%)	Solubility in water	Solubility in ethanol	Solubility in ethyl acetate
BGA	11.74 ± 0.04^{a}	6.44 ± 0.28^{a}	29.64 ± 0.13^{a}	35.58 ± 0.16^{a}	6.24 ± 01^{a}	5.64 ± 00^{a}	Slightly soluble	Soluble	Slightly soluble
BGB	11.50 ± 0.01^{b}	6.92 ± 0.01^{b}	27.46 ± 0.11^{b}	32.12 ± 0.42^{b}	6.17 ± 0.01^{b}	5.61 ± 0.00^{a}	Slightly soluble	Soluble	Soluble

Note: Values are expressed as mean ± SEM; for each row, values having different superscript letters are significantly different ($p < 0.05$).

The macronutrients generated energy values of 372.84 kcal/100 g and 402.54 ± 1.94 kcal/100 g for BGA and BGB, respectively. The vitamin C content was found to be 6.03 mg/100 g for BGA and 5.40 ± 0.63 mg/100 g for BGB. Improving the quality of life for patients means addressing these needs through a nutritional approach. Opting for a diverse intake of multiple nutrients, targeting the replenishment of energy, protein, and other essential components, proves more beneficial than relying on a singular nutrient intake [71]. Moreover, Guo et al. [72] suggested that incorporating vegetable oils rich in bioactive compounds, such as phenolic compounds, phytosterols, and tocopherols, into the daily diet can offer substantial physiological benefits, such as a decrease in inflammatory response [73]. Higher dietary intake of vitamin C, carotenoids, and α-tocopherol has been associated with a reduced risk of cardiovascular disease, total cancer, and age-period-cohort mortality [74]. Markovic and Natoli [75] also highlight that patients with chronic diseases require an increased intake of protein. Regular consumption of plant-based proteins has been shown to help combat these chronic conditions [76]. This is synergistically supported by the presence of plant-derived bioactive compounds such as tannins and flavonoids, which may potentiate the health benefits of the protein matrix through antioxidative and anti-inflammatory mechanisms [77]. Fibers are regarded as bioactive compounds due to their capacity to inhibit the digestion of triglycerides and sugars within the intestinal lumen. When consumed in adequate amounts, fibers can also enhance the serum lipid profile [78] and boost immune functions [79]. The vitamin C concentrations, although marginally higher in BGA (6.03 mg/100 g), were statistically comparable ($p < 0.05$), reinforcing both products' roles in supporting antioxidant defenses, collagen synthesis, and iron absorption [80]. Given vitamin C's pivotal role in modulating oxidative stress and inflammation, its presence complements the phytochemical antioxidants to potentially enhance the formulations' preventive efficacy against oxidative damage and chronic disease progression.

3.4. Mineral Content of BGA and BGB. The macrominerals such as magnesium, phosphorus, sulfur, chlorine, and potassium and microminerals such as iron, zinc, manganese, and copper were detected in BGA and BGB, while none of the heavy metals like mercury, cadmium, arsenic, and lead were identified in either formulation (Table 7). Copper, phosphorus, and sulfur were significantly greater ($p < 0.05$) in BGA (6293.33 ± 115.66 μg/100 g, 249.67 ± 2.03 μg/100 g, and 238.33 ± 2.91 μg/100 g, respectively) than in BGB (150.00 ± 0.00 μg/100 g, 198.00 ± 2.00 μg/100 g, and 149.33 ± 1.67 μg/100 g, respectively).

The mineral profiles of BGA and BGB demonstrate the presence of several macro- and microminerals essential for physiological homeostasis and disease modulation. Notably, both formulations were free of toxic heavy metals such as lead, mercury, cadmium, and arsenic, suggesting a favorable safety profile compliant with WHO guidelines for mineral and contaminant thresholds in dietary substances [81]. Significant quantitative differences were observed between the formulations. BGA exhibited notably higher levels of copper, phosphorus, and sulfur compared to BGB, with copper content in BGA (6293.33 ± 115.66 μg/100 g) exceeding that of BGB (150.00 ± 0.00 μg/100 g) by more than 40-fold. This elevated copper level may contribute substantially to the antioxidant and enzymatic potential of BGA, considering copper's central role as a cofactor in critical antioxidant enzymes such as Cu/Zn superoxide dismutase (SOD) and ceruloplasmin [82]. These enzymes are known to regulate ROS and protect against oxidative stress, a hallmark of chronic inflammation and cancer pathogenesis. Similarly, the higher phosphorus content in BGA supports key metabolic functions such as ATP synthesis, cellular signaling via phosphorylation cascades, and nucleic acid stability. Given the energy-demanding nature of immune modulation and tumor suppression, this elevation in phosphorus may enhance the bioenergetic efficiency of immune or epithelial cells exposed to BGA. The increased sulfur content in BGA (238.33 ± 2.91 μg/100 g vs. 149.33 ± 1.67 μg/100 g in BGB) is also of interest. Inorganic sulfur serves as a precursor for various sulfur-containing amino acids and antioxidants like glutathione and directly participates in detoxification and DNA protection through its ability to neutralize electrophilic free radicals [83]. This makes it potentially beneficial in nutraceutical formulations aimed at chemoprevention or mitigation of oxidative cellular damage. Among other essential minerals, magnesium, although not statistically different between the formulations—was found in high concentrations in both BGA (61,606.67 ± 3837.37 μg/100 g) and BGB (50,606.67 ± 3276.92 μg/100 g). Magnesium's well-established involvement as a cofactor in over 300 enzymatic systems, including those regulating glucose metabolism, DNA/RNA synthesis, and neural signaling, positions it as a cornerstone mineral for systemic resilience against metabolic and neurodegenerative disorders [72]. Zinc and manganese, also critical enzymatic cofactors, were slightly elevated in BGA. Zinc is vital for DNA repair, immune modulation, and enzymatic defense mechanisms including those mediated by metallothioneins and antioxidant proteins such as SOD. Elevated zinc levels have been positively correlated with reduced tumor progression in certain cancers, including mammary carcinoma, partly due to their role in apoptosis regulation and cell cycle control through zinc-finger transcription factors [84]. Interestingly, potassium and chlorine levels were slightly but significantly higher in BGB, suggesting subtle variations in electrolyte balance between the formulations. Potassium is indispensable for membrane potential regulation and mitochondrial bioenergetics. Elevated potassium in BGB may modulate mitochondrial redox potential and contribute to neuroprotective effects. Iron concentrations were comparable in both BGA and BGB. As a central component of hemoproteins (e.g., cytochromes, catalase, and peroxidase), iron is essential for oxygen transport, mitochondrial respiration, and host defense. Maintenance of appropriate iron levels without excess is crucial, as dysregulated iron homeostasis can trigger oxidative damage [85].

Table 6: Macronutrient, crude fiber, and vitamin C contents of BobyGuard A and BobyGuard B.

Formulation	Proximate composition (g/100 g DM)					
	Protein	Carbohydrate	Lipid	Crude fiber	Energy (kcal/100 g DM)	Vitamin C (mg/100 g DM)
BGA	29.07 ± 0.08^{a}	44.72 ± 0.00^{a}	8.63 ± 0.21^{a}	7.07 ± 0.07^{a}	372.84 ± 2.21^{a}	6.03 ± 0.63^{a}
BGB	32.08 ± 0.00^{b}	46.17 ± 0.38^{a}	9.95 ± 0.05^{b}	8.28 ± 0.08^{b}	402.54 ± 1.94^{b}	5.40 ± 0.63^{b}

Note: Values are expressed as mean ± SEM; for each row, values having different superscript letters are significantly different ($p < 0.05$).

Table 7: Minerals and heavy metal contents of BobyGuard A and BobyGuard B.

Mineral (μg/100 g)	BGA	BGB
Magnesium	$61{,}606.67 \pm 3837.37^{a}$	$50{,}606.67 \pm 3276.92^{a}$
Manganese	$16{,}326.67 \pm 277.21^{a}$	$15{,}050.00 \pm 742.99^{b}$
Zinc	$16{,}163.33 \pm 204.80^{a}$	$14{,}973.33 \pm 648.49^{a}$
Copper	6293.33 ± 115.66^{a}	150.00 ± 0.00^{b}
Potassium	6458.00 ± 68.60^{a}	7363.00 ± 5.77^{b}
Chlorine	594.67 ± 5.81^{a}	646.67 ± 5.36^{b}
Iron	391.00 ± 2.65^{a}	382.33 ± 2.73^{a}
Phosphorus	249.67 ± 2.03^{a}	198.00 ± 2.00^{b}
Sulfur	238.33 ± 2.91^{a}	149.33 ± 1.67^{b}
Lead	ND	ND
Mercury	ND	ND
Cadmium	ND	ND
Arsenic	ND	ND

Note: Values are expressed as mean ± SEM; for each column, values having different superscript letters are significantly different ($p < 0.05$).
Abbreviation: ND = not detected.

3.5. Phytochemical Quantification of BGA and BGB. Phytochemical characterization of BGA and BGB reveals that BGB is richer in certain compounds than BGA (Table 8). Significantly higher ($p < 0.05$) contents of flavonoids (6093.01 ± 182.29 mg eq QuE/100 g DM), flavonols (56.04 ± 1.97 mg eq QuE/100 g DM), carotenoids (403.52 ± 5.35 mg/100 g DM), and phytate (1.20 ± 0.10 mg eq phytic acid/100 g DM) were found in BGB compared to BGA (718.15 ± 2.51 mg eq QuE/100 g DM, 47.07 ± 2.37 mg eq QuE/100 g DM, 384.91 ± 4.16 mg/100 g DM, and 0.89 ± 0.06 mg eq phytic acid/100 g DM, respectively). Secondary metabolites, particularly flavonoids and phenolic compounds, are recognized for their antioxidant and antiradical activities. Both types exert scavenging effects through the stabilization of free radicals via their hydroxyl groups and conjugated ring structures [86]. The high contents of phenols, alkaloids, carotenoids, and flavonoids in both formulations contribute to their substantial antioxidant properties. Carotenoids exhibit antioxidant activity through various mechanisms such as hydrogen atom transfer (HAT), single-electron transfer (SET), and chelation of transition metals. They play a crucial role in redox defense mechanisms, protecting against oxidative stress-related diseases like cancer, skin problems, and diabetes. Carotenoids, with their antioxidant capacity, are suggested to provide protective effects, potentially reducing cancer cell growth or inducing apoptosis [87]. Alkaloids can exhibit potent antioxidant properties through free radical quenching activity driven by proton affinity, SET reactions, and deprotonated species that show significant antioxidant effects by favoring electron abstraction at specific positions, leading to the stability of free radicals and resonance structures. They also directly scavenge free radicals and upregulate antioxidant pathways like Nrf2/HO-1 [88].

3.6. Identification of Bioactive Compounds in BGA and BGB Using LC–MS/MS. BGA and BGB were analyzed using reversed-phase ultra-high-performance liquid chromatography (UHPLC) coupled with electrospray ionization ion trap mass spectrometry (ESI-MS) detection in positive ion mode. This method enabled the identification of more than 50 compounds, as detailed in Table 9, along with their biological activities and corresponding chromatogram (Figure 2). BGA is characterized by the presence of ascorbalamic acid, catechin, germacrone-13-aL, quercetin 7-xyloside, and deacylmaomailiaoine I, while BGB is characterized by the presence of sesquisabinene, coumaperine, canthaxanthin, palmitone, and chlorophyll a. Both nutraceuticals contain more than 40 common compounds, including curcumin, piperine, piperdardine, turmeronol A, demethoxycurcumin, and piperyline. The most prevalent classes of metabolites are amide alkaloids (22 and 21 compounds for BGA and BGB, respectively), chlorophyll derivatives (6 and 3 compounds for BGA and BGB, respectively), carotenoids (5 and 4 compounds for BGA and BGB, respectively), curcuminoids (3 and 6 compounds for BGA and BGB, respectively), sesquiterpenes (4 and 5 compounds for BGA and BGB, respectively), nucleosides (1 and 2 compounds for BGA and BGB, respectively), retinoids (1 and 1 compounds for BGA and BGB, respectively), and vitamin C derivatives (0 and 1 compounds for BGA and BGB, respectively). Based on peak height, the 5 most abundant compounds in BGA are piperine, piperettine I, curcumin, piperic acid, and artumerone, while less abundant compounds include apo-12-violaxanthal, pipercide, bisdemethoxycurcumin, 2′-O-β-D-ribofuranoside, and dihydroferuperine. In BGB, the 5 most abundant compounds are piperine, piperettine I, dipiperamide A, piperic acid, and curcumin, with less

Table 8: Phytochemical content of BobyGuard A and BobyGuard B.

Formulation	Phenol (mg GAE/100 g DM)	Flavonoids (mg eq QuE/100 g DM)	Flavonols (mg eq QuE/100 g DM)	Alkaloids (mg eq berberine/100 g DM)	Tannin (mg eq leucocyanidin/100 g DM)	Oxalate (mg/100 g DM)	Carotenoids (mg/100 g DM)	Phytate (mg eq phytic acid/100 g DM)
BGA	6093.01 ± 182.29^{a}	718.15 ± 2.51^{a}	47.07 ± 2.37^{a}	142.70 ± 0.92^{a}	15.50 ± 1.67^{a}	10.71 ± 0.15^{a}	384.91 ± 4.16^{a}	0.89 ± 0.06^{a}
BGB	6580.36 ± 238.10^{a}	1107.27 ± 9.74^{b}	56.04 ± 1.97^{b}	148.64 ± 1.62^{a}	9.43 ± 0.07^{b}	9.97 ± 0.29^{a}	403.52 ± 5.35^{b}	1.20 ± 0.10^{ab}

Note: Values are expressed as mean ± SEM; for each row, values having different superscript letters are significantly different ($p < 0.05$).

abundant compounds being phytoene, catechin, pipercycliamide, 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, and dihydroferuperine. The processed data were subsequently used to establish correlations with their known biological properties (Table 9). A comparative breakdown of metabolite classes indicates that amide alkaloids are the most predominant group in both formulations. These alkaloids, especially piperine and its analogs, are well-documented for their broad-spectrum bioactivities, including enhancement of bioavailability (via CYP3A4 inhibition), antimicrobial action, and cytotoxicity against tumor cells [89]. This chemical fingerprinting supports the hypothesis that both BGA and BGB exhibit multitarget therapeutic potential, likely driven by synergistic interactions among their metabolites. The diversity of compound classes reflects a polypharmacological strategy characteristic of traditional herbal medicine, wherein multiple signaling pathways are modulated in parallel, an approach well-suited for managing complex chronic conditions such as cancer, inflammatory diseases, and metabolic syndromes.

3.7. Antioxidant and Antiradical Activities of BGA and BGB. The medicinal efficacy of plant-derived formulations is closely linked to their capacity to neutralize oxidative stress, a central mechanism in the pathogenesis of cancer, inflammation, and metabolic disorders. Antioxidant and antiradical assessments are thus pivotal to evaluating the therapeutic relevance of such polyherbal preparations. Given the complexity and heterogeneity of oxidative processes, reliance on a single assay often yields an incomplete or skewed representation of antioxidant potential. To address this, Schlesier et al. [90] advocated for a multiparametric approach incorporating assays with distinct reaction mechanisms. In alignment with this recommendation, the present study employed a panel of complementary in vitro methods—DPPH, FRAP, OH, and TAC—to robustly assess the antioxidant profiles of BGA and BGB (Table 10).

The DPPH assay assesses the ability of compounds to donate hydrogen atoms or electrons to neutralize free radicals. BGB demonstrated a significantly higher scavenging capacity ($91.09 \pm 0.45\%$) compared to BGA ($87.93 \pm 0.60\%$, $p < 0.05$), indicative of a greater abundance or efficacy of hydrogen-donating phytochemicals—likely flavonoids and polyphenols. This superior performance aligns with BGB's richer phenolic fingerprint revealed in earlier phytochemical analyses, reinforcing the hypothesis that antioxidant potential is tightly correlated with total phenolic content. The modest but statistically significant difference suggests that even small compositional variations can produce measurable shifts in radical neutralization dynamics, particularly when multiple synergistic compounds are involved [91, 92].

The FRAP assay measures the electron-donating capacity of antioxidants under acidic conditions, reflecting their ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. BGB again outperformed BGA, with a ferric-reducing capacity more than twice as high (4600.72 ± 31.7 vs. 2157.07 ± 65.19 mg $FeSO_4$ equivalents/100 g DM, $p < 0.05$). This result not only corroborates the DPPH findings but also implies the presence of redox-active molecules with strong electron transfer potential in BGB. The magnitude of difference is particularly noteworthy, suggesting a structurally superior network of conjugated systems or chelating moieties in BGB, capable of stabilizing transition metals and interrupting radical propagation cascades [93].

Hydroxyl radicals are among the most cytotoxic ROS, initiating lipid peroxidation, DNA strand breaks, and protein carbonylation. BGB exhibited a significantly enhanced OH scavenging activity ($86.03 \pm 0.71\%$) compared to BGA ($83.07 \pm 0.30\%$, $p < 0.05$). While the absolute difference appears small, the statistical significance points to the reproducibility and robustness of the effect. The superior hydroxyl radical quenching by BGB suggests a targeted ability to neutralize the most deleterious ROS, potentially via mechanisms involving metal chelation (thereby inhibiting Fenton chemistry) or direct radical interaction by specific polyphenolic substructures such as catechols or *ortho*-dihydroxy phenyl rings [94].

Contrary to the other assays, BGA demonstrated a higher TAC (17.01 ± 0.50 vs. 13.61 ± 0.35 mg ascorbic acid equivalents/100 g DM, $p < 0.05$). TAC integrates both chain-breaking and preventive antioxidant mechanisms under specific assay conditions, including contributions from vitamins, trace elements, and synergistic co-antioxidants not fully captured in radical-based assays. This discrepancy highlights the complexity of antioxidant systems: While BGB excels in specific radical scavenging and reducing functions, BGA may harbor broader antioxidant network components, including lipid-soluble antioxidants, microelements, or polysaccharide complexes that contribute to its global antioxidant potential [95]. BGB showed superior activity in DPPH, FRAP, and OH assays, indicating a high content of fast-acting, redox-active phytochemicals suited for acute oxidative stress. Conversely, BGA's higher TAC reflects a broader, possibly lipophilic or synergistic antioxidant profile more aligned with sustained cellular protection. Together, these complementary profiles support a polyvalent phytotherapy approach, enabling tailored use based on oxidative stress severity and clinical needs.

3.8. Correlation Matrix Between Antioxidant Activities, Nutrient, and Phytochemical Contents of BGA and BGB. A strong positive correlation was observed between some nutrients/phytonutrients and antioxidant and antiradical activities of both polyherbal nutraceuticals (Figures 3 and 4). Although most of the correlations for BGA were not significant, there was a strong positive correlation between TAC and manganese, phytate, and alkaloids; OH and potassium, manganese, sulfur, phytate, and alkaloids; DPPH and manganese, sulfur, alkaloids, and phytate; and FRAP and flavonoids, carotenoids, zinc, flavonols, and vitamin C. Notably, there was a significant strong positive correlation between DPPH and phytate ($R = 1.00$, $p < 0.05$) and

Table 9: Secondary metabolites annotated in positive in BobyGuard A and BobyGuard B.

Peak	RT	M + H (m/z)	M + NA (m/z)	MS/MS fragment ions (m/z)	Formula	Suggested compound	Metabolite class	Biological activity	Height	RDBE
BGA										
1	6.26	264.2068		98.9665 116.9750 134.9829 120.9953	$C_9H_{13}NO_8$	Ascorbalamic acid	Vitamin C derivative	Antioxidant, skin health	4705	5
2	2.30	288.1531		130.9782 139.0035 148.9871 166.9976	$C_{17}H_{21}NO_3$	Piperanine	Amide alkaloid	Antimicrobial, anticancer	5530	9
3	2.37	342.1628		118.9315 234.9276	$C_{21}H_{27}NO_3$	Pipernonaline	Amide alkaloid	Antioxidant, antimicrobial	4232	11
4	2.82	293.1446		116.9735 118.9319 174.9691	$C_{19}H_{16}O_3$	1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one	Curcuminoid	Anti-inflammatory, antioxidant	2034	12
5	3.04	291.1254		174.9687	$C_{15}H_{14}O_6$	Catechin	Flavonoid	Antioxidant, Cardioprotective, anticancer	2642	10
6	3.12	314.1314		106.9812 124.9894 139.0026 166.9960	$C_{19}H_{23}NO_3$	Piperdardine	Amide alkaloid	Antimicrobial, anticancer	8887	10
7	3.22	217.1520		152.9911 187.0095 205.0183 223.0266	$C_{15}H_{20}O$	Ar-tumerone	Sesquiterpene	Anticancer, anti-inflammatory	5622	8
8	3.45	383.1035		234.9285 98.9656	$C_{25}H_{34}O_3$	Apo-12-violaxanthal	Carotenoid	Antioxidant	3440	9
9	3.46	233.1465		116.9740 134.9841 119.0370 147.0287	$C_{15}H_{20}O_2$	Turmeronol A	Sesquiterpene	Antioxidant, anti-inflammatory	4921	6
10	3.48		331.0859	174.9679 128.9715 198.9652 146.0183	$C_{19}H_{16}O_4$	Bisdemethoxycurcumin	Curcuminoid	Anti-inflammatory, anticancer	8593	12
11	3.52		361.0956	147.0290 148.0315 149.0439 177.0378 178.0416 184.9506	$C_{20}H_{18}O_5$	Demethoxycurcumin	Curcuminoid	Anti-inflammatory, antioxidant, anticancer	7272	12

Table 9: Continued.

Peak	RT	M + H (m/z)	M + NA (m/z)	MS/MS fragment ions (m/z)	Formula	Suggested compound	Metabolite class	Biological activity	Height	RDBE
12	3.57	369.1240		136.0473 172.9957 191.0074 209.0122	$C_{21}H_{20}O_6$	Curcumin	Curcuminoid	Anti-inflammatory, antioxidant, anticancer	77,715	12
13	3.68	290.1677			$C_{17}H_{23}NO_3$	Dihydroferuperine	Amide alkaloid	Antimicrobial, antioxidant	1451	8
14	3.88	272.1211		135.0665 102.1182 115.0430	$C_{16}H_{17}NO_3$	Piperyline	Amide alkaloid	Antimicrobial, antioxidant	45,349	9
15	3.95		294.1023	143.0347 159.0288 171.0279 201.0343	$C_{16}H_{17}NO_3$	Piperyline	Amide alkaloid	Antimicrobial, antioxidant	10,735	9
16	3.97	216.1318		187.0096 205.0178 223.0276	$C_{14}H_{17}NO$	N-cinnamoylpiperidine	Amide alkaloid	Antimicrobial, antioxidant	3971	9
17	4.01		247.1435	139.0044 139.9879 187.0098 205.0187 1,209,969	$C_{15}H_{22}O_2$	Tumerone B	Sesquiterpene	Anti-inflammatory, anticancer	8807	5
18	4.11	311.1197		124.9904 148.9867 174.9676 177.1101	$C_{19}H_{18}O_4$	Bisdemethoxydihydrocurcumin	Curcuminoid	Anti-inflammatory, antioxidant	5224	11
19	4.15	309.1048		135.0303 174.9696 197.9834	$C_{19}H_{16}O_4$	Bisdemethoxycurcumin	Curcuminoid	Anti-inflammatory, anticancer	34,713	12
20	4.19	286.1368		120.9955 139.0037 148.9884 166.70	$C_{17}H_{19}NO_3$	Piperine	Amide alkaloid	Bioavailability enhancer, anticancer, antimicrobial	740,693	10
21	4.20	339.1147		147.0293 174.9687 201.031	$C_{20}H_{18}O_5$	Demethoxycurcumin	Curcuminoid	Anti-inflammatory, antioxidant, anticancer	59,029	12
22	4.26		593.2478	135.0303 201.0348 286.1165	$C_{33}H_{30}MgN_4O_4$	Pyrochlorophyllide b	Chlorophyll derivative	Antioxidant, anticancer	5696	22
23	4.37	302.1465		143.0344 201.0349	$C_{18}H_{23}NO_3$	Futoamide	Amide alkaloid	Antimicrobial, anticancer	6320	9

Table 9: Continued.

Peak	RT	M + H (m/z)	M + NA (m/z)	MS/MS fragment ions (m/z)	Formula	Suggested compound	Metabolite class	Biological activity	Height	RDBE
24	4.39		255.1277	115.0426 135.0667 139.0048	$C_{15}H_{20}O_2$	Germacrone-13-aL	Sesquiterpene	Anticancer, anti-inflammatory	14,864	8
25	4.54	312.1509		106.9821 124.9891 148.9847	$C_{19}H_{21}NO_3$	Piperettine I	Amide alkaloid	Antimicrobial, antioxidant	259,103	11
26	4.68	316.1818			$C_{19}H_{25}NO_3$	Piperolein A	Amide alkaloid	Antioxidant, anticancer	57,045	9
27	4.81	224.1794		98.9660 116.9735 127.9889	$C_{13}H_{21}NO_2$	Dihydrodioscorine	Alkaloid (pyridine type)	Antimicrobial, antispasmodic	53,480	5
28	4.87	330.1971		145.0867 141.0556 174.9682	$C_{20}H_{27}NO_3$	Pipercallosine	Amide alkaloid	Antimicrobial, antioxidant	21,678	11
29	4.95	340.1811		102.1196	$C_{21}H_{25}NO_3$	Dehydropipernonaline	Amide alkaloid	Antioxidant, antimicrobial	25,145	10
30	5.00	370.3351		172.99972 191.053	$C_{14}H_{19}N_5O_7$	2′-O-β-D-Ribofuranoside	Glycoside	Antioxidant, Cardioprotective	9646	8
31	5.04	571.2642		121.0888 215.1054	$C_{33}H_{30}MgN_4O_4$	Pyrochlorophyllide b	Chlorophyll derivative	Antioxidant, anticancer	9334	22
32	5.05		593.2450	201.0351 166.0704 225.0351 255.0427	$C_{34}H_{38}N_2O_6$	Dipiperamide A	Amide alkaloid	Anticancer, antimicrobial	159,251	17
33	5.12		364.1783	131.0358 135.0304 138.0773 157.0501	$C_{21}H_{27}NO_3$	Pipernonaline	Amide alkaloid	Antioxidant, antimicrobial	9769	11
34	5.23		239.1329	115.0430 117.0584 120.0771	$C_{15}H_{20}O$	Ar-tumerone	Sesquiterpene	Anticancer, anti-inflammatory	71,636	8
35	5.32		366.1925	136.0339 140.0932 157.0483	$C_{21}H_{29}NO_3$	Piperolein B	Amide alkaloid	Antioxidant, anticancer	27,300	10
36	5.35	356.2112		159.0654 165.0088	$C_{22}H_{29}NO_3$	Pipercide.	Amide alkaloid	Insecticidal, antimicrobial	12,336	12

Table 9: Continued.

Peak	RT	M + H (m/z)	M + NA (m/z)	MS/MS fragment ions (m/z)	Formula	Suggested compound	Metabolite class	Biological activity	Height	RDBE
37	5.84	219.1535		116.9750 127.9891	$C_{12}H_{10}O_4$	Piperic acid	Alkaloid (pyrrole type)	Antimicrobial, anticancer	92,054	9
39	5.92	256.2392		118.1122 105.0606	$C_{14}H_{25}NO_3$	Pipercycliamide	Amide alkaloid	Antioxidant, antimicrobial	2051	4
39	5.85		241.1335	119.0740 121.0894 122.0921	$C_{12}H_{10}O_4$	Piperic acid	Alkaloid (pyrrole type)	Antimicrobial, anticancer	30,035	9
40	6.88	545.3806		525.2457 102.1185	$C_{40}H_{64}$	Phytoene	Carotenoid	Antioxidant, skin protection	2663	9
41	7.27	334.2976		126.0788 138.0891 191.0067	$C_{22}H_{39}NO$	Pipyaqubine	Amide alkaloid	Antimicrobial, antioxidant	5041	3
42	7.31	384.3141		143.0566 244.1681 114.0802	$C_{24}H_{33}NO_3$	Guineensine	Amide alkaloid	Anticancer, antimicrobial	26,008	11
43	7.34		406.2953	121.0904 128.0945 135.1047 149.1184	$C_{24}H_{33}NO_3$	Guineensine	Amide alkaloid	Anticancer, antimicrobial	25,838	11
44	7.42	336.3132		124.0744 138.0874 115.1007	$C_{22}H_{41}NO$	Pipericine	Amide alkaloid	Antimicrobial, antioxidant	23,788	2
45	7.44	925.5491		173.1369 244.1683 255.2069 909.5795	$C_{55}H_{73}MgN_4O_7$	15-OH-Lactone-chlorophyll a	Chlorophyll derivative	Antioxidant, anticancer	23,614	22
46	7.65	529.3839		383.1103 507.2883 509.2195 513.3751	$C_{40}H_{48}$	Octadehydro-β,β-carotene	Carotenoid	Antioxidant	9951	17
47	7.79	435.3940		102.1190 202.1625 314.2745 348.2950	$C_{20}H_{18}O_{11}$	Quercetin 7-xyloside	Flavonoid glycoside	Antioxidant, anti-inflammatory	12,101	12
48	8.17	451.4387		149.0087 192.9969 350.3112	$C_{31}H_{62}O$	Palmitone	Fatty acid derivative	Antimicrobial, antifungal	13,193	1
49	8.70	885.4872		202.1627 865.5628 866.5608	$C_{55}H_{72}N_4O_6$	Pheophytin b	Chlorophyll derivative	Antioxidant, anticancer	5740	22

Table 9: Continued.

Peak	RT	M + H (m/z)	M + NA (m/z)	MS/MS fragment ions (m/z)	Formula	Suggested compound	Metabolite class	Biological activity	Height	RDBE
50	8.93	547.3719		177.0381 525.2454	$C_{40}H_{50}O$	Hexadehydro-β,β-caroten-3-ol	Carotenoid	Antioxidant	34,726	16
51	9.05	317.2158		124.0731 142.1448	$C_{20}H_{28}O_3$	7-Hydroxyretinoic acid	Retinoid	Skin health, antioxidant	33,566	7
52	9.06	343.2408		136.0729	$C_{20}H_{22}O_5$	Deacylmaomailiaoine I	Amide alkaloid	Antimicrobial, anticancer	3950	10
BGB										
1	2.04	205.0325		101.0032 119.0148 137.0269 147.0001	$C_{15}H_{24}$	Sesquisabinene	Sesquiterpene	Antimicrobial, anti-inflammatory	31,552	4
2	2.37	356.1815		104.9941 118.9317 165.0093 175.0292	$C_{22}H_{29}NO_3$	Pipercide	Amide (alkaloid)	Insecticidal, antimicrobial	2149	9
3	2.92	314.1351		163.0033 139.0052 124.9901 106.9827 182.9742	$C_{19}H_{23}NO_3$	Piperdardine	Amide (alkaloid)	Antimicrobial, anticancer	5381	9
4	3.12		336.1155	174.9692 156.9601 145.0147 121.0537	$C_{19}H_{23}NO_3$	Piperdardine	Amide (alkaloid)	Antimicrobial, anticancer	17,294	9
5	3.42	383.1061		234.9315 116.9743	$C_{25}H_{34}O_3$	Apo-12-violaxanthal	Carotenoid	Antioxidant, anti-inflammatory	2442	9
6	3.43	233.1495		98.9661 134.9827	$C_{15}H_{20}O_2$	Turmeronol A	Sesquiterpene	Antimicrobial, anti-inflammatory	5568	6
7	3.51		361.0980	117.0230 134.0239 145.0150 177.0382 198.9676	$C_{20}H_{18}O_5$	Demethoxycurcumin	Curcuminoid	Anti-inflammatory, antioxidant, anticancer	5152	12
8	3.52	339.1155		174.9697 156.9597 138.9497 118.9319	$C_{20}H_{18}O_5$	Demethoxycurcumin	Curcuminoid	Anti-inflammatory, antioxidant	47,659	12
9	3.55		391.1077	117.0231 134.0235 145.0147 177.0390	$C_{21}H_{20}O_6$	Curcumin	Curcuminoid	Anti-inflammatory, antioxidant, anticancer	8975	12

Table 9: Continued.

Peak	RT	M + H (m/z)	M + NA (m/z)	MS/MS fragment ions (m/z)	Formula	Suggested compound	Metabolite class	Biological activity	Height	RDBE
10	3.57	369.1265		136.0484 172.9978 191.0065 209.0129 234.9299	$C_{21}H_{20}O_6$	Curcumin	Curcuminoid	Anti-inflammatory, antioxidant, anticancer	81,332	12
11	3.65	258.1412		98.9676 116.9756 134.9837 152.9930 187.0123	$C_{16}H_{19}NO_2$	Coumaperine	Coumarin alkaloid	Antioxidant, anticancer	12,541	8
12	3.69	288.1527		120.9966 130.9804 139.0049 148.9875	$C_{17}H_{21}NO_3$	Piperanine	Amide (alkaloid)	Antimicrobial, insecticidal	5858	8
13	3.74	290.1677		116.9749 135.0682 163.0980 181.1056	$C_{17}H_{23}NO_3$	Dihydroferuperine	Amide (alkaloid)	Antimicrobial, antioxidant	1451	7
14	3.87	272.1221		135.0670 115.0442	$C_{16}H_{17}NO_3$	Piperyline	Amide (alkaloid)	Antimicrobial, anticancer	39,291	9
15	3.88		294.1039	135.0317 159.0300 171.0288 275.1372	$C_{16}H_{17}NO_3$	Piperyline	Amide (alkaloid)	Antimicrobial, anticancer	10,998	9
16	3.94	565.2187		201.9359 163.0964 149.0462 107.0400 98.9680	$C_{40}H_{52}O_2$	Canthaxanthin	Carotenoid	Antioxidant, anti-inflammatory	5678	15
17	3.97	216.1335		116.9754 134.9840 187.0112 205.0191 223.0288	$C_{14}H_{17}NO$	N-cinnamoylpiperidine	Amide (alkaloid)	Antimicrobial, anticancer	3246	7
18	4.09	309.1151		106.9834 120.9962 148.9889 156.9590 174.9694	$C_{19}H_{16}O_4$	Bisdemethoxycurcumin	Curcuminoid	Anti-inflammatory, antioxidant	1996	12

Table 9: Continued.

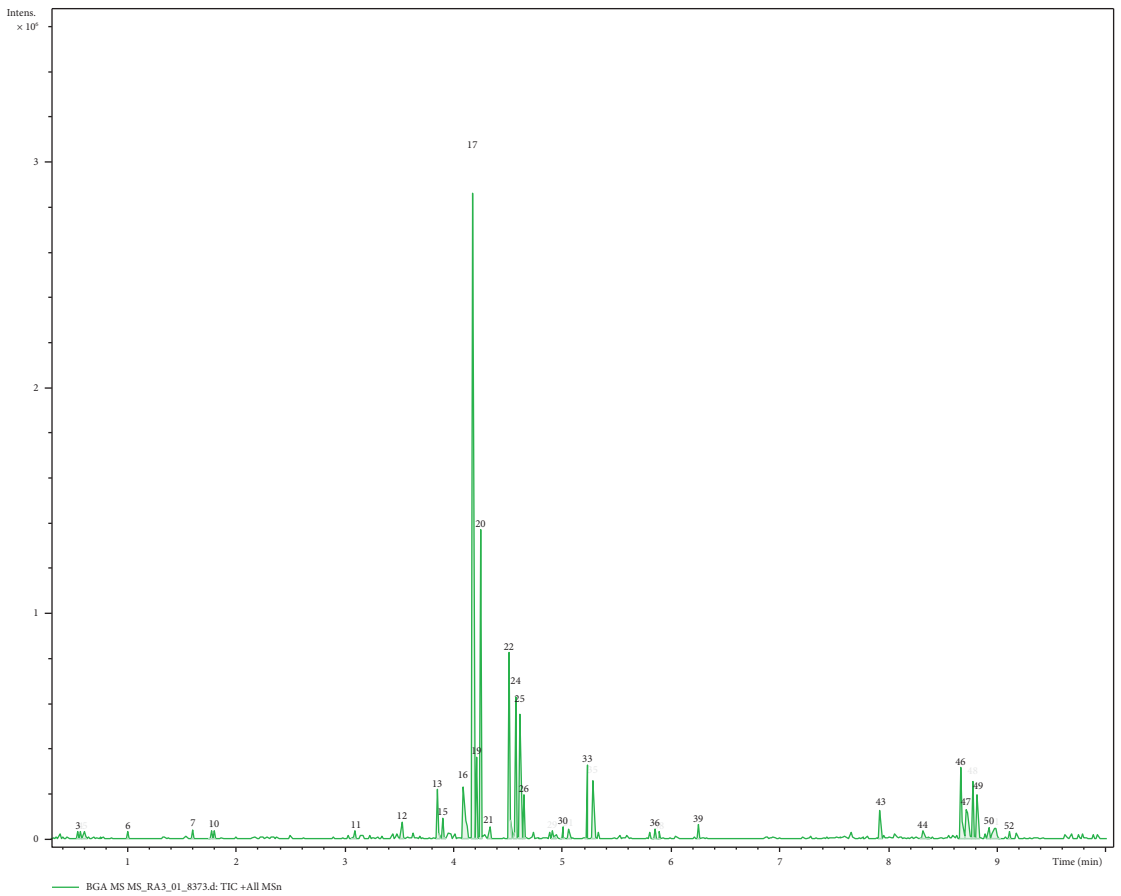
Peak	RT	M + H (m/z)	M + NA (m/z)	MS/MS fragment ions (m/z)	Formula	Suggested compound	Metabolite class	Biological activity	Height	RDBE
18	4.00		257.1457	116.9752 120.9969 134.9845 157.9984	$C_{15}H_{22}O_2$	Dehydrocurdione	Sesquiterpene	Anti-inflammatory, antimicrobial	19,762	5
20	4.11		331.0876	107.0408 119.0389 131.0375 147.0307	$C_{19}H_{16}O_4$	Bisdemethoxycurcumin	Curcuminoid	Anti-inflammatory, antioxidant	5820	12
21	4.18	286.1379		119.0753 120.9972 139.0054 148.9897	$C_{17}H_{19}NO_3$	Piperine	Alkaloid	Antioxidant, anti-inflammatory, bioavailability enhancer	757,279	9
22	4.20		593.2491	112.0664 135.0319 143.0362 151.0848 159.0301 173.0444	$C_{34}H_{38}N_2O_6$	Dipiperamide A	Amide (alkaloid)	Antimicrobial, insecticidal	5275	17
23	4.23	*593.2758*		112.0664 135.0314 143.0383 151.0848 173.0444	$C_{35}H_{36}N_4O_5$	Pheophorbide a	Chlorophyll derivative	Photodynamic therapy, anticancer	7216	24
24	4.28		308.1193	115.0446 135.0319 143.0363 151.0850 159.02999 171.0293	$C_{17}H_{19}NO_3$	Piperine	Alkaloid	Antioxidant, anti-inflammatory, bioavailability enhancer	90,059	9
25	4.35	302.1685		115.0443 135.0317 143.0359 151.0856 159.0300 171.0291	$C_{18}H_{23}NO_3$	Futoamide	Amide (alkaloid)	Antimicrobial, insecticidal	5290	8
26	4.52	312.1526		106.9826 124.9907 177.1104	$C_{19}H_{21}NO_3$	Piperettine I	Amide (alkaloid)	Antimicrobial, anticancer	191,452	10

Table 9: Continued.

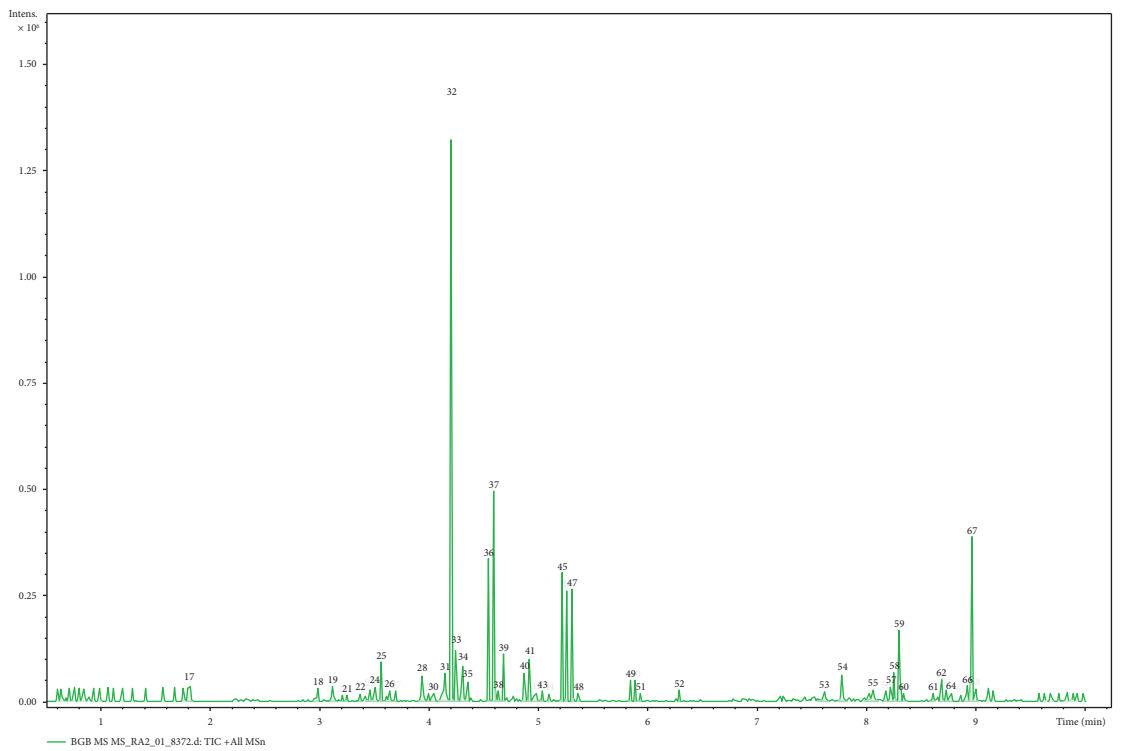
Peak	RT	M + H (m/z)	M + NA (m/z)	MS/MS fragment ions (m/z)	Formula	Suggested compound	Metabolite class	Biological activity	Height	RDBE
27	4.59		334.1333	115.0445 131.0374 141.0573	$C_{19}H_{21}NO_3$	Piperettine I	Amide (alkaloid)	Antimicrobial, anticancer	36,356	10
28	4.67		338.1650	169.0503 135.0320	$C_{19}H_{25}NO_3$	Piperolein A	Amide (alkaloid)	Antioxidant, anticancer	16,213	8
29	4.71	316.1841		106.9831 120.9970 124.9913 148.9896	$C_{19}H_{25}NO_3$	Piperolein A	Amide (alkaloid)	Antioxidant, anticancer	45,204	8
30	4.86	330.1990		141.0570 169.0506 174.9696	$C_{20}H_{27}NO_3$	Pipercallosine	Amide (alkaloid)	Antimicrobial, anticancer	19,813	12
31	4.90		352.1803	131.0367 135.0322 136.0359 147.0003	$C_{20}H_{27}NO_3$	Pipercallosine	Amide (alkaloid)	Antimicrobial, anticancer	10,043	12
32	4.93		362.1646	135.0322 136.0622 137.0707	$C_{21}H_{25}NO_3$	Dehydropipernonaline	Amide (alkaloid)	Antimicrobial, insecticidal	7004	10
33	4.99	370.3368		172.9976 191.0044	$C_{14}H_{19}N_5O_7$	2′-O-β-D-Ribofuranoside	Nucleoside	Antiviral, anticancer	8654	8
34	5.01	571.2663		318.24	$C_{33}H_{30}MgN_4O_4$	Pyrochlorophyllide b	Chlorophyll derivative	Anticancer, photodynamic therapy	9599	21
35	5.08		364.1792	103.0463 135.0322 157.0513	$C_{21}H_{27}NO_3$	Pipernonaline	Amide (alkaloid)	Antimicrobial, insecticidal	7303	9
36	5.25		239.1344	119.0758	$C_{15}H_{20}O$	Ar-tumerone	Sesquiterpene	Anti-inflammatory, neuroprotective	61,238	6
37	5.30		366.1956	135.0324	$C_{21}H_{29}NO_3$	Piperolein B	Amide (alkaloid)	Antioxidant, anticancer	23,464	8
38	5.87		241.1350	105.0616 121.0907	$C_{12}H_{10}O_4$	Piperic acid	Amide (alkaloid)	Antioxidant, antimicrobial	25,618	8
39	5.91	219.1553		116.9751 134.9841	$C_{12}H_{10}O_4$	Piperic acid	Amide (alkaloid)	Antioxidant, antimicrobial	79,536	8
40	6.06		392.1880	153.0323 166.1090	$C_{14}H_{19}N_5O_7$	2′-O-β-D-Ribofuranoside	Nucleoside	Antiviral, anticancer	1531	6

Table 9: Continued.

Peak	RT	M + H (m/z)	M + NA (m/z)	MS/MS fragment ions (m/z)	Formula	Suggested compound	Metabolite class	Biological activity	Height	RDBE
41	6.88	609.2485		140.1299 244.1691	$C_{35}H_{36}N_4O_6$	13-OH-Pheophorbide a	Chlorophyll derivative	Anticancer, photodynamic therapy	3928	20
42	6.91	256.2417		102.1209 118.1135	$C_{14}H_{25}NO_3$	Pipercycliamide	Amide (alkaloid)	Antimicrobial, anticancer	2602	3
43	7.25	368.3648		140.1301 143.0571	$C_{23}H_{29}NO_3$	Piperundecalidine	Amide (alkaloid)	Antimicrobial, anticancer	6476	10
44	7.29	384.3170		244.1688 368.3976	$C_{24}H_{33}NO_3$	Guineensine	Amide (alkaloid)	Antioxidant, antinociceptive	27,083	9
45	7.34		406.2968	114.0817 121.0914 135.1054	$C_{24}H_{33}NO_3$	Guineensine	Amide (alkaloid)	Antioxidant, antinociceptive	27,941	9
46	7.44	925.5009		115.1033 133.0760 140.1302	$C_{55}H_{73}MgN_4O_7$	15-OH-Lactone-chlorophyll a	Chlorophyll derivative	Anticancer, photodynamic therapy	28,415	22
47	7.60	617.4139		190.0654 595.3376 597.2666 601.4201	$C_{40}H_{56}O_5$	Vaucheriaxanthin	Carotenoid	Antioxidant, anti-inflammatory	16,569	13
48	7.65	529.3665		128.1070 187.0594 327.0508 383.1097 439.1693 507.2896	$C_{40}H_{48}$	Octadehydro-β,β-carotene	Carotenoid	Antioxidant, anti-inflammatory	13,820	17
49	7.69	336.3130		124.0756 138.0897 191.0076 206.0164	$C_{22}H_{41}NO$	Pipericine.	Amide (alkaloid)	Antimicrobial, insecticidal	11,390	3
50	7.82	334.2966		102.1209 124.0749 138.0906	$C_{22}H_{39}NO$	Pipyaqubine	Amide (alkaloid)	Antimicrobial, anticancer	30,004	4
51	8.16	557.3955		102.1203 140.1310	$C_{33}H_{32}MgN_4O_3$	Pyrochlorophyllide a	Chlorophyll derivative	Anticancer, photodynamic therapy	29,736	20
52	8.20	451.4426			$C_{31}H_{62}O$	Palmitone	Ketone	Antimicrobial, anticancer	8678	1
53	8.60	893.5870		133.0756 177.0949 215.1071	$C_{55}H_{72}MgN_4O_5$	Chlorophyll a	Chlorophyll	Anticancer	11,849	22
54	8.93	547.3733		102.1204 146.1045	$C_{40}H_{50}O$	Hexadehydro-β,β-caroten-3-ol	Carotenoid	Antioxidant, anti-inflammatory	32,137	16
55	9.04	317.2146		142.1466	$C_{20}H_{28}O_3$	7-Hydroxyretinoic acid	Retinoid	Anticancer, anti-inflammatory	31,723	7



(a)



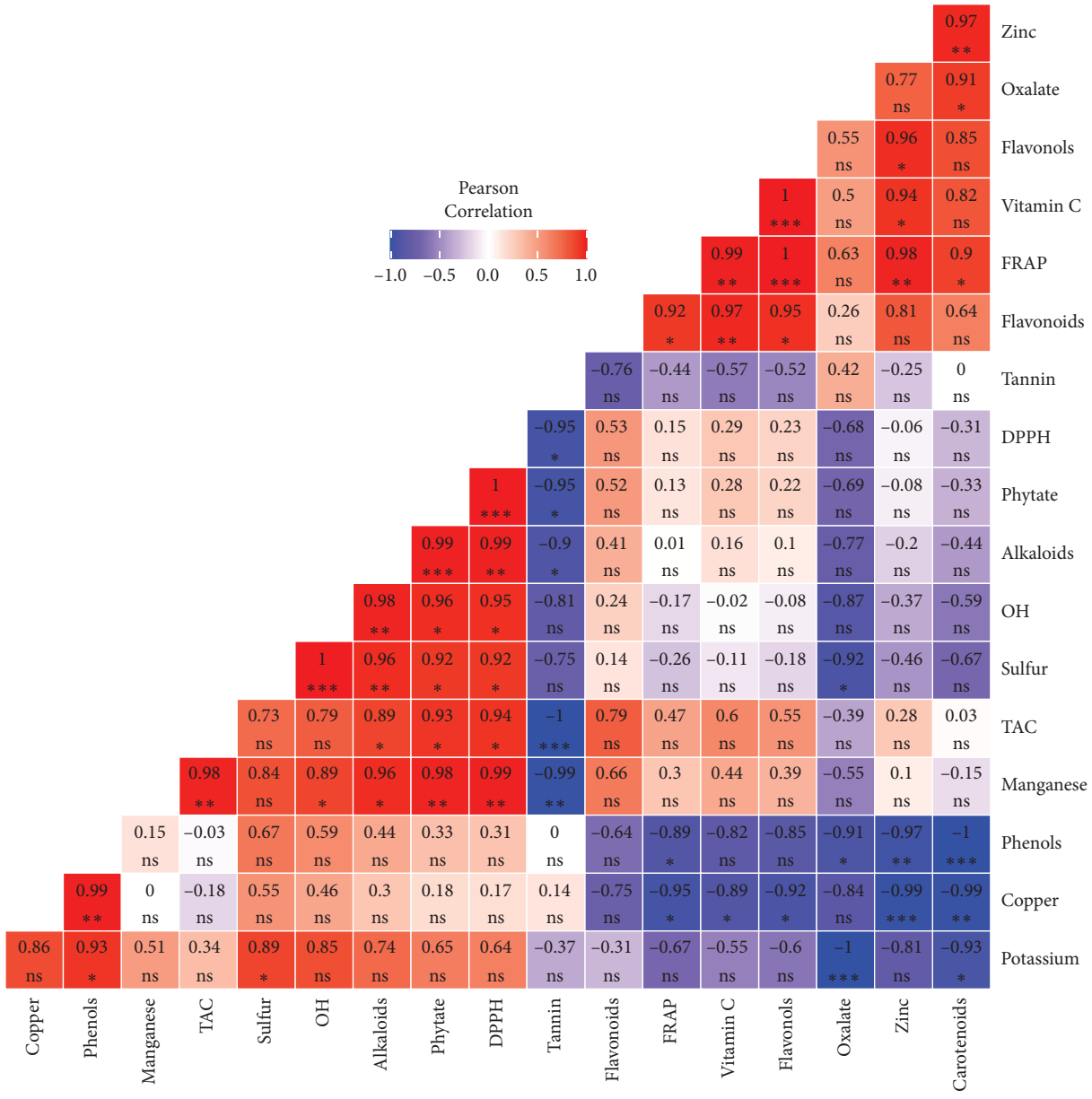
(b)

Figure 2: Base peak chromatogram for methanol extract in (+)-ESI mode, portraying annotated compounds from BGA (a) and BGB (b).

Table 10: Antioxidant and antiradical activities of BobyGuard A and BobyGuard B.

Formulation	DPPH (%)	FRAP (mg eq $FeSO_4$/100 g DM)	OH (%)	TAC (mg eq ascorbic acid/100 g DM)
BGA	87.93 ± 0.60^a	2157.07 ± 65.19^a	83.07 ± 0.30^a	17.01 ± 0.50^a
BGB	91.09 ± 0.45^b	4600.72 ± 31.7^b	86.03 ± 0.71^b	13.61 ± 0.35^b

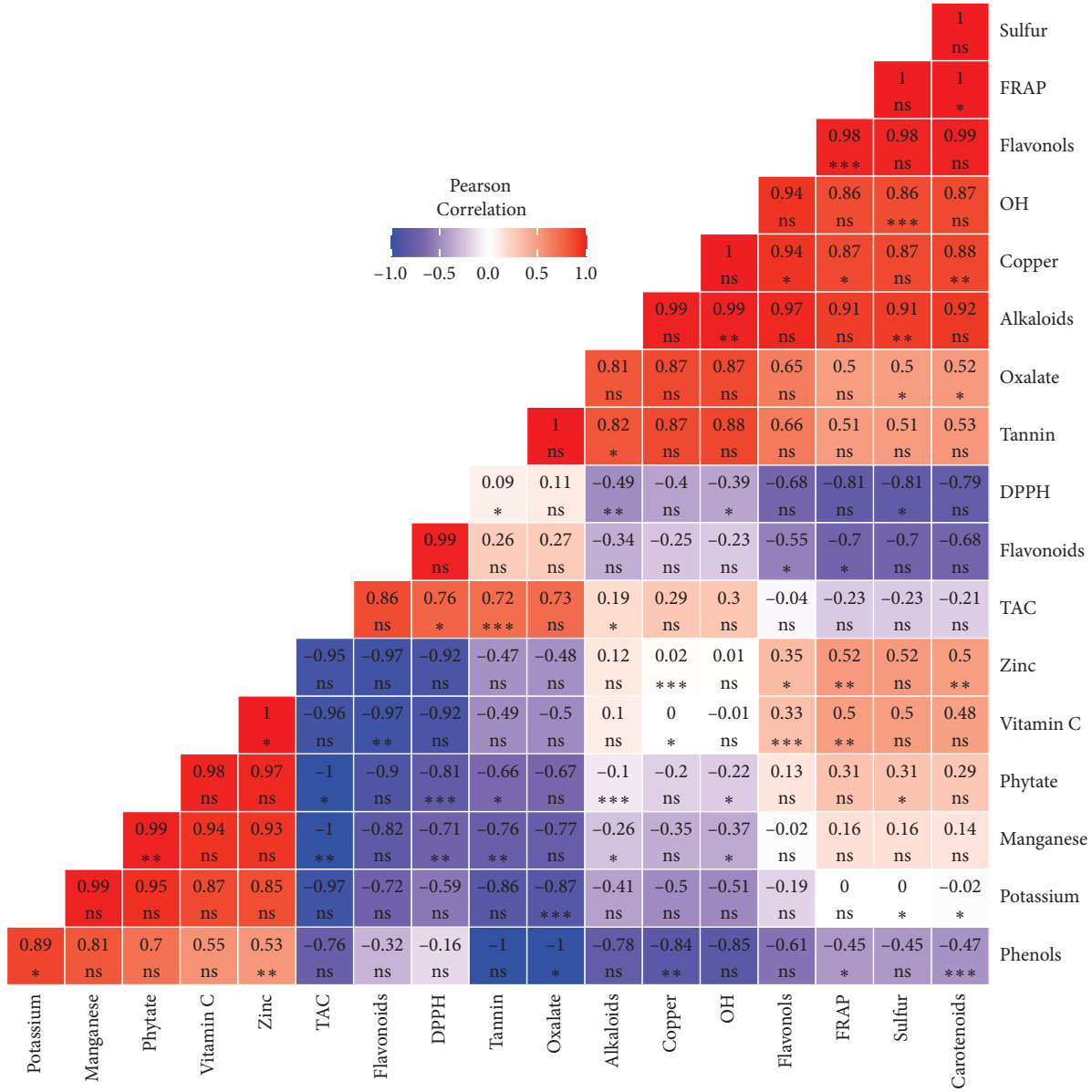
Note: Values are expressed as mean ± SEM; for each row, values having different superscript letters are significantly different ($p < 0.05$).



Significance levels: ns $p \geq 0.05$, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Figure 3: Pearson's correlation diagram of phytochemicals, nutrients, and antioxidant activities of BGA.

a significant strong negative correlation between TAC and tannins ($R = -1.00$, $p < 0.05$). For BGB, strong positive correlations were found between DPPH and flavonoids; OH and tannins, oxalate, alkaloids, copper, sulfur, carotenoids, and flavonoids; FRAP and alkaloids, copper, flavonols, carotenoids, and sulfur; and TAC and flavonoids. Significant strong correlations were observed between TAC and manganese ($R = -1.00$, $p < 0.01$), and FRAP and carotenoids ($R = 1.00$, $p < 0.05$) in BGB. The correlations between these assays and nutrients/phytonutrients indicate their major



Significance levels: ns $p \geq 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Figure 4: Pearson's correlation diagram of phytochemicals, nutrients, and antioxidant activities of BGB.

contributions to the antioxidant and antiradical activities in these assays. Indeed, the antioxidant action of these nutrients/phytonutrients present in these formulations is attributed to diverse mechanisms, including preventing chain initiation, decomposing peroxide, hindering continued hydrogen abstraction, and radical scavenging action [96]. Additionally, strong positive correlations were observed between DPPH and TAC ($R = 0.94$), DPPH and OH ($R = 0.95$) for BGA, and FRAP and OH ($R = 0.95$) for BGB, suggesting that these three methods have similar predictive capacities for the antioxidant activities of the formulations.

4. Conclusion

This study successfully developed and evaluated the quality of two polyherbal nutraceutical formulations, BGA and BGB, emphasizing their potential as dietary interventions for managing and preventing chronic diseases. Both formulations exhibited rich phytochemical profiles, featuring key bioactive compounds such as curcumin, piperine, quercetin, and chlorophyll a, which contribute to their significant antioxidant properties. Notably, BGB demonstrated superior antioxidant activities with higher flavonoid and carotenoid contents, showcasing higher inhibition in the

DPPH assay and a ferric-reducing capacity. Conversely, BGA provided higher TAC and superior mineral content, especially copper. The synergistic effects of the combined phytochemicals in BGA and BGB highlight their potential for enhancing nutritional intake and supporting health in individuals suffering from chronic diseases. Future research should focus on the valorization of residues after extraction of bioactive compounds, determining the action mechanism of each nutraceutical, preclinical research to validate the health benefits of these formulations individually and further explore their efficacy in vivo.

Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

Informed written consent was obtained from the participants before the sensory evaluation of the polyherbal nutraceutical samples was conducted in compliance with ethical standards. The goal of the study and its methods were explained to participants beforehand.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

B.M. participated in conceptualization, data curation, formal analysis, investigation, methodology, validation, writing – original draft, and writing – review and editing; S.Z. participated in conceptualization, supervision, and writing – review and editing; C.K.T. participated in formal analysis, investigation, and methodology; M.D. participated in writing – review and editing. F.A.F.F. participated in formal analysis, investigation, and methodology; R.P. participated in conceptualization, supervision, and writing – review and editing; E.F. participated in conceptualization, supervision, and writing – review and editing.

Funding

No funding was received for this manuscript.

Acknowledgments

The authors are grateful to their respective institutions for the material support.

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