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Research Article

PHYTOCHEMICAL PROFILES AND RADICAL-SCAVENGING ACTIVITY OF ETHNOBOTANICALLY PRIORITIZED MEDICINAL PLANTS FROM PARTIDO DISTRICT, PHILIPPINES

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ABSTRACT

This study quantified major phytochemical classes and radical-scavenging activity in ten medicinal plants prioritized from documented traditional healing practices in Partido District, Camarines Sur, Philippines. Ethanolic extracts were prepared from authenticated plant materials and evaluated in triplicate for total alkaloids, total phenolics, total flavonoids, saponins and 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity. Variance-robust comparisons and multivariate analysis were used to characterize differences among taxa. The extracts showed pronounced and statistically supported heterogeneity. *Artemisia vulgaris* had the highest total phenolic content and strongest radical-scavenging activity; *Zingiber zerumbet* had the highest total flavonoid content; *Caesalpinia sappan* had the highest alkaloid estimate; and *Rauvolfia serpentina* had the highest saponin content. Total phenolic content showed a moderate inverse but nonsignificant association with the concentration required for 50% radical inhibition, whereas the other phytochemical classes showed weak associations. Principal component analysis further demonstrated distinct phytochemical profiles rather than a uniform pattern of chemical richness. The findings provide quantitative follow-through to ethnobotanical prioritization and identify chemically distinctive extracts for chromatographic characterization, complementary antioxidant assays, safety testing and mechanism-relevant biological evaluation. The results support research prioritization but do not establish clinical efficacy or safety of the traditional remedies.

Keywords: Antioxidant capacity, Ethnobotany, Flavonoids, Phenolics, Secondary metabolites.

INTRODUCTION

Medicinal-plant research is most informative when traditional knowledge is used to generate testable priorities rather than treated as evidence of efficacy. Ethnopharmacological field methods can identify culturally salient taxa and improve the efficiency of downstream chemical and biological studies (Heinrich *et al*., 2009; Andrade-Cetto & Heinrich, 2011). Philippine investigations have similarly documented substantial medicinal-plant knowledge among geographically and culturally distinct communities (Abe & Ohtani, 2013; Balangcod & Balangcod, 2011; Antonio & Tuason, 2022; Naive *et al*., 2021; Ong & Kim, 2014). Partido District in eastern Camarines Sur retains an active tradition of folk healing. Cerio (2020) documented the cultural beliefs and healthcare practices of Partido albularyos, and Rebuya *et al*. (2020a) described the persistence of medical pluralism in the district. The wider cultural setting of Camarines Sur also includes strong place-based religious and traditional practices (Rebuya *et al*., 2020b). More recently, Cerio (2024) documented 153 medicinal plant species from 60 families used for 17 disease categories by Partido folk healers and recommended pharmacological and chemical evaluation of plants with higher ethnobotanical importance. The present study selected its ten-plant panel from that published ethnobotanical dataset. Quantitative phytochemical screening provides an intermediate step between ethnobotanical prioritization and compound-specific pharmacology. Plant secondary metabolites include chemically diverse alkaloids, phenolic compounds,

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flavonoids and saponins, whose abundance can vary with species, organ, developmental stage, environment and extraction conditions (Harborne, 1998; Balasundram *et al*., 2006; D'Archivio *et al*., 2007; Kennedy & Wightman, 2011; Panche *et al*., 2016). Saponins and alkaloids encompass broad structural classes with biological activities that extend beyond direct antioxidant behavior (Cowan, 1999; Francis *et al*., 2002; Güçlü-Üstündağ & Mazza, 2007; Sparg *et al*., 2004). Phenolics and flavonoids are frequently studied in antioxidant screening because hydroxylated structures can participate in electron- and hydrogen-transfer reactions (Rice-Evans *et al*., 1997). The 2,2-diphenyl-1-picrylhydrazyl assay is widely used as a rapid cell-free measure of radical-scavenging capacity (Brand-Williams *et al*., 1995; Molyneux, 2004; Prior *et al*., 2005). However, Folin-Ciocalteu values reflect overall reducing capacity rather than an exclusive inventory of phenolics, and different antioxidant assays may rank plant extracts differently (Everette *et al*., 2010; Thaipong *et al*., 2006). Accordingly, radical-scavenging results should be interpreted as comparative chemical activity under defined assay conditions rather than as direct evidence of in vivo therapeutic efficacy. The present study therefore aimed to quantify total alkaloid content, total phenolic content, total flavonoid content and saponin content in ten ethnobotanically prioritized medicinal plants; determine their 2,2-diphenyl-1-picrylhydrazyl radical-scavenging IC_{50} values; compare analytical profiles among taxa using variance-robust statistics; and explore whether phytochemical-class abundance was associated with radical-scavenging activity.

MATERIALS AND METHODS

Study design, period and plant selection

Candidate medicinal plants were selected from the ethnobotanical survey of Partido albularyos reported by Cerio (2024), which used informant consensus factor, use value and fidelity level to characterize traditional plant use. Samples were collected from sources within the fourth legislative district of Camarines Sur and submitted to the José Vera Santos Memorial Herbarium, University of the Philippines Diliman, for botanical identification and certification. Leaves were analyzed for nine taxa and the rhizome for *Zingiber zerumbet*.

Table 1. Ethnobotanically prioritized medicinal plants and plant parts analyzed.

Local name	Scientific name	Part analyzed	Ethnobotanical basis
Artamesa	*Artemisia vulgaris*	Leaf	Infectious/parasitic diseases
Esensya	*Rauvolfia serpentina*	Leaf	Neoplasms/metabolic diseases
Sibukaw	*Caesalpinia sappan*	Leaf	Blood/blood-forming organ diseases
Lakad Bulan	*Blumea balsamifera*	Leaf	Nervous-system diseases
Tawa-tawa	*Euphorbia hirta*	Leaf	Visual-system diseases
Tanglad	*Cymbopogon citratus*	Leaf	Circulatory-system diseases
Herba buena	*Mentha arvensis*	Leaf	Respiratory-system diseases
Bayawas	*Psidium guajava*	Leaf	Digestive-system diseases
Kalawag	*Zingiber zerumbet*	Rhizome	Skin diseases
Lino	*Morinda* sp.	Leaf	Musculoskeletal/connective-tissue disorders

Preparation of ethanolic extracts

Plant materials were cleaned, dried and ground to a fine powder. Approximately 100 g of powdered material was soaked in 200 mL ethanol and filtered through Whatman No. 1 filter paper. Filtrates were concentrated using a rotary evaporator at a 40 °C water-bath temperature and 120 rpm under reduced pressure until solvent removal and constant extract mass. Concentrated extracts were transferred to airtight containers and refrigerated until analysis. The extraction workflow was based on standard phytochemical procedures (Harborne, 1998).

Total alkaloid content

A 5-g portion of sample was mixed with 200 mL of 20% acetic acid in ethanol and allowed to stand for 4 h. The mixture was filtered, and the extract was concentrated to approximately one-quarter of the original volume. Concentrated ammonium solution was added dropwise until precipitation was complete. The precipitate was collected, dried and weighed following the gravimetric procedure of Obadoni and Ochuko (2002). Each determination was performed in triplicate and expressed as mg alkaloid fraction per g sample.

Total phenolic content

Total phenolic content was determined using a modified Folin-Ciocalteu method (Singleton *et al*., 1999; Aiyegoro & Okoh, 2010). One milliliter of extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2.0 mL of 2% sodium carbonate. After 15 min at room temperature, absorbance was measured at 765 nm. Gallic acid was used

for calibration. Triplicate results were expressed as mg gallic acid equivalents per g extract. The assay was interpreted as an operational phenolic-equivalent measure because nonphenolic reductants can also react with the reagent (Everette *et al*., 2010).

Total flavonoid content

Total flavonoid content was quantified by aluminum-chloride colorimetry (Chang *et al*., 2002; Aiyegoro & Okoh, 2010). One milliliter of extract was mixed with 3.0 mL methanol, 0.2 mL 10% aluminum chloride, 0.2 mL 1 M potassium acetate and 5.6 mL distilled water. After 30 min at room temperature, absorbance was measured at 420 nm. Quercetin served as the calibration standard. Triplicate values were expressed as mg quercetin equivalents per g extract. Structural differences among flavonoids can influence colorimetric response (Miean & Mohamed, 2001).

Saponin content

Five grams of sample were dispersed in 100 mL of 20% ethanol and heated with continuous stirring for 4 h at approximately 55 °C. The residue was re-extracted with another 100 mL of 20% ethanol. The combined extracts were reduced to approximately 40 mL, washed with diethyl ether, partitioned with n-butanol and washed twice with 5% aqueous sodium chloride. The remaining solvent was removed, and the residue was dried to constant mass. Triplicate saponin results were expressed as percentage by mass using the gravimetric procedure of Obadoni and Ochuko (2002).

Radical-scavenging assay

Radical-scavenging activity was assessed using the stable 2,2-diphenyl-1-picrylhydrazyl radical following Braca *et al*. (2001) and the general framework of Brand-Williams *et al*. (1995). Plant extract (0.1 mL) was added to 3.0 mL of 0.004% 2,2-diphenyl-1-picrylhydrazyl in methanol. Absorbance was measured at 517 nm after 30 min. Each concentration was analyzed in triplicate. Percentage inhibition was calculated using Equation 1, and concentration-response curves were used to estimate the IC_{50}, defined as the concentration producing 50% inhibition.

$$\text{Radical scavenging (\%)} = [(A_0 - A_1) / A_0] \times 100 \quad (1)$$

where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of extract.

Statistical analysis

All quantitative determinations were performed in triplicate and are reported as mean ± standard deviation. Because dispersions differed among taxa, alkaloid, total phenolic, total flavonoid, saponin and IC_{50} outcomes were compared using Welch one-way analysis of variance followed by Games-Howell pairwise comparisons at $\alpha = 0.05$. The triplicates represent repeated analytical determinations of the tested extracts; therefore, the inferential tests are interpreted as analytical separation under the study conditions rather than estimates of natural population-level variability. Exploratory Pearson correlations related each phytochemical measure to mean IC_{50} across the ten taxa. Ninety-five percent confidence intervals were calculated using Fisher z transformation, and correlation P values were adjusted by the Holm procedure. Principal component analysis was applied to standardized species means for the four phytochemical variables to visualize multivariate profile differences.

RESULTS AND DISCUSSION

The ten extracts showed marked quantitative heterogeneity (Table 2). Welch analysis of variance detected analytical differences for alkaloids ($F = 904.52$), total phenolics ($F = 6348.48$), total flavonoids ($F = 1157.01$), saponins ($F = 828.61$) and IC_{50} ($F = 237.28$); all P values were < 0.001. Games-Howell comparisons resolved overlapping and distinct groups while accommodating unequal variances.

Table 2. Quantitative phytochemical contents and radical-scavenging activity of the selected medicinal plants.

Plant	Part	Alkaloids (mg/g)	TPC (mg GAE/g)	TFC (mg QE/g)	Saponins (%)	IC50 (µg/mL)
Artemisia vulgaris	Leaf	0.323 ± 0.012^{cd}	190.324 ± 1.355^{a}	68.325 ± 0.988^{b}	0.679 ± 0.038^{f}	22.695 ± 0.578^{e}
Rauvolfia serpentina	Leaf	0.353 ± 0.008^{c}	26.126 ± 0.012^{h}	5.510 ± 1.164^{h}	3.363 ± 0.034^{a}	32.150 ± 1.225^{d}
Caesalpinia sappan	Leaf	1.381 ± 0.017^{a}	121.828 ± 1.286^{b}	17.589 ± 1.131^{g}	2.355 ± 0.103^{bc}	29.901 ± 1.302^{d}
Blumea balsamifera	Leaf	0.706 ± 0.052^{b}	74.247 ± 0.554^{d}	21.543 ± 0.405^{g}	1.973 ± 0.092^{cd}	25.394 ± 2.342^{de}
Euphorbia hirta	Leaf	1.284 ± 0.097^{a}	85.511 ± 1.084^{c}	32.076 ± 0.809^{f}	2.672 ± 0.015^{b}	42.770 ± 0.320^{ab}
Cymbopogon citratus	Leaf	0.267 ± 0.030^{cde}	45.382 ± 1.875^{fg}	46.743 ± 1.100^{e}	1.523 ± 0.081^{e}	38.034 ± 0.756^{c}
Mentha	Leaf	0.273 ± 0.007^{e}	$56.543 \pm$	$63.386 \pm$	0.804 ± 0.081^{f}	$34.067 \pm$

arvensis			2.366^{e}	1.104^{c}		0.572^{d}
Psidium guajava	Leaf	0.684 ± 0.012^{b}	53.326 ± 0.987^{ef}	54.211 ± 1.222^{d}	0.933 ± 0.113^{f}	41.198 ± 1.156^{bc}
Zingiber zerumbet	Rhizome	0.381 ± 0.014^{c}	90.207 ± 2.101^{c}	89.922 ± 1.441^{a}	0.977 ± 0.092^{f}	44.196 ± 0.871^{ab}
Morinda sp.	Leaf	0.278 ± 0.010^{de}	41.469 ± 1.071^{g}	44.998 ± 0.767^{e}	1.835 ± 0.103^{de}	49.673 ± 1.414^{a}

Note. Values are mean ± SD of three analytical determinations. Within each column, means sharing at least one superscript letter were not significantly different by Games-Howell comparison at $P < 0.05$. Lower IC_{50} indicates stronger radical-scavenging activity. TPC, total phenolic content; TFC, total flavonoid content; GAE, gallic acid equivalents; QE, quercetin equivalents.

Caesalpinia sappan had the highest alkaloid content (1.381 ± 0.017 mg/g) and overlapped statistically with *Euphorbia hirta* (1.284 ± 0.097 mg/g). *Artemisia vulgaris* had the highest total phenolic content (190.324 ± 1.355 mg GAE/g), followed by *Caesalpinia sappan* (121.828 ± 1.286 mg GAE/g). *Zingiber zerumbet* had the highest total flavonoid content (89.922 ± 1.441 mg QE/g), followed by *Artemisia vulgaris* and *Mentha arvensis*. *Rauvolfia serpentina* had the highest saponin content (3.363 ± 0.034%), followed by *Euphorbia hirta*. Radical-scavenging IC_{50} values ranged from 22.695 ± 0.578 to 49.673 ± 1.414 µg/mL. *Artemisia vulgaris* showed the strongest activity, followed by *Blumea balsamifera* and *Caesalpinia sappan*. *Morinda* sp. showed the highest IC_{50} and therefore the weakest activity in the tested panel. The mean total phenolic content showed the strongest association with IC_{50} ($r = -0.538$, 95% CI -0.872 to 0.138), but the relationship was not statistically significant ($P = 0.109$; Holm-adjusted $P = 0.434$). Correlations for alkaloids, total flavonoids and saponins were weak and nonsignificant (Table 3).

Table 3. Exploratory Pearson correlations between phytochemical measures and radical-scavenging IC_{50} across species means.

Predictor	Pearson r	95% CI	P value	Holm-adjusted P
Alkaloids	-0.074	-0.672 to 0.583	0.839	1.000
Total phenolics	-0.538	-0.872 to 0.138	0.109	0.434
Total flavonoids	0.275	-0.429 to 0.771	0.442	1.000
Saponins	0.003	-0.628 to 0.631	0.994	1.000

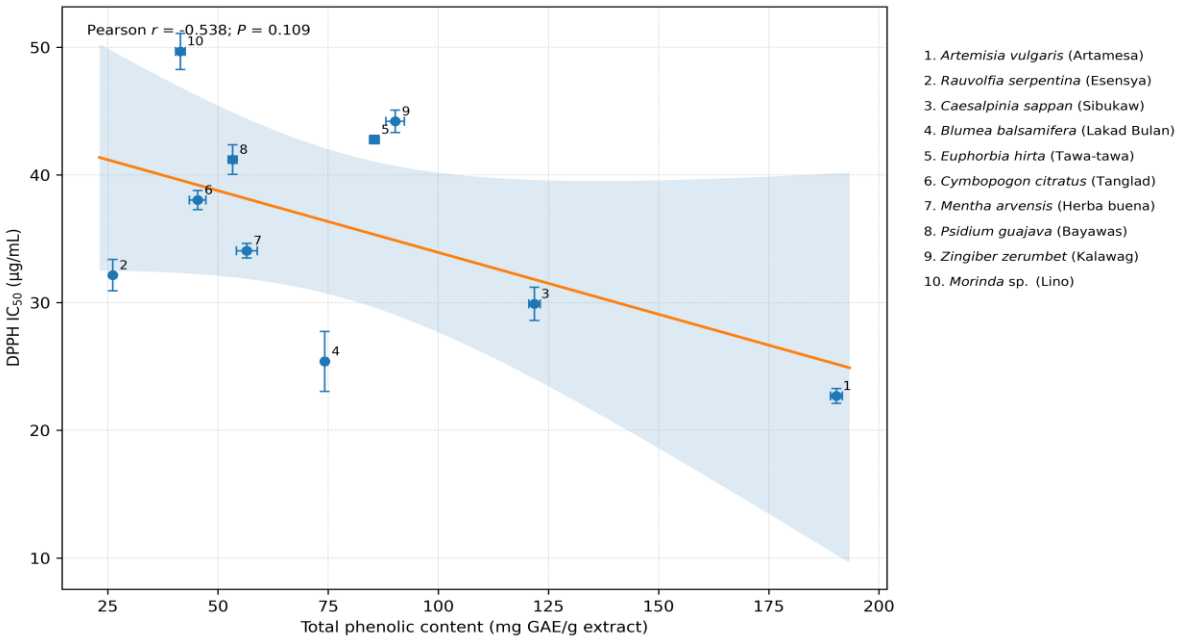


Figure 1. Relationship between total phenolic content and radical-scavenging IC_{50} across the ten species. Error bars show standard deviations of triplicate analytical determinations. The line represents a least-squares fit and the shaded region is the 95% confidence band. Numbered points correspond to the species key shown in the figure.

The principal component analysis of the four quantitative phytochemical variables explained 88.1% of total standardized variance on the first two components (Figure 2). Principal component 1 explained 57.0% and separated flavonoid-rich/low-saponin profiles from alkaloid- and saponin-rich profiles, while principal component 2 explained 31.1% and further separated the phenolic- and alkaloid-associated patterns. The ordination reinforced that the ten taxa did not share a uniform phytochemical signature.

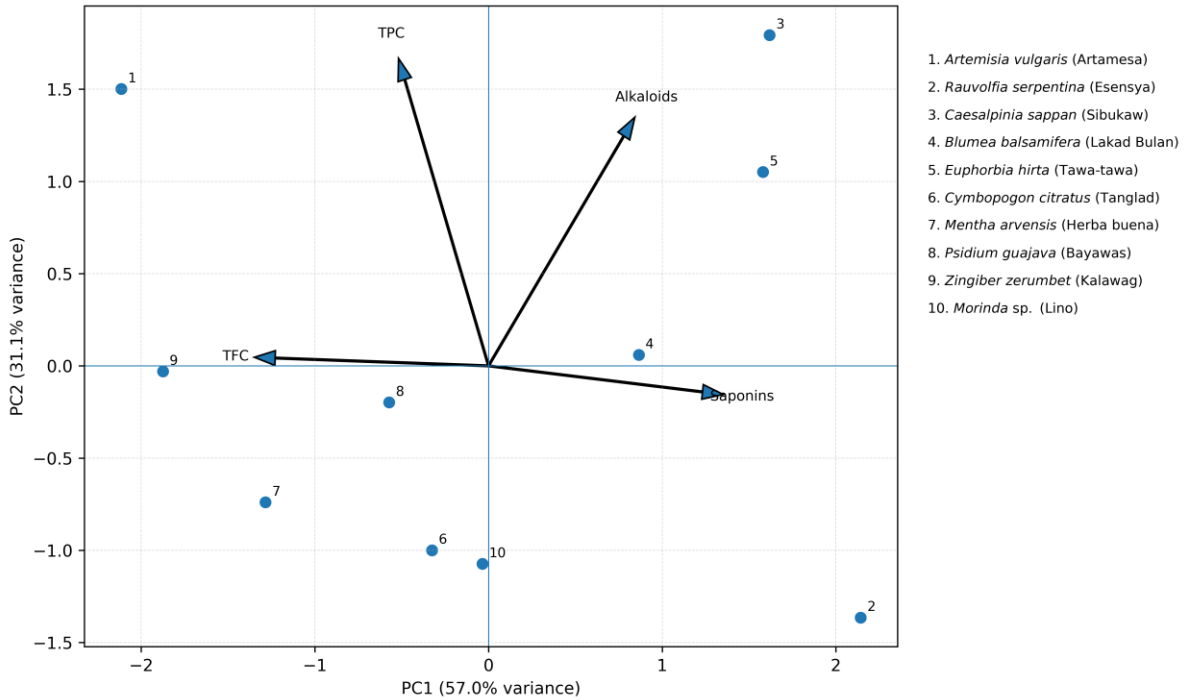


Figure 2. Principal component biplot of the four quantitative phytochemical measures. Variables were standardized before analysis. The first two components explained 88.1% of the total variance. Numbered points correspond to the species key shown in the figure.

The study translates ethnobotanical prioritization into a quantitative chemical comparison of medicinal plants used in Partido District. The primary finding is not that one plant was uniformly superior, but that the taxa separated into distinct phytochemical phenotypes. *Artemisia vulgaris* was strongly phenolic and had the lowest IC_{50}; *Zingiber zerumbet* was flavonoid-rich; *Caesalpinia sappan* and *Euphorbia hirta* were alkaloid-rich; and *Rauvolfia serpentina* was distinguished by its saponin fraction. Such divergence is expected because secondary metabolism is species-, organ-, environment- and extraction-dependent (Balasundram *et al*., 2006; D'Archivio *et al*., 2007; Kennedy & Wightman, 2011). The combination of high total phenolic content and strong radical scavenging in *Artemisia vulgaris* is chemically plausible. Artemisia species contain phenolic acids, flavonoids, coumarins and volatile constituents, and comparative work has demonstrated substantial interspecific variation in phenolic composition and antioxidant capacity (Abad *et al*., 2012; Carvalho *et al*., 2011). Nevertheless, the Folin-Ciocalteu assay is not chemically specific to phenolics, and a low IC_{50} in a cell-free radical system should not be equated with clinical antioxidant efficacy (Everette *et al*., 2010; Prior *et al*., 2005). *Caesalpinia sappan* was also a high-priority extract because it combined the highest alkaloid estimate, the second-highest total phenolic content and strong radical-scavenging activity. Brazilin-rich *Caesalpinia sappan* preparations have documented antioxidant, antibacterial and anti-inflammatory properties (Nirmal & Panichayupakaranant, 2015). The present study analyzed leaves rather than the more commonly investigated heartwood, so direct numerical comparisons are inappropriate; the leaf results instead justify chromatographic investigation of the local material.

Blumea balsamifera ranked second for radical-scavenging potency despite intermediate total phenolic and relatively low total flavonoid values. Studies of *Blumea balsamifera* leaves demonstrate solvent- and maturity-dependent differences in phenolics, flavonoids and antioxidant activity, while high-resolution mass spectrometry has identified diverse constituents in the species (Jirakitticharoen *et al*., 2022; Dai *et al*., 2023). This supports the interpretation that antioxidant behavior depends on compound identity and relative abundance rather than on a single total-class measurement. *Euphorbia hirta* showed high alkaloid and saponin estimates and relatively high total phenolics, yet its radical-scavenging

potency was weaker than several extracts with lower total phenolic content. Independent studies confirm that *Euphorbia hirta* contains phenolics and flavonoids and can exhibit antioxidant activity, but magnitude varies with solvent and analytical method (Kumar *et al*., 2010; Basma *et al*., 2011; Mahomoodally *et al*., 2020). Such variation cautions against treating IC_{50} values produced under different extraction and assay conditions as directly interchangeable. *Zingiber zerumbet* rhizome had the highest total flavonoid content but only moderate radical-scavenging activity within the panel. Growth stage strongly influences phenolic content, flavonoid content and antioxidant activity in *Zingiber zerumbet* (Ghasemzadeh *et al*., 2016), illustrating why a high bulk flavonoid-equivalent value does not necessarily predict the lowest IC_{50}. This pattern supports compound-specific profiling rather than reliance on total flavonoid content as a surrogate for antioxidant potency.

The profiles of *Mentha arvensis*, *Psidium guajava* and *Cymbopogon citratus* further illustrate the influence of chemical composition and method. Antioxidant-active polyphenols have been reported from herbaceous extracts including Mentha species (Lin *et al*., 2013; Tupe *et al*., 2015). *Psidium guajava* leaves contain diverse phenolics and flavonoids, and their measured antioxidant capacity varies with cultivar, extraction solvent and assay system (Gutiérrez *et al*., 2008; Camarena-Tello *et al*., 2018; Hartati *et al*., 2020; Mazumder *et al*., 2023). *Cymbopogon citratus* contains radical-scavenging phenolics such as orientin and related compounds, with antioxidant performance differing among extract systems (Cheel *et al*., 2005; Pereira *et al*., 2009). *Rauvolfia serpentina* requires a separate interpretation because its pharmacological identity is historically associated with indole alkaloids, including reserpine, whereas the present bulk alkaloid estimate was modest and its saponin value was highest. A gravimetric total-alkaloid fraction is not equivalent to targeted reserpine quantification; targeted chromatography would be required before compound-specific conclusions are made (Lobay, 2015). The moderate inverse total phenolic content-IC_{50} relationship did not reach statistical significance, and the other phytochemical classes showed weak associations. Broader medicinal-plant surveys often report phenolic-antioxidant relationships (Cai *et al*., 2004), but antioxidant assays do not necessarily rank samples consistently because they probe different reaction mechanisms (Thaipong *et al*., 2006). Free-radical chemistry is also only one dimension of biological antioxidant effects (Lobo *et al*., 2010). Accordingly, the present screening should be used to prioritize extracts for orthogonal antioxidant assays, chromatographic characterization, toxicity evaluation and mechanism-relevant bioassays rather than to claim therapeutic validation. Natural products remain important sources of pharmacologically useful chemical diversity, but progression from ethnobotanical use to drug development requires staged chemical and biological validation (Newman & Cragg, 2016). The study has several limitations. The colorimetric and gravimetric assays are class-level operational measurements and do not identify individual metabolites. Analytical triplicates improve precision but do not represent independent biological populations; future work should incorporate separately collected biological replicates across locations or seasons. Only one principal extraction workflow and one antioxidant assay were applied. Morinda was identified only to genus in the available record. These constraints define the study as comparative quantitative screening and prioritization rather than definitive pharmacological validation.

CONCLUSION

The ten ethnobotanically prioritized medicinal plants from Partido District displayed distinct quantitative phytochemical profiles and radical-scavenging activities. *Artemisia vulgaris* combined the highest total phenolic content with the strongest radical scavenging, *Caesalpinia sappan* showed the highest alkaloid estimate and high phenolic content, *Zingiber zerumbet* had the highest total flavonoid content, and *Rauvolfia serpentina* had the highest saponin content. The strongest phytochemical-radical scavenging relationship was moderate but nonsignificant, confirming that bulk class abundance alone did not predict antioxidant behavior across the panel. These results identify chemically distinctive extracts for compound-resolved profiling and complementary biological testing and should be interpreted as research prioritization rather than clinical validation.

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CONFLICT OF INTERESTS

The author declares no financial or personal conflict of interest related to this work.

ETHICS APPROVAL

This laboratory study did not involve human participants or experimental animals. Ethnobotanical information was used only to prioritize the plant panel and was obtained from a previously published study.

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AI TOOL DECLARATION

The author declares that no AI and related tools were used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request.

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