Research Article

Preliminary Phytochemical Screening of Selected Medicinal Plants Traditionally Used as Anthelmintics by Native Chicken Farmers in Agusan Del Norte, Philippines

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Abstract | The persistent threat of gastrointestinal helminthiasis in native chickens raised under free-range and semi-scavenging systems has led many smallholder poultry farmers to rely on traditional plant-based remedies, despite limited scientific validation. This study aimed to identify and phytochemically screen eight ethnoveterinary plants commonly used by native chicken raisers in Agusan del Norte, Philippines including *Basella alba*, *Carica papaya*, *Allium sativum*, *Tinospora rumphii*, *Azadirachta indica*, *Plectranthus amboinicus*, *Piper betel*, and *Areca catechu*. The traditionally used plant parts, such as leaves, stems, bulbs, and fruits, were collected and taxonomically verified. Aqueous extracts of these ethnomedicinal components were prepared through maceration to replicate local decoction practices, followed by qualitative phytochemical screening. The results revealed that most plants possessed a diverse range of bioactive compounds. Flavonoids were strongly detected (+++) in *B. alba, C. papaya, A. sativum, T. rumphii, P. betel,* and *A. catechu*, while tannins were abundant (+++) in *T. rumphii, A. indica, P. betel,* and *A. catechu*. Moreover, alkaloids showed strong (+++) presence in *C. papaya* and *T. rumphii*, and moderate (++) levels in *A. sativum, P. betel,* and *A. catechu*. Saponins were strongly present (+++) in *B. alba, P. betel,* and *A. catechu*. Steroids were also consistently detected at strong (+++) levels in all species except *P. amboinicus*, which had moderate (++) presence. On the other hand, anthraquinones and cyanogenic glycosides were absent (–) across all samples. Among the tested species, *T. rumphii*, *P. betel, A. indica,* and *A. catechu* emerged as the top selections in terms of phytochemical abundance, offering an essential scientific justification for their persistent role in ethnoveterinary medicine. Further studies are recommended to isolate active constituents and assess their biological efficacy and safety through *in vitro* and *in vivo* trials.

Keywords | Anthelmintics, Bioactive compounds, Ethnoveterinary medicine, Native chicken, Phytochemical screening, Plant secondary metabolites

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INTRODUCTION

Gastrointestinal helminthiasis remains a notable health issue among free-range and backyard poultry production worldwide (Shifaw *et al.*, 2021). This challenge is particularly pronounced across Asia, where the warm and humid tropical climate favors helminth development and survival (Dube *et al.*, 2010; Shifaw *et al.*, 2021). This has been well-documented in several countries including Bangladesh, Thailand and the Philippines, where high prevalence of helminth infections in small-scale and free-range chickens has been reported (Ybañez *et al.*, 2018; Wuthijaree *et al.*, 2019; Abdullah *et al.*, 2021; Wuthijaree *et al.*, 2024). Consequently, this ecological advantage boosts the dynamics of parasite populations as parasite eggs may build up and potentially develop infectivity in the soil, litter, pasture, or *via* animal vectors (Thapa *et al.*, 2015; Wongrak *et al.*, 2015). In such systems, native chickens in semi-scavenging systems are highly susceptible to soil-transmitted helminths such as *Ascaridia spp.* and *Heterakis spp.* (Mubarokah *et al.*, 2019; Zalizar *et al.*, 2021; Ritu *et al.*, 2024). These parasites can lead to stunted growth, poor nutrient absorption, inefficient feed conversion, reduced egg production, and in severe cases, result in mortality (Zalizar *et al.*, 2021; Zirintunda *et al.*, 2022; Hazarika *et al.*, 2023). While synthetic anthelmintics are routinely used, their frequent use has raised concerns regarding anthelmintic resistance and accumulation of drug residues (Kabir *et al.*, 2004; Kaplan, 2004; Goetting *et al.*, 2011; Escosteguy, 2014; Sattar *et al.*, 2014; Mund *et al.*, 2016; Owusu-Doubreh *et al.*, 2023; Mesfin *et al.*, 2024). In rural settings, limited access to veterinary services and high costs of commercial dewormers further hinder effective parasite control (Permin and Hansen, 1998; Jamil *et al.*, 2022).

Over the past decades, there has been a growing scientific interest on investigating the anthelmintic potential of medicinal plants traditionally used in ethnoveterinary practices (Mali and Mehta, 2008; Raza *et al.*, 2015; Kuralkar and Kuralkar, 2021; Ahmad *et al.*, 2023). In various parts of the Philippines, native chicken growers commonly administer crude aqueous extracts of indigenous plants such as *Azadirachta indica* (Neem), *Areca catechu* (Betel Nut), *Tinospora rumphii* (Makabuhay), *Basella alba* (Alugbati) and *Piper betel* (Betel Leaf) to combat worm infections; however, despite their widespread use, farmers largely remain unaware of the specific bioactive compounds responsible for their therapeutic effects (Dela Vega and Aban-Gonzales, 2019; Nadela, 2020). Recent studies have begun to elucidate the pharmacological basis of these practices. For instance, *A. catechu* extract has demonstrated significant *in vitro* and *in vivo* activity against *A. galli*, with its anthelmintic properties potentially attributed to the presence of alkaloids and tannins and saponins which can cause morphological damage in parasites (Mubarokah *et al.*, 2019).

Moreover, *A. indica* which are found to contain tannins, alkaloids, and flavonoids, has demonstrated significant anthelmintic activity against *A. galli* in both *in vitro* and *in vivo* studies (Raza *et al.*, 2015). These metabolites exert their effects by damaging the parasite's cuticle, inhibition of neuromuscular and mitochondrial functions (Stephen *et al.*, 2022; Gasaliyu *et al.*, 2022; Tiuria *et al.*, 2024).

Similarly, *Plectranthus amboinicus* (Indian borage) has demonstrated significant *in vitro* anthelmintic activity against *Pheretima posthuma* and *A. galli* (Wadekar *et al.*, 2011). This therapeutic effect is primarily attributed to tannins, which bind to glycoproteins on the parasite cuticle and disrupt oxidative phosphorylation, leading to structural damage, energy depletion, and eventual paralysis and death (Wadekar *et al.*, 2011; Raza *et al.*, 2015; Stephen *et al.*, 2022; Gasaliyu *et al.*, 2022; Tiuria *et al.*, 2024). Given these pharmacological insights, the present study conducted a preliminary phytochemical screening of medicinal plants traditionally used by native chicken farmers in Agusan del Norte, Philippines. By identifying the presence of key secondary metabolites, this study provides foundational evidence to support the scientific validation and future development of these ethnoveterinary botanicals as accessible, safe, and sustainable alternatives to conventional anthelmintics.

MATERIALS AND METHODS

Geographical Sampling and Ethnoveterinary Survey

This study adopted a purposive geographical strategy to identify focal areas with the highest populations of native chicken raisers in Agusan del Norte (Figure 1). Preliminary data on chicken population densities were obtained from the Provincial Veterinary Office, while the Municipal Agriculture Offices provided barangay-level inventory of farmers actively engaged in native chicken production. Barangays exhibiting the highest flock densities were selected as study sites. Structured, face-to-face interviews were conducted using primarily close-ended questionnaires, supplemented with open-ended items, and delivered in the local dialect to accurately capture comprehensive ethnoveterinary knowledge related to medicinal plant use for deworming. Inclusion criteria for respondents included active management of native chickens under a semi-scavenging system and personal experience in the use of plant-based anthelmintics. To ensure methodological rigor, the required sample size was calculated using Cochran's formula for large populations (adopted from Cochran, 1963; Singh and Masuku, 2014).

$$n_0 = \frac{Z^2 \times p \times q}{e^2}$$

Where, n_0 is the required sample size, Z is the z-score corresponding to the desired confidence level, p is the estimated proportion of an attribute present in the population, q = 1 – p, and e is the desired level of precision. The methodological approach adheres to standards established in ethnobotanical and ethnoveterinary studies (Giday and Ameni, 2003; Lans *et al.*, 2007; Bekalo *et al.*, 2009). A total sample size of 189 respondents was selected to ensure representative coverage of native chicken farmers across major production areas in Agusan del Norte.

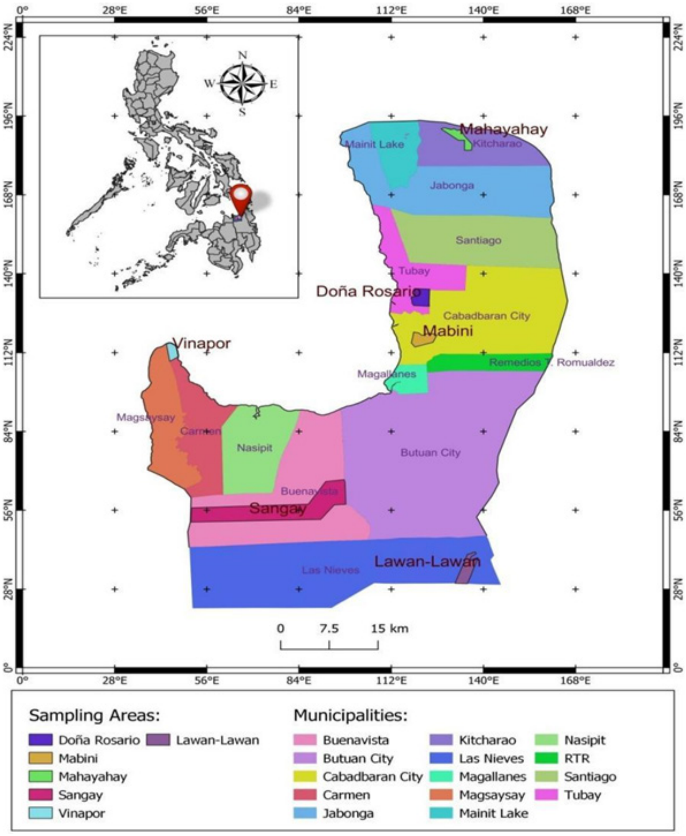

Figure 1: Map showing the geographic distribution of barangays with the highest concentrations of native chicken farmers in Agusan del Norte, Philippines.

Selection and Identification of Plant Materials

The selection of plant species for phytochemical screening was based on data obtained through ethnoveterinary interviews. These species were shortlisted based on the frequency of mention, consensus among informants, and prior corroboration with peer-reviewed ethnobotanical and ethnoveterinary literature. A total of eight plant species and their traditionally used parts were identified and collected from multiple locations in six municipalities: Cabadbaran City, Tubay, Kitcharao, Las Nieves, Buenavista, and Carmen. Collection per species ranged from 3-5 replicates per site with a total of 24-30 samples. These included the leaves and vines of *B. alba* (Alugbati), leaves of *C. papaya* (Papaya), bulbs of *A. sativum* (Garlic), stems of *T. rumphii* (Panyawan), leaves of *A. indica* (Neem), leaves of *P. amboinicus* (Indian Borage), leaves of *P. betel* (Betel Leaf), and the fruit (nut) of *A. catechu* (Betel Nut).

Voucher specimens of the identified plant species were prepared and subjected to taxonomic verification by a qualified botanist using standard morphological descriptors. The specific plant parts reportedly used were documented during the interviews and substantiated with photographic records to ensure scientific validation and reproducibility.

Sample Preparation

Fresh plant materials were thoroughly washed with clean water to remove soil and debris, then air-dried in a well-ventilated shaded area for 5 to 7 days to prevent degradation of heat-sensitive phytochemicals. Once fully dried, the samples were ground into a fine powder using a mechanical grinder and stored in airtight, labeled containers under dry conditions to avoid moisture-induced degradation.

Aqueous extraction was performed by soaking 10 g of each powdered plant material in 100 mL of distilled water (1:10 w/v) at room temperature (~25°C) for 24 hours with occasional stirring. This maceration procedure was designed to replicate traditional decoction practices commonly used by rural poultry farmers in the preparation of herbal anthelmintics. After extraction, the mixtures were filtered through Whatman No. 1 filter paper to obtain clear filtrates, which were subsequently used for phytochemical screening. The use of water as an extraction solvent was intended to mimic traditional ethnoveterinary practices, where water-based preparations are most preferred.

Phytochemical Screening Methods

A qualitative phytochemical screening was carried out to detect major classes of secondary metabolites present in the aqueous extracts of the selected medicinal plants. The screening focused on the identification of alkaloids, anthraquinones, flavonoids, cyanogenic glycosides, saponins, steroids, and tannins, based on their established veterinary ethnopharmacological relevance in animal health (Harborne, 1998; Trease and Evans, 2002). Each test was selected based on its diagnostic specificity and was performed under controlled laboratory conditions. Observable color changes or precipitate formations served as qualitative indicators of compound presence.

All assays were conducted in duplicate to ensure consistency and reliability of observations, following methodological standards adopted in veterinary phytochemical and ethnomedicinal research (Ayoola *et al.*, 2008; Doughari, 2012). Table 1 summarizes the phytochemical groups evaluated, the specific tests employed, and the diagnostic criteria used for interpretation.

Table 1: Preliminary phytochemical screening and diagnostic observations.

Phytochemical Group	Test Applied	Observation
Alkaloids	Culvenor–Fitzgerald	Formation of a precipitate or turbidity (orange-brown complex).
Anthraquinones	*Modified* Borntrager's	Pink to red coloration in ammoniacal layer.
Flavonoids	Bate-Smith and Metcalf	Red or violet coloration upon acid hydrolysis.
Cyanogenic glycosides	Guignard's picrate paper	Sodium picrate paper turns from yellow to brick-red.
Saponins	Froth (foam)	Persistent froth formation (>1 cm)
Steroids	Keller–Kiliani	Reddish-brown ring at interface with bluish-green upper layer.
Tannins	Ferric chloride	Blue-black or green coloration upon addition of $FeCl_3$.

RESULTS AND DISCUSSION

Phytochemical Profiles and Ethnopharmacological Implications

The phytochemical screening revealed distinct qualitative profiles across eight indigenous plant species. Seven key phytochemical groups were evaluated such as alkaloids, anthraquinones, flavonoids, cyanogenic glycosides, saponins, steroids (deoxysugars), and tannins. The qualitative presence was categorized based on standard diagnostic observations as not detected (–), trace/weak (+), moderate (++), or strong (+++), as illustrated in Figure 2.

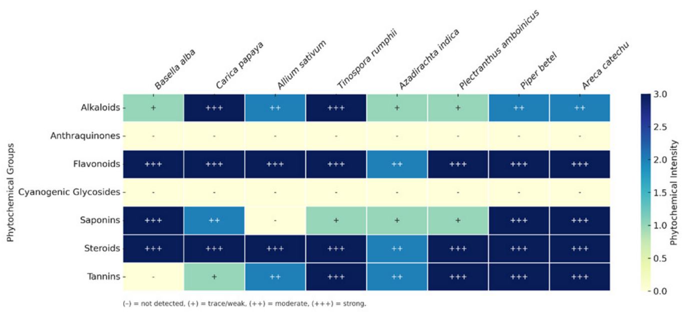

Figure 2: Qualitative intensity of seven phytochemical groups detected across eight medicinal plants traditionally used as anthelmintics.

All plant samples exhibited the presence of at least five phytochemical groups. Notably, *T. rumphii*, *P. betel*, *A. indica*, and *A. catechu* emerged as the most phytochemically rich species. Flavonoids were strongly present (+++) in *B. alba, C. papaya, A. sativum, T. rumphii, P. betel*, and *A. catechu*, while tannins were also abundantly detected (+++) in *T. rumphii, A. indica, P. betel*, and *A. catechu*. Alkaloids were most prominent in *C. papaya* and *T. rumphii*, and moderately present in *A. sativum, P. betel*, and *A. catechu*. Saponins were strongly detected in *B. alba, P. betel*, and *A. catechu*, while steroids were consistently present at strong (+++) levels in all plants except *P. amboinicus*, which exhibited moderate levels (++). The phytochemical profiles observed in this study largely corroborate earlier findings on the abundant presence of alkaloids, flavonoids, tannins, and saponins in *T. rumphii, P. betel, A. indica*, and *A. catechu* (Mubarokah *et al*., 2019; Jamil *et al*., 2022; Ahmad *et al*., 2023). However, anthraquinones and cyanogenic glycosides were undetectable (–) in all tested extracts. This is possibly due to the differences in solvent polarity, where aqueous maceration excludes less polar metabolites which are often better extracted with methanol or chloroform (Ayoola *et al*., 2008). Variability in chemotypes, soil conditions, light exposure, and seasonality may further account for differences in phytochemical expression (Tchetan *et al*., 2022).

Structural and Digestive Disruption by Tannins and Saponins

High levels of tannins in *T. rumphii, A. indica, P. betel*, and *A. catechu* indicate potential for disrupting nematode cuticle integrity. Tannins bind to glycoproteins and digestive enzymes on the parasite surface, impairing nutrient uptake and energy metabolism. Mubarokah *et al*. (2019) and Stephen *et al*. (2022) demonstrated significant cuticle damage and death in *Ascaridia galli* following exposure to tannin-rich extracts. Similarly, saponins, detected strongly in *B. alba, P. betel*, and *A. catechu*, possess amphipathic properties that cause cytoplasmic leakage and parasite membrane rupture. These surfactant-like actions can potentiate synergistic interactions with other bioactives and have been linked to significant worm burden reduction in chickens treated with *A. catechu* (Mubarokah *et al*., 2019).

Neuromuscular and Metabolic Inhibition by Flavonoids and Alkaloids

Flavonoids which are abundant in *C. papaya*, *T. rumphii*, *A. sativum*, and *P. betel* reportedly interfere with ATPase function, calcium ion pumps, and mitochondrial metabolism, ultimately impairing motility and reproduction (Ahmad *et al*., 2023). A recent study of Tiuria *et al*. (2024) reported that flavonoid-rich *P. betel* extracts caused complete *in vitro* mortality of *A. galli* through paralysis and structural collapse. Moreover, alkaloids such as arecoline and piperine mimic or block neurotransmitters, leading to helminth paralysis. These alkaloids, found in *T. rumphii*, *C. papaya*, and *A. sativum*, mimic the mode of action of synthetic agents like levamisole due to their neurotoxicity and anti-reproductive properties (Jamil *et al*., 2022; Ahmad *et al*., 2023; Zirintunda *et al*., 2025).

Reproductive Interference and Immunomodulation by Steroidal Glycosides

Steroidal glycosides were consistently detected in all tested species except *P. amboinicus*. These compounds have been reported to interfere with mitochondrial membrane potential, which disrupts energy-dependent processes vital for helminth development. As a result, larval viability and egg hatching are significantly impaired, reflecting their ovicidal and larvicidal potential (Ahmad *et al*., 2023). In a study of Tchetan *et al*. (2022), extracts from *Dioscorea spp.*, rich in steroidal glycosides, significantly suppressed egg production and larval development in *H. contortus*, which may be extrapolated to *A. galli* due to conserved metabolic pathways. Moreover, these compounds can enhance host immunity by promoting lymphocyte activity, boosting macrophage function, and increasing antibody production, which may improve resistance to reinfection (Kuralkar and Kuralkar, 2021).

CONCLUSIONS AND RECOMMENDATIONS

This present study validated the presence of key anthelmintic compounds in medicinal plants traditionally used for native chickens in Agusan del Norte. *T. rumphii*, *P. betel*, *A. indica*, and *A. catechu* exhibited the richest phytochemical profiles. These findings substantiate the ethnopharmacological relevance of these plants as a potential source of plant-based anthelmintics. Future studies should include quantitative phytochemical assays and controlled *in vitro* and *in vivo* studies to isolate active compounds, determine dose-dependent efficacy, and assess safety. Limitations of this study include its reliance on qualitative screening methods, potential seasonal variability in phytochemical expression, and the absence of biological efficacy testing.

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NOVELTY STATEMENTS

This study provides a region-specific phytochemical profile of eight ethnoveterinary plants used against gastrointestinal helminths in native chickens in Agusan del Norte, Philippines. Although their ethnoveterinary use has been previously reported in various contexts, limited data exist on their comparative phytochemical composition using water-based extractions that mimic conventional decoction techniques. By coupling farmer-derived knowledge with laboratory evidence, the study provides a data-driven shortlist of botanical leads for dose-response, mechanism-of-action, and safety trials aimed at sustainable parasite control in low-input poultry systems.

AUTHOR'S CONTRIBUTIONS

All authors contributed substantially to the study's conception and design, data collection, and analysis or interpretation of results. They were involved in drafting the manuscript or revising it critically for important intellectual content. All authors approved the final version for publication and agreed to be accountable for all aspects of the work.

Conflict of Interest

The authors have no conflict of interest to declare.

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