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NUTRITIONAL COMPOSITION OF HOOPVINE, *Trichostigma octandrum* (L.), DRY LEAF AND ITS BIOLOGICAL ACTIVITIES

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ABSTRACT

Trichostigma octandrum (L.) (hoopvine) is a plant native to tropical and subtropical regions that is traditionally valued for its medicinal and nutritional properties. This study was conducted to investigate the nutritional composition and biological activities of hoopvine leaf powder. Therefore, the proximate analysis, secondary metabolites, antimicrobial activity, antioxidant activity, and total polyphenol and flavonoid contents of aqueous and ethanolic leaf extracts were evaluated. Hoopvine leaf powder contained high levels of crude protein (24.46%), ether extract (3.66%), ash (21.74%), crude fiber (10.69%), and energy content (3365.50 kcal/kg), along with essential vitamins and minerals. The amino acid profile was dominated by glutamic acid (2.46%) followed by aspartic acid (1.86%), and lysine and methionine contents were 1.09% and 0.26%, respectively. Gas chromatography–mass spectrometry analysis revealed fifteen bioactive constituents in the methanolic extract, among which *Ethylamine, bis-N-(trimethylsilyl)* (86% similarity) was the predominant compound, contributing 46.65% of the total chromatographic area. Stock concentrations of hoopvine (250 mg/mL for the aqueous extract and 50 mg/mL for the ethanolic extract) demonstrated antimicrobial activity against *Staphylococcus aureus*. Antimicrobial activity and the polyphenol and flavonoid contents of both aqueous and ethanolic hoopvine extracts increased in a concentration-dependent manner ($p \leq 0.05$). Comparative analysis of the two solvent extracts revealed that at concentrations of 0.5 and 1.0 mg/mL, the aqueous extract exhibited stronger antioxidant activity than the ethanolic extract ($p \leq 0.05$). In addition, at 0.5 mg/mL, the flavonoid content of the aqueous extract was significantly higher than that of the ethanolic extract ($p \leq 0.05$). These findings suggest that hoopvine leaf powder has potential applications as a functional ingredient in animal feed and human food owing to its high protein content, rich mineral composition, energy value, and antioxidant properties.

Keywords: Antioxidant activity, Biological activity, Flavonoid, Hoopvine, Polyphenol, Nutritional composition.

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INTRODUCTION

Functional foods derived from natural or processed plant sources have been widely consumed in recent years due to their high biological activity and health benefits (Mohamad *et al.*, 2019). The risk of several diseases, including cardiovascular disorders and cancer, can be reduced through the intake of plant-based functional foods (Kapinova *et al.*, 2017, Mehmood *et al.*, 2019). Recognizing the great potential of natural plants, research on immune enhancement through plant-derived functional foods has garnered a significant attention (Davoodvandi *et al.*, 2019).

Trichostigma octandrum (L.), commonly known as hoopvine, is a fast-growing shrub native to the tropical and subtropical regions of the Americas. It is widely distributed in forested areas and has been domesticated in Haiti, where it is cultivated in rural and urban settings (Francis, 2004). Hoopvine can reach up to 10 m in height, with twining stems, glabrous leaves, and long petioles. It produces narrow flower clusters and red to red–purple fleshy berries, with a biomass yield of approximately 61 g/m² under both sunny and shaded conditions (Matsubara *et al.*, 2009). Due to its rapid growth, adaptability to varied light conditions, and established cultivation in diverse environments, hoopvine shows potential for

agricultural domestication and sustainable utilization (Francis, 2004; Pumijumnong and Buajan, 2013).

Hoopvine has diverse applications in different cultures. Its stems are traditionally used in construction of barrel hoops, baskets, bent furniture and handicrafts, and its leaves are used for their medicinal properties, particularly in treating wounds in Colombia. In Haiti, hoopvine leaves are consumed as vegetables and believed to alleviate anemia, asthma and heart palpitations. In Cuba, this plant is used as a laxative, and its bark is used to treat cold symptoms and for water retention (Cano and Volpato, 2004). Hoopvine has long been used in traditional medicine and nutrition to alleviate symptoms such as heart palpitations, colds, and water retention (Francis, 2004). Despite its long-standing use in traditional medicine and nutrition, scientific research on the nutritional composition and bioactive compounds of hoopvine is limited. Preliminary studies have suggested the presence of secondary metabolites and pigments in its leaves. For instance, Matsubara *et al*. (2009) identified chlorophyll, carotenoids, neoxanthin, violaxanthin, antheraxanthin, zeaxanthin, lutein, lutein epoxide, α-carotene, and β-carotene in hoopvine leaves. Gaitán *et al.* (2011) reported that hoopvine leaf extracts exhibited antifungal activity against *Fonsecaea pedrosoi* (MIC: 12.5 µg/mL).

Owing to its potential nutritional benefits and bioactive properties, hoopvine offers promising opportunities for broad agricultural domestication and commercial applications. However, comprehensive data on the nutritional profile and functional properties of hoopvine are lacking, largely due to the absence of quantitative analyses and limited comparative evaluations assessing its antioxidant and antimicrobial potential. This may be attributed to the fact that hoopvine has not been widely domesticated or recognized as a conventional food or feed crop, resulting in limited research interest and a lack of standardized analytical approaches. Therefore, this study aimed to evaluate the nutritional composition including proximate components, vitamins, amino acid profile, and mineral content, and bioactive properties such as antioxidant activity, total polyphenol flavonoid contents and antimicrobial effects of hoopvine leaf extracts to explore its potential as functional food ingredients.

MATERIALS AND METHODS

Plant sample collection: Fresh hoopvine (*Trichostigma octandrum* (L.)) leaves were collected from Tabarre, Haiti, by Nazaire Fouquet, and a voucher specimen was deposited in the herbarium of the College of Agronomy and Veterinary Medicine (FAMV), State University of Haiti (UEH). Plant parts were cut approximately 20–40 cm from the stem using sterile shears. The collected samples were initially shade-dried for 4–6 days at ambient temperature, followed by oven drying at 70–80 °C for 7 hours per day over a period of 3–4 days to ensure complete dehydration. Dried samples were ground into a fine powder using a mechanical grinder at the Faculty of Agronomy and Veterinary Medicine, State University of Haiti (UEH/FAMV, Haiti). The powdered plant material was then sealed in sterile polyethylene bags and transported to the Feed Biotechnology Laboratory, Konkuk University (Seoul, South Korea), where it was stored at room temperature (20 ~ 25 °C) in a dark, dry environment until further use.

Nutrient composition of hoopvine leaf powder: The proximate composition was analyzed at the Animal Resources Research Center of Konkuk University using standard procedures recommended by the Association of Official Analytical Chemists (AOAC) (Baur and Ensminger, 1997) (Table 1). Moisture content was determined by oven-drying at 105 °C, crude protein by the Kjeldahl method, crude fat by ether extraction using a Soxhlet apparatus, crude fiber by acid and alkali digestion, and ash by incineration at 550 °C in a muffle furnace. Energy content was determined using a bomb calorimeter. Dried and homogenized hoopvine leaf samples were combusted in an oxygen-rich environment, and the heat released was measured and expressed as kilocalories per kilogram (kcal/kg). The vitamin concentrations were also analyzed at the Animal Resources Research Center of Konkuk University, using high-performance liquid chromatography (HPLC; 1260 Infinity II, Agilent Technologies, USA) with UV or fluorescence detection, following AOAC protocols. The Amino acid and mineral contents were determined by the Feed Industry Research Institute of the Korea Feed Association (Seocho-gu, Seoul, Korea). The amino acid composition was determined using an automatic amino acid analyzer (L-8900, Hitachi, Tokyo, Japan) after hydrolysis with 6 N HCl at 110 °C for 24 h. Methionine and cysteine were determined after performic acid oxidation. Mineral concentrations were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES; Optima 8300, PerkinElmer, USA) after nitric acid digestion (Olesik, 1991).

Quantitative analysis of secondary metabolites in hoopvine: Gas chromatography (GC) coupled with mass spectrometry (MS) was used to analyze the secondary metabolite profile of plant samples. Leaf powder (10 g) and methanol (90 mL) were mixed for 3 h at 60 °C. After evaporating the sample, the residue was diluted in ethyl acetate (EA) and water, followed by liquid–liquid separation. NaCl was added to accelerate phasing. The nonpolar phase was evaporated using a rotary evaporator (N-1000; Eyela, Tokyo, Japan) at 35 °C in a water bath (Digital Water Bath SB-1000; Eyela, Tokyo, Japan). Once the EA completely evaporated, 200 µL of tetra-methyl silane and 800 µL of pyridine were added, and

samples were incubated for 1 h at 70 °C prior to GC–MS analysis.

Table 1. Proximate composition of hoopvine dried leaves

Nutrient	Content
Moisture content (%)	4.70
Crude protein (%)	24.46
Fat (%)	3.66
Crude fiber (%)	10.69
Ash (%)	21.74
Carbohydrate (%)	34.75
Energy (Kcal/kg)	3365.50

Experimental design and treatments: Two types of extracts (aqueous and ethanolic) were prepared from dried leaf powder of hoopvine to evaluate their antioxidant activity, total phenolic content, and total flavonoid content. A total of 50 g of plant powder was mixed with 450 mL of distilled water or ethanol (1:9, w/v) and heated for 3 h in a water bath at 100 °C (aqueous) or 60 °C (ethanol), as described by Sharma *et al*. (1997). It should be noted that heating the aqueous extract at 100 °C for 3 h may cause degradation of heat-sensitive compounds such as vitamin C and carotenoids. The mixtures were filtered through Whatman No. 2 filter paper (Advantec Toyo, Tokyo, Japan), and the resulting filtrates were concentrated under reduced pressure using a rotary evaporator (Rotavapor RE 111, Büchi, Switzerland) at the respective extraction temperatures.

The concentrated extracts were reconstituted in a solvent mixture and adjusted to final concentrations of 250 mg/mL for the aqueous extract (10% dimethyl sulfoxide (DMSO) and 90% distilled water) and 50 mg/mL for the ethanolic extract (50% DMSO and 50% distilled water). All extracts were stored at −70 °C (MDF-U53V; SANYO, Osaka, Japan) until further analysis. Working dilutions were freshly prepared with distilled water prior to each assay. The study was laid out following completely randomized design and all analyses were performed in triplicate.

Antimicrobial activity: The pathogenic microbes used in this study were obtained from and stored in the Feed Biotechnology Laboratory Stock at Konkuk University. Microbes were revived three times in Luria broth (LB) plates within 3 d and incubated (BF-150LI, BioFree, Bucheon, South Korea) at 37 °C for 12–14 h each time. Colonies were then moved to LB broth followed by incubation in a shaking incubator (VS-8480SF; Vision Scientific Co., Ltd., Korea) at 140 rpm and 37 °C. Using a sterilized cotton loop, the bacteria were charged and smeared on LB plates under the bell shape of an ethanol flame. Four 6-mm holes (2 controls and 2 treatments) were made in the LB plate, and 100 µL of sample was poured into each hole. The plates were then incubated at 37 °C for 12–14 h. Controls consisted of a 1:1 DMSO:DW ratio, equal to the solvent in the ethanol extract. Similarly, another control was prepared at a 1:9 ratio with the aqueous extract to reflect the DMSO:DW ratio in the aqueous extract stock concentration. All measurements were performed in triplicate.

Antioxidant activity: A modified version of the 2,2-dimethyl-1-picrylhydrazyl (DPPH) method, as described by Saeed *et al.* (2012) was used to estimate antioxidant activity. Briefly, extracts were diluted to 0.25, 0.5, and 1 mg/mL. Then, 200 µL of extract was mixed with 200 µL of 1.5 × 10-4 M DPPH solution for each treatment. The mixtures were incubated for 15 min at room temperature and optical density was determined at 515 nm using a 96-well ELISA plate reader (Synergy 2, Biotek. Vermont, USA). Ascorbic acid was used at the following concentrations: 0, 0.02, 0.04 and 0.06 mg/mL as a standard curve. All measurements were performed in triplicate, and antioxidant activity was determined using the following formula:

DPPH radical scavenging activity (%) = (1 - control absorbance/ sample absorbance) × 100.

Finally, the concentration inhibiting 50% of the free radicals or activity in an assay (IC_{50}) was determined using the equations found in the regression curves at concentrations of 0.25, 0.5, and 1 mg/mL.

Total polyphenol contents: The total polyphenol content was determined using a modified method described by Dudonne *et al.* (2009). Briefly, 50 µL methanolic solution of gallic acid (1 mg/mL) and 200 µL of sodium carbonate (7.5% w/v) dissolved in DW were added to 250 µL of Folin–Ciocalteu's phenol reagent (1 N) diluted in DW to constitute the standard curve. Then, 50 µL of extract was mixed with the same agents as described previously. All mixtures were incubated for 1 h at room temperature. Optical density was measured at 765 nm using a 96-well ELISA plate reader (Synergy 2, BioTek. Vermont, USA). All detections were performed in triplicate. Results were expressed as milligrams of gallic acid equivalent (GAE)/g of extract and were calculated according to the following equation: TP = (C*V)/M

TP is the total polyphenol content, C is the concentration (mg/mL) obtained from the standard curve, V is the extract volume (mL), and M is the extract weight (g).

Total flavonoid content: The total flavonoid content was determined according to the modified method of Ordonez *et al.* (2006). To determine the total flavonoid contents, 180 µL of sample was added to test tubes, followed by addition of 10 µL of aluminum chloride and 10 µL of potassium acetate. The tubes were then gently vortexed and incubated at room temperature for 30 min before measuring absorbance at 415 nm using a 96-well ELISA

plate reader (Synergy 2, Biotek. Vermont, USA). Quercetin solutions diluted in ethanol at different concentrations were used to generate standard curves. The results were expressed as total flavonoid content in milligrams of quercetin equivalent per gram of extract (mg QE/g) according to the following equation: TF = (C*V)/M

TF is the total flavonoid content, C is the concentration (QE)/g obtained from the standard curve, V is the extract volume (mL), and M is the extract weight (g).

Statistical analysis: Antioxidant activity, total phenolic and flavonoid contents were analyzed in triplicate and presented as mean values. Triplicate data were submitted to the Statistical Analysis System (SAS Institute, 9.3 version, NC, U.S.A.) using a general linear model (GLM). The model utilized was as follows:

$Y_{ij} = \mu + \alpha_i + \beta_j + E_{ij}$

Y_{ij} is the response variable for the ith extract, μ is the grand mean, α_i is the ith extract (1, 2: aqueous or ethanolic), βj is the effect of the jth concentration level, and E_{ij} is the experimental error. Significant differences were determined using Duncan's multiple range test at $p \leq 0.05$ (Duncan, 1955). The results are presented as mean values and standard error of means (SEM) for triplicate detections, or simply as mean values without SEM for duplicate detections, for biological activity and proximate analyses, respectively.

RESULTS

Nutrient composition of hoopvine leaf powder: The proximate composition of hoopvine is shown in Table 1. The results revealed that the dry hoopvine leaves, with a moisture content of 4.70%, contained 24.46% crude protein (CP), 10.69% crude fiber, 3.66% fat (ether extract), 21.74% ash, and 34.75% carbohydrates. The crude fat content of the hoopvine leaf powder used in this study was 3.66%. The ash content of hoopvine leaves was 21.74%, indicating the mineral composition of the leaves. The crude fiber content of dry hoopvine leaves was 10.69%. The amino acid profile of hoopvine leaves was dominated by glutamic acid (2.46%) followed by aspartic acid (1.86%) (Table 2). The essential amino acids were lysine (1.09%), leucine (1.39%), isoleucine (0.74%), valine (0.97%), threonine (0.84%), methionine (0.26%), cysteine (0.53%), histidine (0.62%), alanine (1.01%), and phenylalanine (1.05%). The measured vitamin A content was 940.93 IU/kg, which is equivalent to 0.28 µg retinol equivalent per gram, or 0.56 µg β-carotene per gram. Hoopvine leaf powder contained vitamins B1 (34.30 mg/kg), B2 (0.36 mg/kg), B3 (66.49 mg/kg), B5 (21.03 mg/kg), B6 (6.93 mg/kg), B9 (3.51 mg/kg), and B12 (15.57 mg/kg). The vitamin C concentration in hoopvine leaf powder was 23,265.6 IU/kg (i.e., 1,163.28 mg/kg). The vitamin D_3 content in hoopvine leaf powder was 149,556.64 IU/kg (i.e., 3.74 µg/g). The mineral composition of hoopvine leaves revealed the notable presence of macrominerals, including calcium (3.13%), phosphorus (0.24%), sodium (0.02%), potassium (5.4%), and magnesium (0.95%).

Table 2. Amino acid, vitamin, and mineral profiles of hoopvine dried leaves

Item	Content
Amino acids (%)	
Aspartic acid	1.86
Threonine	0.84
Serine	0.86
Glutamic acid	2.46
Glycine	1.45
Alanine	1.01
Valine	0.97
Isoleucine	0.74
Leucine	1.39
Tyrosine	0.76
Phenylalanine	1.05
Lysine	1.09
Histidine	0.62
Arginine	0.92
Cystine	0.53
Methionine	0.26
Proline	1.1
Vitamins	
Vitamin A (IU/kg)	940.93
Vitamin B1 (mg/kg)	34.3
Vitamin B2 (mg/kg)	0.36
Vitamin B6 (mg/kg)	6.93
Vitamin B12 (mg/kg)	15.57
Pantothenic acid (B5) (mg/kg)	21.03
Folic acid (B9) (mg/kg)	3.51
Nicotinic acid (B3) (mg/kg)	66.49
Vitamin C, IU/kg	23,265.60
Vitamin D, IU/kg	149,556.64
Minerals (%)	
Ca	3.13
P	0.24
Fe	0.03
Na	0.02
K	5.4
Mg	0.95

Abbreviations: IU, International Unit; Ca, calcium; P, phosphorus; Fe, iron; Na, sodium; K, potassium; Mg, magnesium

Quantitative analysis of secondary metabolites in hoopvine: Methanol was used for the quantitative analysis of secondary metabolites due to its ability to dissolve polar compounds and preserve biological activity and volatile constituents. A total of 15 peaks

corresponding to distinct bioactive compounds were identified in the methanolic extract of hoopvine using GC–MS (Table 3). The identified compounds included amides (formamide, acetamide), amino acids (glycine, L-valine), amines (ethylamine, bis-N-trimethylsilyl), carboxylic acids (malonic acid, acetic acid), aldehydes (benzaldehyde, 2-methyl-(CAS) p-toluene), and a fatty acid (hexadecanoic acid). Compound identification was based on peak area, area percentage, homology (similarity) scores, and retention time. The compound corresponding to peak No. 1 showed 93% similarity to N,N-diethylformamide and accounted for approximately 4.38% of the total chromatographic area (Fig. 1). The major compound, ethylamine (peak No. 3), exhibited 86% similarity to bis-N-(trimethylsilyl) and represented 46.65% of the total area.

Table 3. Qualitative analysis of compounds found in hoopvine leaf extracts using GS-MS.

No.[1]	RT[2]	Compound name	Area	Area (%)	Hom.[3], %	M. W.[4]
1	5.531	Formamide, N,N-diethyl- (CAS) N,N-Diethylformamide	510675	4.38	93	101
2	5.840	Glycine, N-(1-oxopropyl)-, trimethylsilyl ester	322813	2.77	72	203
3	6.042	Ethylamine, Bis-N-(Trimethylsilyl)	5432053	46.65	86	189
4	6.279	L-Valine, N-(trimethylsillyl)-, trimetylsilyl ester	1293878	11.11	77	261
5	6.715	2,2-Dielthylacetamide	271298	2.33	75	115
6	7.771	Acetamide, N,N-diethyl- (CAS) N,N-diethylacetamide	1264015	10.85	97	115
7	8.778	2,2-Diethylacetamide	1081672	9.29	85	115
8	9.695	Malonic acid 2MTS	463817	3.98	76	248
9	12.515	Benzaldehyde, 2-methyl- (CAS) p-Tolualdehyde	117065	1.00		120
10	15.731	Acetic acid, bis[(trimethylsilyl)oxyl]-, trimethylsilyl ester	179959	1.54	85	308
11	18.188	Ethanedioic acid, bis(trimethylsilyl)ester (CAS) OXALIC ACID-DITMS	84251	0.72	75	234
12	19.422	3,6,9-Trioxa-2,10-disilaundecane, 2,2,10,10-tetramethyl- (CAS) Diethylene Glycol Bistrimethylsilyl Ether	81713	0.70	92	250
13	33.325	Borneol, tert-buthyldimethylsilyl ether	276080	2.37	68	268
14	34.609	Neophytadiene	97776	0.84	90	278
15	38.701	Hexadecanoic acid, trimethylsilyl ester (CAS) Palmitic Acid-Monotms	171753	1.47	94	328

[1]No.: Peak number; [2]RT, Retention time; [3]Hom. Homogeneity or similarity; [4]M.W.: Molecular weight

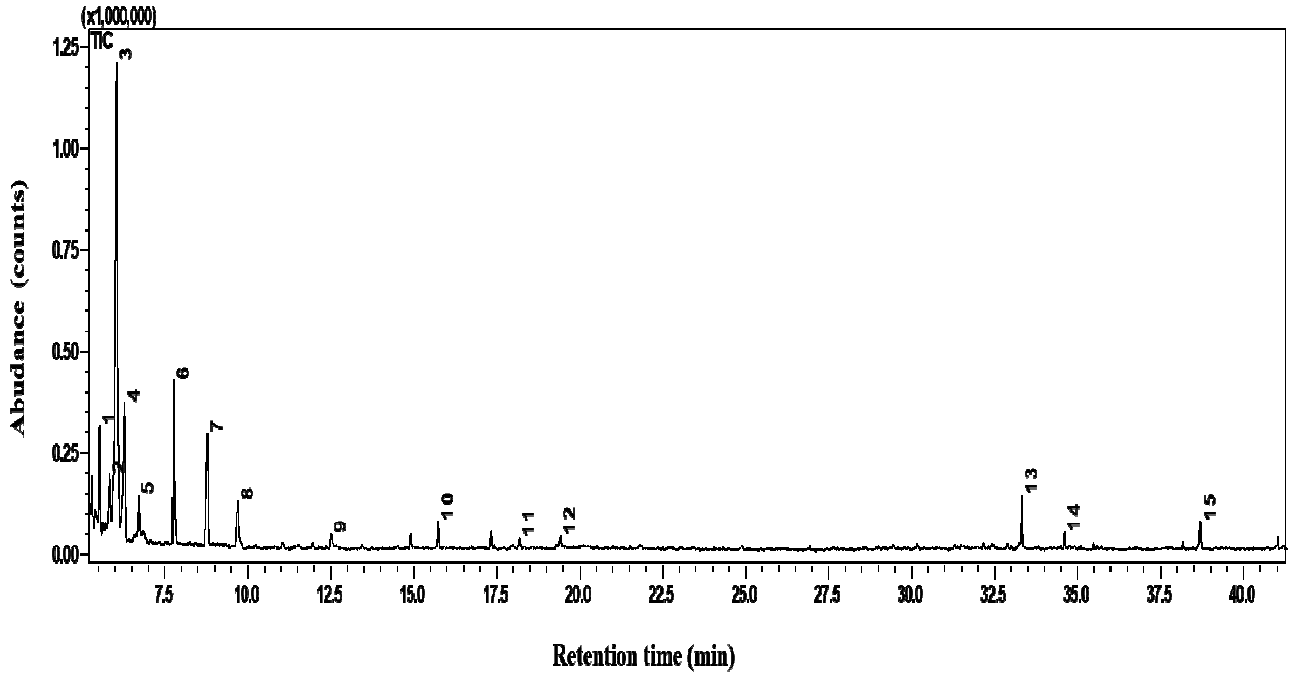

Fig. 1. GC–MS chromatogram of the methanolic extract of hoopvine leaves.

Compounds were identified based on retention time and mass spectral data. Peak assignments and compound information are summarized in Table 3.

Table 4. Extraction yield of hoopvine leaves.

	Solvent	
	Water	Ethanol
Leaf weight (g)	50	50
Solvent volume (mL)	450	450
Solute:solvent ratio	1:9	1:9
Extraction yield (%)	21.76	3.34
Stock concentration (mg/mL)	250	50

Extracted from 50 g leaf powder in 450 mL solvent (1:9, w/v). Stock concentration used for assays.

Antimicrobial activity: Extraction yields of hoopvine leaves were 21.76% for the aqueous extract and 3.34% for the ethanolic extract (Table 4). Antibacterial activity was evaluated against six bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella gallinarum*, *Listeria monocytogenes*, and *Salmonella typhimurium* (Table 5). No inhibition was observed at working concentrations (0.25, 0.5, and 1 mg/mL); therefore, stock concentrations (250 mg/mL for the aqueous extract and 50 mg/mL for the ethanolic extract) were tested. At stock concentrations, hoopvine exhibited antimicrobial activity only against *S. aureus.* The aqueous extract produced a clear zone averaging 13.33 ± 0.58 mm in diameter, and the ethanolic extract resulted in a 7.33 ± 0.58 mm clear zone.

Table 5. Antimicrobial activity of hoopvine leaf extracts.

Pathogenic strain	Stock no.	Aqueous extract	Ethanol extract
		250 mg/mL	50 mg/mL
Escherichia coli	SK833	-[1]	-
Staphyloccocus aureus	SK741	+++[2] (13.33 ± 0.58)[4]	+[3](7.33 ± 0.58)[4]
Pseudomonas aeruginosa	SK804	-	-
Salmonella gallinarum ATCC 9184	SK3359	-	-
Listeria monocytogenes KCCM 40307	SK1060	-	-
Salmonella typhimurium KCTC 2515	SK859	-	-

[1]- No antimicrobial activity detected
[2]+++ Antimicrobial activity was relatively high
[3]+ Relatively low antimicrobial activity
[4]Mean ± SD: standard deviation
(Hole diameter = 6 mm)

Antioxidant activity: Antioxidant activity was analyzed at three concentrations of each extract (Table 6). The results demonstrated that the antioxidant effect increased with an increase in extract concentration ($p \leq 0.05$). Notably, the aqueous extract exhibited a stronger antioxidant effect than the ethanol extract at the same concentrations (0.5 mg/mL and 1 mg/mL) ($p \leq 0.05$). In this study, IC_{50} values obtained for ethanol (0.50 mg/mL) and aqueous (0.38 mg/mL) extracts were within the range observed for other leaf extracts.

Table 6. Antioxidant activity of hoopvine leaf extracts (%).

	Concentration (mg/mL)			
	0.25	0.5	1	SEM[1]
Aqueous extract	$^{A}33.86^{c}$	$^{A}60.34^{b}$	$^{A}67.22^{a}$	0.51
Ethanolic extract	$^{A}27.42^{b}$	$^{B}49.83^{a}$	$^{B}54.46^{a}$	1.76
SEM[1]	2.42	1.28	0.31	

Different lowercase letters in the same row and uppercase letters in the same column indicate significant differences ($p \leq 0.05$)
[1]SEM: Standard error of the mean

Total polyphenol content: In this study, the total polyphenol content of hoopvine leaves was quantified and expressed as gallic acid equivalents (GAE, mg/g extract) (Table 7). The results demonstrated a significant variation in total polyphenol content depending on the extract concentration, regardless of the extraction method. A concentration-dependent increase in polyphenol content was observed, with the aqueous extract containing 23.62 mg GAE/g at 1 mg/mL, higher than that containing 14.10 mg GAE/g at 0.5 mg/mL ($p \leq 0.05$), and undetectable at 0.25 mg/mL. Similarly, the ethanol extract exhibited 19.50 mg GAE/g at 1 mg/mL, higher than that of 11.20 mg GAE/g at 0.5 mg/mL ($p \leq 0.05$), and lowest at 6.81 mg GAE/g at 0.25 mg/mL ($p \leq 0.05$).

Table 7. Total phenolic content of hoopvine leaf extract.

	Concentration (mg/mL)			
	0.25	**0.5**	**1**	**SEM[1]**
Aqueous extract	ND[2]	$^{A}14.10^{b}$	$^{A}23.62^{a}$	0.87
Ethanolic extract	6.81^{c}	$^{A}11.20^{b}$	$^{A}19.50^{a}$	0.73
SEM[1]	1.51	0.57	0.93	

Different lowercase letters in the same row and uppercase letters in the same column indicate significant differences ($p \leq 0.05$)
[1]SEM: Standard error of the mean
[2]ND: Not detected

Total flavonoid content: The total flavonoid content of hoopvine leaf extracts, expressed as quercetin equivalents (QE, mg/g extract), varied significantly depending on the extraction method and concentration (Table 8). The flavonoid content increased with concentration in both aqueous and ethanolic extracts ($p \leq 0.05$). At a concentration of 0.5 mg/mL, the aqueous extract exhibited a notably higher flavonoid content (35.64 mg QE/g) than the ethanolic extract (12.62 mg QE/g) ($p \leq 0.05$).

Table 8. Total flavonoid content of hoopvine leaf extracts.

	Concentration (mg/mL)			
	0.25	**0.5**	**1**	**SEM[1]**
Aqueous extract	7.69^{c}	$^{A}35.64^{b}$	$^{A}46.89^{a}$	0.71
Ethanolic extract	ND[2]	$^{B}12.62^{b}$	$^{A}44.391^{a}$	1.06
SEM[1]	0.58	1.42	1.15	

Different lowercase letters in the same row and uppercase letters in the same column indicate significant differences ($p \leq 0.05$)
[1]SEM: Standard error of the mean; [2]ND: Not detected

DISCUSSION

The protein content of 24.46% crude protein (CP) observed in this study categorizes hoopvine leaves as a potential protein supplement, comparable to other leguminous plants within the Petiveriaceae family. This level is consistent with protein contents reported in other legume leaves, such as *Moringa oleifera*, *Vigna unguiculata* (cowpea), and *Lactuca sativa*. Although further investigation into its digestibility is required, the protein content in dried hoopvine leaves could be sufficient to meet the daily protein requirement of a three-year-old child, based on WHO recommendations. Proteins are essential nutrients for growth and development, and the present findings highlight hoopvine's potential as a valuable dietary protein source (Corkins *et al*., 2016). In contrast, the crude fat content was lower than that reported for *M. oleifera* leaves (Witt, 2013). *Moringa oleifera*, commonly known as the drumstick tree, is a nutrient-dense plant widely consumed for its high protein, vitamin, and antioxidant content. In contrast, the fat content of hoopvine has been reported to be comparable to that of *Vigna unguiculata* L. Walp. (cowpea), a leguminous plant recognized for its protein-rich seeds and edible leaves (Nielsen *et al*., 1997). The fat content of hoopvine can be considered typical and within the range reported for other legume leaves. However, the overall fat content alone is not a key determinant of its nutritional or functional value. More importantly, the fatty acid composition, particularly the balance between saturated and unsaturated fats, should be considered. Excessive consumption of saturated fatty acids has been linked to various metabolic disorders (Iggman and Risérus, 2011); whereas, unsaturated fatty acids, such as omega-3 and omega-6, offer health benefits, particularly in reducing inflammation (Coniglio *et al.*, 2023). Among these, conjugated linoleic acid is of particular interest due to its functional properties (Collomb *et al.*, 2006). Thus, although the crude fat content of hoopvine was within the expected range for legume leaves, further analysis is needed to determine its lipid composition, including the specific fatty acid types and profiles. This study clarifies its potential as a functional food source. The ash content observed in this study was notably higher than that of other commonly consumed dried plant materials, such as *Moringa oleifera*, *Colocasia esculenta*, and *Solanum melongena* fruits. According to Platace *et al*. (2015), ash content in plants can vary depending on factors such as species, soil characteristics, moisture levels, and harvesting time. Considering that the samples in this study were air-dried prior to storage, the high ash content may have been influenced by dust accumulation during the drying process. The crude fiber content of hoopvine leaves was slightly lower than that reported for other leafy vegetables, such as *Jatropha curcas* and species of *Brassica*. Dietary fiber is known to improve bowel movement, relieve constipation (Slavin and Jacobs, 2010), and lower the risk of colon cancer (Zeng *et al*., 2014).

Traditionally, hoopvine has been used in Cuba to treat constipation (Cano and Volpato, 2004), supporting its potential functional benefits.

Although *Vigna unguiculata* leaves have been reported to contain high concentrations of essential amino acids (Nielsen *et al*., 1997), hoopvine leaves appear to have relatively lower levels of these nutrients. This may limit their nutritional value when compared to other leafy vegetables with more balanced amino acid profiles. Further research is needed to explore processing methods, such as fermentation or enzymatic treatment, to improve amino acid bioavailability and overall protein quality in hoopvine. As reported by Abuye *et al*. (2003) and Akubugwo *et al*. (2007), *Moringa stenopetala* and *Amaranthus hybridus* contain higher levels of β-carotene than those found in hoopvine in this study. The lower vitamin A content may have been due to the drying method used, as Sachdeva *et al.* (2019) demonstrated that vitamin A is heat sensitive. According to the Food and Agriculture Organization and World Health Organization (Joint, 2002), vitamin A is crucial for the visual system, growth, immune function, and reproduction. According to Miyamoto *et al.* (2005), most plant vegetables contain low levels of vitamin B12, typically > 1 µg/kg. However, in the present study, the B12 content was higher than that in leaves of other plants, such as broccoli, water shield, asparagus, and mung bean sprouts. Vitamin B plays a vital role in carbohydrate, amino acid, and fatty acid metabolisms. In developing countries, where diets are often based on polished cereals, B vitamins are commonly deficient, making hoopvine a potential source of these vitamins. According to Akubugwo *et al*. (2007), the concentration in *Amaranthus hybridus* leaves is 254 mg/kg, which is significantly lower than the value observed in this study. Vitamin C enhances iron absorption in the intestinal tract (Deng *et al.*, 2023) and may contribute to the potential of hoopvine to combat anemia. In addition, vitamin C acts as a powerful antioxidant by reducing free radicals in the body (Padayatty *et al.*, 2003). The vitamin D content in hoopvine appears to be higher than that reported for commonly consumed vegetables such as *Solanum lycopersicum* and *Cucurbita pepo*. Vitamin D plays a critical role in calcium absorption in the small intestine (Holick, 2007), and its deficiency has been linked to various health conditions, including cancer, diabetes, cardiovascular disease, and osteoporosis (Wang *et al*., 2008). These findings suggest that hoopvine leaves may serve as a relatively rich source of vitamin D, underscoring their potential as a functional food ingredient.

The calcium-to-phosphorus (Ca:P) ratio in hoopvine (13.04) was significantly higher than the 2.16 ratio found in bone hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$) (Zaichick and Tzaphlidou, 2002). Because a low dietary Ca:P ratio has been associated with increased bone resorption and the risk of osteoporosis (Kemi *et al.*, 2010, Zaichick and Tzaphlidou, 2002), the high calcium content and Ca:P ratio of hoopvine suggested potential benefits for bone health. Hoopvine leaves also contained essential trace minerals including manganese, zinc, copper, and fluoride. Notably, the iron content (0.03%) was more than 4-times higher than that of carrot roots and more than 8-times higher than that of carrot leaves (Warman and Havard, 1997). Given that excessive calcium intake can inhibit iron absorption, the Fe/Ca ratio of hoopvine (0.009) is of particular interest. Although high calcium levels typically reduce heme and total iron absorption, non-heme iron absorption remains less affected (Roughead *et al.*, 2005). This raises two key hypotheses for future investigation: (a) the low Fe/Ca ratio in hoopvine may not significantly impair iron bioavailability, and; (b) if iron absorption remains efficient, hoopvine could serve as a functional food to help prevent iron deficiency and anemia. Further research on mineral bioavailability and its potential dietary applications is warranted to fully assess its nutritional value.

The GC–MS analysis of the methanolic extract of hoopvine revealed a diverse profile of secondary metabolites, indicating its potential as a source of bioactive compounds. Methanol has been widely recognized as an effective solvent for extracting and analyzing plant-derived organic compounds, as demonstrated in several previous studies (Nga *et al*., 2022; Trang *et al*., 2015). The production of secondary metabolites in plants can be influenced by various factors, including physiological conditions, environmental stimuli, geographic origin, and genetic variability, all of which contribute to chemical diversity (Figueiredo *et al*., 2008). Notably, several compounds identified in the methanolic extract, such as amides, amino acid derivatives, and volatile aldehydes like benzaldehyde, have been reported to exhibit antioxidant and antimicrobial activities (Aljaafari *et al*., 2022; Monteiro and Paiva-Martins, 2022). Their presence in hoopvine suggests that its biological effects may be closely associated with its secondary metabolite composition. In particular, ethylamine derivatives and hexadecanoic acid, which have been associated with reactive oxygen species modulation in previous studies, may contribute to the antioxidant activity (Kareem *et al*., 2021; Ganesan *et al*., 2024). While this study identified several putative bioactive compounds through GC–MS analysis, further quantitative validation using authentic standards is necessary. In addition, targeted investigation of key metabolites, especially ethylamine-related compounds, is needed to clarify their pharmacological significance. Future studies employing advanced analytical techniques such as high-performance liquid chromatography or tandem mass spectrometry may improve the precision of compound identification and quantification.

The significantly higher yields observed in aqueous extracts than in ethanolic extracts suggested that water was more effective than ethanol at extracting soluble compounds from hoopvine leaves. Water, as a polar solvent, is highly effective in extracting hydrophilic compounds such as polysaccharides, flavonoid glycosides, tannins, and certain phenolic acids, which are abundant in many plant materials. In contrast, ethanol, despite being a commonly used solvent for bioactive compound extraction, has lower polarity than water, which may limit its ability to dissolve certain water-soluble constituents. The ethanolic extract yield of hoopvine was comparable to that of *Piper sarmentosum*, a plant with similar extraction characteristics, but lower than yields reported for other species from related plant families such as *Petiveria alliacea* and *Rivina humilis* (Tachakittirungrod *et al*., 2007; Blainski *et al*., 2010; Ajaib *et al*., 2013). It was also lower than that of several commonly used medicinal plants, including *Mentha cordifolia*, *Cymbopogon citratus*, and *Cocos nucifera*. These differences in extraction yields can be attributed to variations in plant composition, such as fiber content, cell wall structure, and the specific distribution of bioactive compounds across plant species. Further research is needed to optimize the extraction conditions and identify the key bioactive components present in hoopvine leaf extracts.

In this study, antimicrobial activity of hoopvine was confirmed at high extract concentrations. Notably, the aqueous extract exhibited stronger antimicrobial effects than the ethanol extract, suggesting that water-soluble compounds may contribute more significantly to the observed activity. According to Ochoa Pacheco *et al.* (2013), *Petiveria alliacea* L. (anamu), a species in the same family as hoopvine, also exhibited antibacterial activity against S. aureus, although to a lesser extent. This difference may be attributed to the use of hydroalcoholic extracts in anamu versus aqueous and ethanolic extracts in the present study. These findings are consistent with those of previous studies on plant-derived antimicrobial compounds, in which polar solvents such as water facilitated extraction of bioactive substances, including polyphenols and flavonoids, which possess strong antibacterial properties (Manso *et al.*, 2021). In contrast, ethanol extraction may favor nonpolar compounds, which may have different antimicrobial mechanisms or lower solubilities in aqueous environments (Dhayalan *et al.*, 2018). The stronger antimicrobial effect observed in the aqueous extract may therefore be attributed to a higher abundance of hydrophilic bioactive molecules. Overall, hoopvine extracts, particularly those obtained through aqueous extraction, demonstrated promising antimicrobial potential. These findings suggest that water-based extraction may be an effective approach for isolating functional compounds from hoopvine and support its potential application in food preservation or natural antimicrobial formulations. Further studies are required to evaluate its efficacy across various microbial systems and to clarify the underlying mechanisms of its antimicrobial activity.

Although hoopvine extracts exhibited antimicrobial activity against *S. aureus* at stock concentrations (250 mg/mL for the aqueous extract and 50 mg/mL for the ethanolic extract), such high levels are unlikely to be physiologically relevant through dietary intake. Recent studies suggest that for plant-derived compounds to exert antimicrobial effects *in vivo*, they must demonstrate efficacy at concentrations that are more easily achievable within the body, considering factors like limited intestinal absorption and significant first-pass metabolism (Lippolis *et al*., 2023). Therefore, the antimicrobial activity observed in this study may not be achievable under normal dietary conditions. Further experiments are necessary to assess whether hoopvine-derived compounds can exert biological effects at concentrations that are relevant to physiological contexts, including in vivo studies to evaluate their bioavailability and effectiveness in living organisms. The IC_{50} value reflects the antioxidant potency of the extract, with lower values indicating stronger activity. In previous studies, *Stevia rebaudiana* and *Moringa oleifera* extracts have demonstrated strong antioxidant capacities, with *M. oleifera* showing particularly high activity across various solvent extracts (Shukla *et al*., 2009; El Sohaimy *et al*., 2015). Plants exhibit high antioxidant activity owing to the presence of bioactive compounds, such as polyphenols, flavonoids, anthocyanins, and vitamins C and E, which help protect the plant from oxidative stress and environmental damage (Muscolo *et al.*, 2024) and neutralize reactive oxygen species and free radicals. The extraction method and solvent used can influence antioxidant activity, as some solvents are more effective in extracting specific bioactive compounds (Sobuj *et al.*, 2021). For example, hot water extracts more water-soluble antioxidants, whereas ethanol is more efficient at extracting fat-soluble compounds (Cheng *et al.*, 2023). Different plants exhibit varying antioxidant potentials based on their composition, and the extraction process plays a critical role in maximizing their bioactivities (Zaky *et al.*, 2024). Further in-*vivo* antioxidant analyses are required to evaluate the effectiveness of hoopvine extracts under physiological conditions. The antioxidant activity observed in this study was likely due to the presence of various bioactive compounds, including phenolic compounds, anthocyanins, tocopherols, ascorbic acid, β-carotene, flavonoids, and other molecules in plant leaves. Total polyphenol and flavonoid contents were also evaluated, as these compounds significantly contribute to the antioxidant potential of plants, highlighting the importance of hoopvine as a potential source of natural antioxidants.

In terms of total polyphenol content, previous studies have reported varying levels across plant species and extraction methods. For instance, *Carica papaya* and *Moringa oleifera* have shown notable polyphenol contents, with some studies indicating higher concentrations in ethanol extracts compared to aqueous ones (Dudonne *et al*., 2009; Siddhuraju and Becker, 2003; El Sohaimy *et al*., 2015). The variation in extraction efficiency between ethanol and aqueous solvents highlighted the complexity of polyphenol solubility, which can be influenced by the polarity of the solvents, extraction conditions, and structural diversity of polyphenolic compounds. Although ethanol is generally considered more effective than water for extracting a broad spectrum of phenolic compounds owing to its intermediate polarity, certain highly polar polyphenols may be more effectively extracted with water (Radzali *et al.*, 2020). The contrasting findings between studies suggest that extraction efficiency is not only species-dependent but is also influenced by solvent composition and specific extraction methodologies. Further research should explore optimization of extraction parameters, including solvent ratio, temperature, and time, to maximize the polyphenol yield while preserving bioactivity. Future studies should investigate the bioavailability and functional implications of extracted polyphenols to improve understandings of their potential health benefits.

In the present study, the significantly higher flavonoid content in the aqueous extract compared to the ethanol extract suggests that the major flavonoid compounds in hoopvine leaves are predominantly hydrophilic. Flavonoids comprise a diverse group of polyphenolic compounds, with subclasses such as flavonols, flavones, flavanones, and anthocyanins exhibiting varying solubilities depending on their structural characteristics (Dias *et al.*, 2021). Higher flavonoid extraction efficiency in water may have been attributed to the presence of glycosylated flavonoids, which are more soluble in polar solvents (Plaskova and Mlcek, 2023). Conversely, ethanol tended to extract more aglycone flavonoids, which have lower water solubility. Flavonoids play a crucial role in antioxidant defense mechanisms owing to their ability to scavenge free radicals, chelate metal ions, and modulate antioxidant enzyme activities (Kumar and Pandey, 2013). The higher flavonoid content in aqueous extract likely contributed to the superior antioxidant activity observed in the present study. Several studies have demonstrated a strong correlation between the flavonoid concentration and antioxidant potential, with flavonoid-rich plant extracts exhibiting enhanced radical scavenging and reducing power (Jennings *et al.*, 2024, Lu *et al.*, 2024). In addition to their direct antioxidant properties, flavonoids can exert protective effects through the upregulation of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Simos *et al.*, 2012). These findings highlight the importance of optimizing extraction conditions to maximize flavonoid yield and bioactivity. The differences in extraction efficiency between the solvents further emphasize the need for targeted extraction strategies when isolating bioactive compounds for functional applications. Future research should focus on identifying the specific flavonoid compounds present in hoopvine leaves and evaluating their individual contributions to the antioxidant and other biofunctional properties. In addition, studies assessing the bioavailability and stability of these flavonoids in food and feed matrices may provide valuable insights into their potential applications in nutrition and health.

Conclusion: Hoopvine leaves possess significant nutritional and functional potential, with aqueous extraction showing enhanced antioxidant activity and greater retention of polyphenols and flavonoids compared to ethanolic extraction. These results highlight their applicability as a protein-rich dietary component and functional feed additive. Further studies are needed to assess bioavailability, safety, and scalability for practical use in food and feed systems.

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