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Untapped potentials of *Ganoderma* species: a neglected fungal resource in Tanzania

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Abstract

Ganoderma species are common wood-decaying and medicinally important macrofungi utilized by Miombo woodland communities, yet the linkage between their ethnomycological values and nutraceutical profile remains poorly explored in Tanzania. This study integrated ethnomycological surveys and nutraceutical profiling to assess their medicinal and nutritional relationship. We documented eight species with 1828 individuals. *Ganoderma lucidum* was the most abundant and culturally important species, while *Ganoderma mbrekobenum* was highly localized. Ethnomycological surveys revealed that 95.7% recognized *Ganoderma* species being used for local medicinal purposes, particularly for treating pain, digestive disorders, respiratory infections, kidney complications, and inflammatory conditions, whereas 4.3% reported food-related utilization. The highest ethnomedicinal significance was recorded for *G. lucidum* and *Ganoderma applanatum*, both of which were associated with elevated content of K, Fe, Zn, vitamin C, flavonoids, crude fibre, and phenolic compounds, indicating notable antioxidant, immunomodulatory, and anti-inflammatory potential. Furthermore, *Ganoderma arbiforme* exhibited the highest Ca, Mg, and total phenolic content, whereas *Ganoderma australe* had the highest protein content. The observed interspecific variation in nutritional and antinutritional composition was strongly aligned with patterns of ethnomycological utilization. Principal component and cluster analyses further demonstrated strong associations between ethnomedicinal utility and phytochemical, proximate composition and antinutritional compounds, particularly among phenolic and flavonoid-rich species, suggesting potential trade-offs between ethnomedicinal utility, medicinal value and nutrient bioavailability. Integrating ethnomycological knowledge with nutritional and phytochemical profiles highlights their substantial nutraceutical and pharmacological potential, providing a scientific basis for therapeutic development and incorporation into local healthcare systems.

Key words: *Ganoderma* species, Miombo woodlands, mushrooms, medicinal value, antinutritional profile.
Abbreviations: FC, frequency of citation; FR, forest reserve; QE, quercetin equivalent; RFC, relative frequency of citation; TTC, tannin content.

Introduction

Miombo woodlands form the largest dry forest ecosystem in sub-Saharan Africa, extending over approximately 2.7 million km^2 across southern and central Africa, including large areas of Tanzania (Mlambo, Maphosa 2022). These woodlands are dominated primarily by leguminous tree species belonging to the genera *Brachystegia, Julbernardia*, and *Isoberlinia* (Chelela et al. 2014; Moura et al. 2017). Beyond their botanical importance, Miombo ecosystems support a rich diversity of macrofungi that contribute significantly to ecosystem functioning through nutrient cycling, decomposition of woody material, and maintenance of forest productivity (Sileshi et al. 2023; Schulze et al. 2025; Pampolina et al. 2026). Among these fungi, species of the genus *Ganoderma* are particularly abundant and commonly occur on dead or decaying trunks of Miombo tree species due to their strong lignin-degrading capabilities (Schulze et al. 2025; Pampolina et al. 2026). Miombo woodlands also provide diverse edible and medicinal mushrooms that are widely utilized by surrounding communities as food resources, traditional remedies, and they are sources of household income (Kamalebo, De Kesel 2020; Vicente-Pérez et al. 2024; Kibona et al. 2025; Malunguja et al. 2025).

Mushrooms are recognized for their high nutritional value because they contain essential minerals, vitamins, antioxidants, and other bioactive compounds important for human health (Tibuhwa 2013; Orywal et al. 2022; Kibona et al. 2025; Malunguja et al. 2025). Their nutritional and antinutritional properties have been associated with improved dietary diversity, food security, and medicinal benefits, particularly in regions where access to conventional food resources is limited (Mwamatope et al. 2023; Sharma et al. 2024; Tesfay et al. 2024; Ade-ogunnowo et al. 2024). Consequently, interest in mushroom utilization and cultivation has increased in several African countries, including Tanzania, Rwanda, Kenya, Nigeria, South Africa, and Lesotho (Tibuhwa 2013; Nteziryayo et al. 2024). This growing demand is largely linked to their recognized antioxidant and therapeutic potential (Lyamuya et al. 2023;

Mwamatope et al 2023). Within this broader fungal diversity, *Ganoderma* species (Basidiomycota, Polyporaceae) have gained considerable scientific and commercial attention because of their ecological importance and medicinal value. These fungi are predominantly saprophytic and are easily recognized by their glossy reddish-brown basidiocarps growing on decaying wood. Globally, more than 250 taxonomic names of *Ganoderma* have been reported, including *Ganoderma lucidum, Ganoderma applanatum, Ganoderma australe, Ganoderma resinaceum, Ganoderma tsugae*, and *Ganoderma sinense* among others (Josiah et al. 2019; Moncalvo et al. 1995).

Previous studies have demonstrated that *Ganoderma* species contain diverse bioactive compounds such as triterpenoids, polysaccharides, and peptidoglycans that contribute to their pharmacological properties (Asad et al. 2025). These compounds exhibit antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, antidiabetic, hepatoprotective, cytotoxic, and anticancer activities, thereby enhancing their value in nutraceutical and pharmaceutical applications (Chelela et al. 2014; Ngurthankhumi et al. 2024; Taskozhina et al. 2024; Asad et al. 2025; da Silva et al. 2025; Kibona et al. 2025; Razzaque, Wimalawansa 2025). Their medicinal use has a long history in traditional healthcare systems, where they have been associated with health promotion and longevity, and they are currently marketed worldwide as medicinal supplements and functional foods (Orywal et al. 2022; Konara et al. 2025).

In several countries, *Ganoderma* has already been developed into high-value medicinal and biotechnological products contributing substantially to the nutraceutical industry (Atri et al. 2019; Mlambo, Maphosa 2022; Mafe et al. 2025). Despite their abundance within Tanzanian Miombo woodlands and their recognized traditional importance, studies on the diversity, utilization, and medicinal potential of *Ganoderma* species in Tanzania remain limited and fragmented (Brady 2024). As a result, these fungi continue to be underutilized and are often regarded mainly as ecological components rather than valuable bioresources. This reflects the broader challenge across Africa, where many wild mushroom species remain neglected despite their ecological significance (Sileshi et al. 2023). Consequently, limited scientific attention to Miombo-associated *Ganoderma* species represents a missed opportunity for biodiversity utilization, bioprospecting, and socio-economic development in Tanzania. Therefore, an integrative field and laboratory-based investigation was conducted to generate scientific evidence suggesting the potential incorporation of *Ganoderma* species into nutraceutical applications. The study was guided by the hypotheses that: (i) the distribution patterns of *Ganoderma* species differ significantly among the studied forest reserves resulting from variations in habitat structure and ecological conditions; and (ii) interspecific variation in nutritional and phytochemical composition is associated with the ethnomedicinal importance and traditional therapeutic utilization within local healthcare systems. By integrating ecological, biochemical, and ethnomycological approaches, the study aimed to establish a baseline dataset on the nutraceutical, pharmacological, and therapeutic potential of *Ganoderma* species. The findings are expected to contribute to evidence-based promotion of their pharmaceutical and functional food applications, while also providing scientific guidance for policymakers and stakeholders on the sustainable utilization and integration of these valuable fungal bioresources into traditional and complementary healthcare systems.

Materials and methods

Description of the study area

The study was carried out in four forest reserves (FRs) within the Miombo woodlands of the Mbeya region in northern Tanzania. These FRs include Ituli FR, Mdabulo FR, Lila FR, and Lualage FR, which are located at 8°18’54.00”S and 33°29’09.60”E. Notably, the selected FRs extend across administrative boundaries, linking Mbeya region with Singida and Tabora regions to the north, Iringa to the east, Songwe to the south, and Rukwa to the west. This broad spatial coverage characterizes the study area as representing both Central and Southern Miombo woodlands of Tanzania. The FRs are defined by a diverse topography that includes hilly terrain associated with the Mbeya Hills and the Chunya mountain range, gently sloping landscapes largely covered by dense Miombo woodland, flat lowland areas along the Lake Rukwa basin, and a plateau situated between the Ibagu plains and Lake Rukwa. The hydrological network is dominated by permanent rivers Songwe, Lupa, and Zira, all of which originate from the Mbeya Hills. Vegetation within the study sites is primarily composed of tree species belonging to the genera *Brachystegia, Julbernardia*, and *Isoberlinia*, which are known to form ectomycorrhizal associations with a wide range of mushroom-producing fungi.

Within these FRs, *Ganoderma* species represent a prominent group of wood-decaying macrofungi, mainly colonizing dead and decaying tree trunks throughout the Miombo woodlands. Beyond their ecological importance, particularly in nutrient cycling, these fungi are valued by nearby communities for their medicinal uses. Site selection was informed by preliminary reconnaissance surveys that revealed extensive local utilization of wild mushrooms, especially species of the genus *Ganoderma*, for therapeutic purposes. The reported abundance of *Ganoderma* species within these reserves further justified their inclusion in the study. The forest reserves are inhabited by four major ethnic groups namely, the Kimbu, Kamba, Sukuma, and Safwa, who possess extensive botanical and ecological knowledge of wild edible resources. These communities have a long-standing tradition of harvesting and using wild plants for food, fibre, fodder, and medicinal purposes, providing a culturally rich context for assessing the nutraceutical potential of *Ganoderma* species.

Respondent demographics

The socio-demographic characteristics of the informants are presented in Table S1. A total of 140 participants were involved in the study, comprising 60 men (42.86%) and 80 women (57.14%), with ages ranging between 18 and 70 years. Informants were selected through purposive sampling from each village, targeting individuals with demonstrated knowledge of mushrooms and relevant experience. Participants were drawn from communities residing in four villages adjacent to the studied forest reserves and included village elders and local leaders. Data collected encompassed age, gender, and knowledge related to the use of *Ganoderma* species. All information was obtained following oral informed consent, after clearly explaining the study objectives and purpose to each respondent.

Field survey for Ganoderma *diversity*

A field survey was conducted between January 2024 and July 2025 to document wild *Ganoderma* species utilized by communities residing adjacent to the studied FRs. To achieve adequate temporal and spatial representation of species occurrence, sampling was performed monthly throughout the study period using systematically established plots distributed along transects within the FRs. In total, 85 circular sampling plots were established, comprising 25 plots in Mdabulo FR and 20 plots each in Ituli FR, Lila FR, and Lualage FR. Each plot covered an area of 314.29 m² with a radius of 10 m, following a modified methodology adapted from Kamalebo and De Kesel (2020). Within each sampling plot, intensive random walks were undertaken to maximize detection and collection of *Ganoderma* fruiting bodies across the entire survey area. Simultaneously, important environmental variables and microhabitats associated with the occurrence and distribution of *Ganoderma* species, hereafter referred to as habitat and ecological drivers, were identified and recorded. All encountered individuals were documented and provisionally identified in the field, while representative voucher specimens were collected and preserved for subsequent laboratory identification and verification. To evaluate the adequacy and representativeness of the sampling effort, species accumulation curves and individual-based rarefaction analyses were performed. The resulting curves approached clear asymptotic trends across all forest reserves, indicating that the sampling effort successfully captured the majority of *Ganoderma* species present within the investigated ecosystems (Fig. 1). The stabilization of both species accumulation and rarefaction curves further suggests that additional sampling would likely yield only a limited number of additional species records, thereby confirming the reliability and completeness of the survey design.

Ethnomycological survey design

Ethnobotanical surveys were conducted through structured engagement with local communities to document traditional knowledge on the utilization of wild mushrooms, particularly the genus *Ganoderma*. Data were collected from communities surrounding the FRs, involving key informants such as traditional healers, village elders, foresters, and local vendors. Participants were selected purposively based on their recognized expertise, experiential knowledge, and long-term interaction with forest ecosystems, as well as their willingness to participate. This purposive approach, while effective for capturing specialized indigenous knowledge, may introduce selection

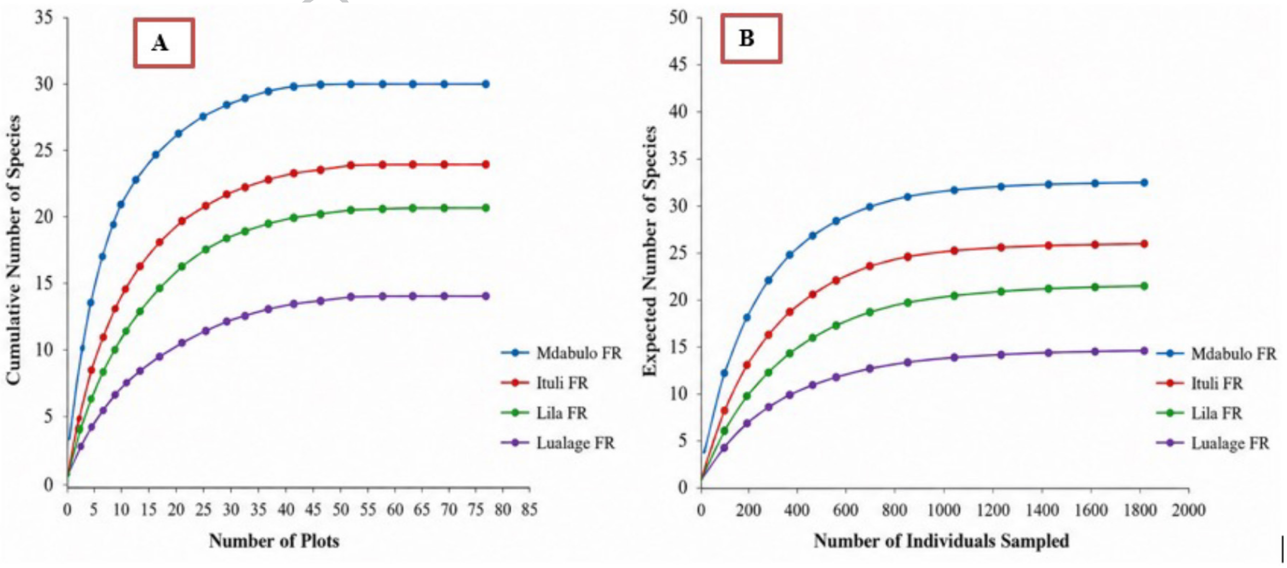

Fig. 1. Species accumulation curves (A) and individual-based rarefaction curves (B) illustrating sampling completeness and adequacy of *Ganoderma* species across the studied FRs.

bias due to the overrepresentation of highly knowledgeable individuals. To reduce response bias, individual interviews were emphasized to minimize peer influence and avoid consensus-driven responses. The interviews focused on local mushroom medicinal uses, preparation methods, perceived efficacy, and cultural significance. Although respondents were interviewed independently, the study did not employ a systematic cross-validation strategy, which may limit the robustness of validation. To address these concerns, information obtained from different respondent groups was compared and cross-referenced with published ethnomycological literature to assess consistency and improve credibility of reported uses (Tardío, Pardo-de-Santayana 2008; Faruque et al. 2018; Nteziryayo et al. 2024; Tibuhwa 2023).

Data collection

Data were collected using a mixed-methods approach that combined structured and semi-structured questionnaires, focus group discussions, and individual interviews. The discussion checklist comprised three components: (i) socioeconomic characteristics of participants (age and gender), (ii) knowledge of known *Ganoderma* species, and (iii) practices and uses, including culinary and medicinal applications. Face-to-face interviews were conducted with elderly participants who were unable to read or write but consented to respond orally, as well as with individuals who were unwilling or unable to complete written questionnaires. Participants were asked about their general knowledge of Ganoderma mushrooms, modes of consumption, and medicinal applications. Interviews were conducted in Kiswahili, Kimbu, Kamba, Sukuma, and Safwa, and subsequently translated into English with the assistance of a trained research team recruited from local communities and fluent in these languages. In each village, a local leader accompanied the research team to facilitate cultural navigation and community engagement. Informed consent was obtained from all participants following a clear explanation of the study objectives.

The ethnobotanical importance of the studied species was quantified using established indices, including Frequency of Citation (FC) and Relative Frequency of Citation (RFC), following Vicente-Pérez et al. (2024) and Faruque et al. (2018). As the study focused on specific use categories (food and/or medicinal), the relative importance of each species was compared using FC and RFC (Eqs. 1 and 2) as described by Vicente-Pérez et al. (2024). RFC was calculated as the proportion of informants who cited a given species relative to the total number of respondents (Tardío, Pardo-de-Santayana 2008). This index reflects the significance of each species based on citation frequency, indicating the number of informants reporting its use. In contrast, FC was determined by dividing the number of times a species was mentioned by the total number of citations for all species (Faruque et al. 2018). Values for both indices range from 0, when no informant reported use of a species, to 1, when all informants cited it as useful. Species diversity within each forest was assessed using the Shannon–Wiener diversity index.

$$RFC = FC / N, \textbf{ (Eq. 1)}$$

where FC represents the number of informants citing the use of a specific species, and N is the total number of informants in the survey.

$$FC = (\text{No. of times a species was mentioned}) / (\text{Total No. of times of all species were mentined}) \times 100.$$

(Eq. 2)

Identification of Ganoderma *species*

Identification of wild *Ganoderma* species in this study was primarily guided by folk taxonomy, which is rooted in culturally embedded knowledge systems and traditional utilization patterns, as described by Tibuhwa (2013). This indigenous classification framework played a foundational role in initial species recognition and facilitated communication with local communities during field surveys. To strengthen this identification, support was obtained from local botanists, experienced mushroom collectors, and officers from the Tanzania Forest Services, alongside the use of standard taxonomic references such as field guidebooks and regional mushroom floras. Each collection was further examined using macro-morphological characteristics and compared with published descriptions for preliminary identification (Nteziryayo et al. 2024; Tibuhwa 2023). Voucher specimens that could not be confidently resolved in the field were collected and assigned voucher numbers (MU001 to MU031) for detailed examination at the National Herbarium of Tanzania. Scientific names were subsequently verified and cross-checked using authoritative taxonomic databases, including Species Fungorum (CABI Bioscience), Fungus Flora of Tropical Africa, Edible Fungi of Tropical Africa, the International Plant Names Index, Plants of the World Online, and The Plant List (Kamalebo, De Kesel 2020; Kibona et al. 2025). Despite these multi-source validation efforts, species identification relied predominantly on macro-morphological traits and ethnotaxonomic knowledge. This approach, while useful for baseline biodiversity assessment, presents limitations given the well-documented taxonomic complexity and frequent morphological overlap within the genus *Ganoderma*, where cryptic species and misidentification of taxa such as *G. lucidum* are common. The absence of molecular confirmation, particularly ITS rDNA sequencing and multilocus phylogenetic analysis, represents a critical limitation of this study. Therefore, future studies should incorporate DNA barcoding approaches to enhance taxonomic accuracy and ensure reliable systematic classification of *Ganoderma* species in the region.

Sample and extract preparation for nutritional analysis

Fresh *Ganoderma* samples were processed using both aqueous and ethanol extraction procedures to preserve nutritional and phytochemical constituents for subsequent analyses. For aqueous extraction, freshly collected *Ganoderma* were homogenized with distilled water under ambient laboratory conditions to minimize degradation of thermolabile compounds, particularly vitamins. The homogenized slurry was filtered through muslin cloth to remove fibrous materials and obtain a clear extract. To improve extraction efficiency, the filtrate was subjected to continuous magnetic stirring at room temperature, followed by centrifugation to separate suspended particles from the soluble fraction. The resulting supernatant was carefully collected and preserved under frozen conditions until nutritional analyses were conducted. This extraction approach was particularly suitable for vitamin determination because water-soluble vitamins are highly sensitive to heat and prolonged processing.

For dry sample preparation, *Ganoderma* were cut into smaller portions and oven-dried at low temperatures to preserve heat-sensitive metabolites while reducing moisture content. The partially dried samples were subsequently air-dried under room conditions until constant weight was achieved. Dried materials were then milled into fine powder using a laboratory blender and sieved through a fine mesh to ensure homogeneity of particle size. The powdered samples were stored in airtight, properly labeled containers before extraction and laboratory analysis. Bioactive compounds were extracted from powdered samples using an ethanol-based extraction method following Kibona et al. (2025). Powdered *Ganoderma* material was immersed in absolute ethanol using an optimized solvent-to-sample ratio and maintained for an extended extraction period to maximize recovery of secondary metabolites. The resulting extracts were filtered through Whatman No. 1 filter paper, after which the solvent was gradually evaporated using a water bath to obtain concentrated extracts. The concentrates were preserved under refrigerated conditions prior to nutritional analyses.

Fresh extracts were prepared by homogenizing 100 g of fresh *Ganoderma* material with 50 mL of distilled water at ambient temperature. The resulting slurry was filtered through muslin cloth to remove fibrous residues, and the procedure was repeated until a final volume of 100 mL of sample extract was obtained. The extract was incubated at room temperature for 1 h under continuous magnetic stirring at 200 rpm, followed by centrifugation at 1000 rpm for 20 min. The supernatant was carefully collected and stored at −20 °C until further analysis. This aliquot was specifically used for nutritional and antinutritional profiling. For dry sample preparation, 5 g of mushroom material was cut into small pieces and oven-dried at 40 – 50 °C until a constant weight was reached, followed by air-drying at room temperature for 2 to 3 days. The dried samples were then ground into a fine powder using a blender, sieved through a 0.5 mm mesh, and stored in airtight, properly labeled containers. Extraction of bioactive compounds was performed using an ethanol-based method as described by Kibona et al. (2025). Briefly, 1 g of powdered mushroom was extracted with 99.9% ethanol at a 1:4 (*w*/*v*) ratio for 72 h. The extracts were filtered using Whatman No. 1 filter paper, and excess solvent was removed using a water bath over 24 – 72 h. The concentrated extracts were deep-frozen at −4 °C prior to nutritional analyses conducted at the Chemistry Laboratory of Mbeya University of Science and Technology, Tanzania. Mineral elements analyzed included potassium, calcium, magnesium, sodium, iron, zinc, copper, and manganese, selected based on their established physiological importance to human health (Orywal et al. 2022; Ngurthankhumi et al. 2024; Malunguja et al. 2025). In addition, vitamins, specifically thiamine (vitamin B_1), riboflavin (vitamin B_2), and ascorbic acid (vitamin C) were analyzed due to their fundamental roles in energy metabolism, growth, development, and cellular function (Orywal et al. 2022; Malunguja et al. 2025). Vitamin C, in particular, is involved in collagen synthesis, L-carnitine production, neurotransmitter function, protein metabolism, and cancer prevention (Taskozhina et al. 2024; da Silva et al. 2025; Razzaque, Wimalawansa 2025).

Quality control and quality assurance protocol

Strict quality control and quality assurance measures were applied throughout the analytical procedures to ensure data reliability and validity. All laboratory analyses were performed using GR-grade analytical reagents and solvents. To prevent contamination and enhance analytical accuracy, all glassware, plasticware, and laboratory vessels were thoroughly cleaned with 1% dilute HNO_3, followed by repeated rinsing with deionized water. Calibration standards and sample dilutions were prepared exclusively using deionized water, and standard solutions were generated at known concentrations from a certified multi-element stock solution. Prior to analysis, samples collected from different locations were washed with running tap water to remove surface debris, cleaned with aqueous ethanol, and finally rinsed with double-distilled water. The samples were then air-dried under a fan to eliminate residual moisture. Procedural blanks were analyzed intermittently to monitor analytical quality, and equipment washings were carried out at regular intervals. Method precision was evaluated by replicate analyses, with each sample measured in triplicate. Elemental concentrations were determined using a Microwave Plasma Atomic Emission Spectrometer (MP-AES; Model 4210, Agilent Technologies, Santa Clara, CA, USA). Instrumental parameters including plasma power, gas flow rates, inlet configuration, and operating pressure were optimized in accordance with the manufacturer's guidelines. Filters and peristaltic pump systems were cleaned with 1% HNO_3 prior to use and properly installed. Method validation was conducted using certified reference material (Lot No. 0011223271, Stevens Creek Blvd, Santa Clara, USA), which was processed using the same procedures as the study samples. Recovery rates for Ca, Mg, Fe, Zn, and Mn were 101.0, 98.7, 104.0, 101.0, and 98.5%, respectively, indicating high analytical accuracy. Method precision for these elements was 3.4% (Ca), 3.6% (Mg), 2.6% (Fe), 2.2% (Zn), and 3.8% (Mn). Method detection limits (MDLs), calculated as three times the standard deviation of procedural blanks (Sharma et al. 2018; Malunguja et al. 2025), were 0.20 mg kg^{-1} for Mn, 0.50 mg kg^{-1} for Fe, and 0.15 mg kg^{-1} for Zn. Calibration curves exhibited strong linearity, with correlation coefficients (R^2) of 0.976 for Mn, 0.967 for Fe, 0.987 for Zn, 0.999 for Na, 0.998 for K, 0.997 for Mg, and 0.9976 for Ca, confirming the robustness and reliability of the analytical methods employed.

Quantification of mineral content

Quantitative determination of mineral content in the mushroom samples was carried out using a Microwave Plasma Atomic Emission Spectrometer (MP-AES; Model 4210, Agilent Technologies, Santa Clara, CA, USA). To optimize extraction of trace elements such as Cu, Fe, Mn, and Zn, a tri-acid wet digestion method was employed, using a mixture of nitric acid, sulfuric acid, and perchloric acid in a 9:4:1 ratio. Briefly, approximately 0.5 g of homogenized sample was placed in a digestion vessel containing 5 mL of freshly prepared aqua regia and heated for ~30 min on a portable stove within a fume hood. Following complete interaction between the acids and the sample, an additional 2 mL of aqua regia was added to dissolve any remaining residues, and the mixture was further heated on a hot plate. The resulting digests were filtered through Whatman No. 1 filter paper and diluted with double-distilled water to a final volume of 50 mL.

For the analysis of Ca, Mg, and K, the digested solutions were further diluted to 100 mL and quantified using an atomic absorption spectrophotometer (UNICAM 919, Spectronic Unicam, Rochester, NY, USA). Specific operating wavelengths were used for each element: Na at 330.2 nm, K at 404.4 nm, Mg at 285.2 nm, and Ca at 422.7 nm. The system's software-controlled temperature regulation ensured precise alignment and optimization of each Hollow Cathode Lamp and adjustment of spectral bandwidth for accurate detection. Calibration standards were prepared from certified stock solutions (1000 mg L^{-1}) to generate working standards for each element. Analytical accuracy was verified using standard reference materials of plant origin (NIST SRM-1515, USA), ensuring the reliability of the quantified mineral concentrations.

Quantification of vitamin content

Vitamin C content was determined using a titrimetric method as described by da Silva et al. (2017). In this procedure, 20 mL of mushroom extract was mixed with three drops of 1% starch indicator and titrated with 20% $CuSO_4$ solution until a dark endpoint was observed. Iodine solution (0.005 mol L^{-1}) was also employed, with 1 mL of iodine corresponding to 0.88 mg of ascorbic acid. The ascorbic acid content in the sample was calculated based on the volume of iodine solution consumed during titration and expressed as mg/100 g of sample.

Thiamine (vitamin B_1), riboflavin (vitamin B_2), and niacin (vitamin B_3) were quantified using colorimetric methods following Al-Ward and Hussein (2016). For vitamin B_1 and B_3 analysis, 10 mL of filtrate was combined with 10 mL of potassium dichromate, and absorbance was measured at 507 nm using a UV-visible spectrophotometer. Calibration curves were prepared using standard solutions at known concentrations, and blank samples were prepared with distilled water.

For vitamin B_2, 10 mL of extract was mixed with 10 mL of 5% potassium permanganate in a 50 mL volumetric flask, followed by the slow addition of 10 mL of 30% H_2O_2. The mixture was heated in a hot water bath for 30 min. Subsequently, 2 mL of 40% sodium sulfate was added, and the final volume was adjusted to 50 mL with deionized water. Blanks were prepared using the same procedure. The absorbance was measured at 450 nm against the respective blanks using a UV-visible spectrophotometer. The content of vitamins B_1 and B_2 was expressed as mg 100 g^{-1} of dry weight.

Quantification of proximate composition

Macronutrient composition including ash content, carbohydrate content, crude fat, crude protein, and calorific value was determined using standard analytical methods. Ash content, representing the inorganic residue remaining after complete combustion of organic matter, was determined following Nielsen (2017). One gram of dried mushroom sample was placed in a clean, pre-weighed porcelain crucible and ashed in a muffle furnace at 500 – 600 °C for approximately 5 h. After cooling to room temperature in a closed desiccator to prevent moisture absorption, the crucible containing the residual greyish-white ash was weighed. The percentage of ash content was calculated using Eq. 3.

Carbohydrate content was quantified using the Anthrone method as described by Ngurthankhumi et al. (2024). Briefly, 100 mg of sample was hydrolyzed in 5 mL of 2.5 N HCl in a boiling tube at a water bath for 3 h. After cooling and neutralization, the hydrolysate volume was adjusted to 100 mL and centrifuged to obtain the supernatant. A 1 mL aliquot of the supernatant was mixed with 4 mL of anthrone reagent, heated in a boiling water bath for 10 min, cooled, and the developed colour intensity was measured at 630 nm using a UV-visible spectrophotometer with a blank as reference.

Crude fat was determined by Soxhlet extraction following Patel et al. (2012). Dried, powdered sample (5 g) was extracted with petroleum ether in a Soxhlet apparatus for 16 h. After solvent evaporation, the residue was oven-dried at 80 °C, cooled in a desiccator, and weighed. Crude fat content was expressed as a percentage of dry weight using Eq. 4.

Crude protein was quantified using the Kjeldahl method. A 0.5 g sample was digested in 10 mL of H_2SO_4 with copper sulfate and potassium sulfate as catalysts.

The digest was distilled with 40% NaOH, and the released ammonia was captured in 4% boric acid and titrated with 0.02 N HCl to determine total nitrogen. Crude protein was calculated using a conversion factor of 6.25 (Eq. 5 & 6).

Calorific value, representing the gross energy content of the mushrooms, was calculated using Eq. 7 and expressed as kilocalories per 100 g of dry weight.

$$AC\ (\%) = (W2 - W0) / W1 \times 100,$$ **(Eq. 3)**

where $W0$ is weight of crucible, $W1$ is weight of the sample, and $W2$ is weight of crucible and ash (final weight).

$$CF\ (\%) = We / Ws \times 100,$$ **(Eq. 4)**

where CF is crude fat (%), We is weight of ether extract (g), Ws is weight of the sample (g).

$$N\ (\%) = (0.014 \times N1 \times N2) / Ws \times 100,$$ (Eq. 5)

where N is nitrogen content (%), $N1$ is N titrants (cm^3), $N2$ is net mL titrant (cm^3), Ws is weight of the sample (g).

$$CP\ (\%) = \%N \times 6.25,$$ **(Eq. 6)**

where CP is crude protein (%).

$$CV\ (\mathrm{kcal}) = (\%CC \times 4) + (\%CF \times 9) + (\%CP \times 4),$$ **(Eq. 7)**

where CC is carbohydrate content (%), CF is crude fat (%), CP is crude protein (%).

Quantification of phytochemical constituents

The present study applied spectrophotometric phytochemical screening techniques to provide baseline information on the bioactive composition of the investigated *Ganoderma* species. These methods are commonly used in preliminary investigations of medicinal macrofungi; however, they are inherently limited in analytical precision and definitive structural identification when compared with advanced chromatographic and spectrometric techniques. Phytochemical constituents of the mushroom samples including total tannin content (TTC), total phenolic content (TPC), and total flavonoid content (TFC), were quantified using standard colorimetric assays (Ade-Ogunnowo et al. 2024). Results were expressed as mg gallic acid equivalent (GAE) per 100 g for TPC, mg quercetin equivalent (QE) per 100 g for TFC, and mg per 100 g for TTC.

Flavonoid content (TFC) was determined using the $AlCl_3$ colorimetric method. Briefly, 1 g of sample was homogenized with 10 mL of deionized water, shaken for 3 h, and filtered through double-layered Whatman No. 41 filter paper. To 1 mL of the extract, 0.3 mL of 5% $NaNO_2$ was added. After 5 min, 0.3 mL of 10% $AlCl_3 \cdot 6H_2O$ was added and incubated for another 5 minutes. Subsequently, 2 mL of 1 M NaOH was added, and the final volume was adjusted to 5 mL with distilled water. After 15 min of incubation, the solution turned pink, and absorbance was measured at 510 nm against a reagent blank using a UV-visible spectrophotometer. Flavonoid content was calculated as mg quercetin equivalent per gram (mg QE g^{-1}) (Eq. 8).

Phenolic content (TPC) was determined using the Folin–Ciocalteu method. One mL of extract was mixed with 2 mL of Folin–Ciocalteu reagent diluted 1:10 with deionized water. After 10 min, 1.6 mL of Na_2CO_3 solution was added. The mixture was vortexed for 15 s and left at room temperature for 30 min to allow colour development. Absorbance was measured at 743 nm, and phenolic content was calculated from a gallic acid standard curve, expressed as mg GAE per gram of sample (Eq. 9).

Tannin content (TTC) was quantified using a modified Folin–Ciocalteu method (Eq. 10). One g of sample was boiled in 20 mL of distilled water and filtered. Three drops of 0.1% ferric chloride were added to confirm the presence of tannins via blue-black colour formation. For analysis, 0.5 g of sample was boiled in 75 mL of water for 30 min and centrifuged at 2000 rpm for 20 min. The supernatant was transferred to a volumetric flask and diluted to 100 mL with distilled water. One mL of this extract was further diluted with 75 mL of water in a volumetric flask, followed by the addition of 5 mL Folin–Denis reagent and 10 mL Na_2CO_3 solution. The solution was made up to 100 mL with distilled water and incubated for 30 min. Absorbance was measured at 700 nm against a blank using a UV-visible spectrophotometer.

While these spectrophotometric approaches provide useful semi-quantitative estimates of total bioactive groups, they remain limited in resolving individual triterpenoids, phenolic derivatives, and other secondary metabolites that are critical in *Ganoderma* pharmacological profiling. Therefore, future work should prioritize high-resolution analytical platforms to enable compound-specific identification, accurate quantification of triterpenoids and phenolics, and comprehensive metabolomic profiling.

$$TFC\ (\mathrm{mg\ QE\ g^{-1}}) = (cQE \times Ve) / Ws,$$ **(Eq. 8)**

where cQE is concentration from standard curve, Ve is volume of extract used, Ws is weight of sample (g).

$$TPC\ (\mathrm{mg\ GAE\ g^{-1}}) = (cGAE \times Ve \times 100) / Ws,$$ **(Eq. 9)**

where $cGAE$ is concentration from standard curve, Ve is volume of extract used, Ws is weight of sample (g).

$$TTC\ (\mathrm{mg\ TAE\ g^{-1}}) = (cTAE \times Ve \times 100) / Ws,$$ **(Eq. 10)**

where $cTAE$ is concentration from std curve, Ve is volume of extract used, Ws is weight of sample (g).

Quantification of anti-nutritional composition

The present study employed a qualitative approach to assess the presence of various antinutritional compounds, including phytates (phytic acid), oxalates, cyanogenic glycosides, lectins, phlobatannins, anthocyanins, betacyanins, quinones, and goitrogens. Evaluating these compounds is essential for ensuring food safety and quality, while minimizing potential toxic effects that could compromise food security. Standard qualitative tests were applied for detection of each compound. Phytates were identified using the ferric chloride test, oxalates with the calcium chloride test, and lectins via the haemagglutination test. Cyanogenic glycosides were detected using the Keller-Killiani test, while phlobatannins were identified with the hydrochloric acid test. Anthocyanins and quinones were assessed using the Borntrager's test, betacyanins with the sodium hydroxide test, and goitrogens were evaluated via the thiourea colour reaction. All analyses were performed on aqueous extracts of the mushroom samples. Results were recorded using a qualitative scale: '+++' for high content, '++' for moderate, '+' for low, and '–' to indicate absence. Detailed procedures for each assay are described by Maheshwaran et al. (2024) and Okuna et al. (2024).

Statistical analysis

Descriptive statistics were employed to summarize the data, with results reported as mean ± standard deviation of three replicates. One-way ANOVA was used to evaluate differences in the nutritional profiles among the studied *Ganoderma* species. Post hoc pairwise comparisons were conducted using Tukey's honestly significant difference tests. Prior to statistical analysis, data were assessed for normality and homogeneity of variance using the Shapiro–Wilk and Levene's tests, respectively. Most variables satisfied the assumptions of normality and homogeneity of variance. For variables exhibiting mild deviations from normality, data transformations were applied to improve adherence to parametric assumptions, rather than adopting a fully non-parametric framework. This approach was preferred due to the higher statistical power of ANOVA, clearer interpretability of mean differences, and methodological consistency across multiple response variables. To explore relationships among nutritional, mineral, vitamin, and phytochemical variables, Pearson correlation analysis was performed, alongside multivariate approaches including principal component analysis and cluster analysis. These methods provided complementary insights into variable interrelationships and species grouping patterns (Malunguja, Devi 2025). Statistical significance was considered at $p < 0.05$. All analyses were conducted using SPSS software (version 26; IBM, NY, USA).

Results

Diversity of Ganoderma *species*

The present study documented eight *Ganoderma* species across the investigated FRs (Table S2), highlighting considerable taxonomic diversity within the studied ecosystems. The identified species included *Ganoderma applanatum, Ganoderma leucocontextum, Ganoderma lucidum, Ganoderma australe, Ganoderma arbiforme, Ganoderma mbrekobenum, Ganoderma gibbosum*, and *Ganoderma resinaceum*. Across the entire study area, a total of 1828 individuals were documented. *Ganoderma* abundance differed significantly among forest reserves ($F = 28.021$, $p < 0.001$). Mdabulo FR contributed the highest proportion of individuals ($n = 712$), followed by Ituli FR ($n = 445$) and Lila FR ($n = 410$). The lowest abundance ($n = 261$) was recorded in Lualage FR. *G. lucidum* was the most abundant and widely distributed species across all FRs. This dominance highlights its broad ecological tolerance and successful establishment across varying forest conditions. *G. applanatum* and *G. leucocontextum* also showed high abundance and relatively consistent distribution. The occurrence of these species across different FRs suggests the ecological suitability of the studied habitats for supporting diverse *Ganoderma* assemblages and underscores their potential significance as valuable fungal bioresources. The patterns of species diversity, assessed using the Shannon–Wiener diversity index (H') revealed notable variation in species diversity across the FRs (Fig. 2). Mdabulo FR exhibited the highest diversity ($H' = 2.179$), significantly exceeding that of Lila FR ($H' = 1.575$, p = 0.003), Ituli FR ($H' = 1.663$, $p = 0.000$), and Lualage FR ($H' = 1.155$, $p = 0.013$). This indicates a more equitable distribution of species and a relatively richer assemblage in Mdabulo FR. In contrast, Ituli and Lila FRs showed moderate diversity with no significant difference between them ($p > 0.05$), suggesting reasonable species richness but increasing dominance by a few taxa. Lualage FR displayed the lowest diversity, reflecting reduced species richness and pronounced dominance.

Habitat and ecological drivers for Ganoderma *distribution*

The distribution and abundance of *Ganoderma* species differed among the studied FRs due to variation in habitat characteristics and ecological conditions. Conditions including soil moisture, canopy cover, deadwood availability, and soil organic matter strongly influenced species occurrence and distribution patterns. The bipartite ecological association network (Fig. S1) demonstrated clear relationships between habitat variables and *Ganoderma* species, identifying soil moisture, canopy cover, and decaying wood resources as the most influential predictors of species presence. The numerical values presented along the connecting lines indicate the relative strength of association between habitat variables and species occurrence. Higher interaction scores (8 – 10) reflected strong positive associations, particularly for *G. lucidum* and *G. applanatum*, which were closely linked to moist soils, dense canopy cover, abundant deadwood, and elevated soil

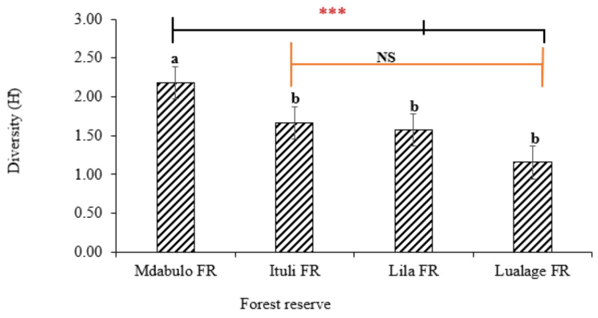

Fig. 2. Variation in species diversity (Shannon diversity index, H') of *Ganoderma* species across different FRs. NS indicates that no statistically significant differences were detected in *Ganoderma* diversity among FRs. Bars sharing the same letter are not significantly different, whereas bars with different letters and marked with *** indicate statistically significant differences at the 95% confidence level ($p < 0.05$).

organic matter. Moderate ecological associations (6 – 7) were observed for *G. leucocontextum, G. australe*, and *G. arbiforme*. The weaker interactions characterized altitude and temperature-sensitive species such as *G. resinaceum* and *G. mbrekobenum*, suggesting narrower ecological tolerance ranges. Forest reserves with favourable microclimatic conditions, including high humidity, dense vegetation cover, nutrient-rich soils, and abundant woody debris, supported comparatively greater *Ganoderma* diversity and abundance. Mdabulo FR exhibited high abundance of most recorded species, indicating ecological conditions suitable for fungal colonization and persistence. The restricted occurrence of *G. mbrekobenum* and *G. resinaceum* suggests stronger habitat specialization. Thus, the findings highlight soil moisture, canopy cover, deadwood abundance, and soil organic matter as key ecological drivers regulating *Ganoderma* distribution and establishment in Miombo woodland ecosystems.

Ethnomedicinal utility of Ganoderma *species*

The ethnomycological survey demonstrated that *Ganoderma* species are highly valued for medicinal purposes and remain important components of traditional healthcare systems within the study area. Respondents identified at least one *Ganoderma* species as therapeutically useful, with reported applications spanning 14 ailment categories (Table 1). The most frequently cited uses were associated with pain management and digestive system disorders, each reported by 81.7% of respondents. Respiratory ailments were also widely mentioned (70.4%), followed by kidney-related disorders (63.5%) and reproductive health conditions (48.7%). These citation patterns suggest that *Ganoderma*-based remedies are mainly relied upon for treating common chronic and recurrent health problems affecting local communities.

Among the documented taxa, *G. lucidum* exhibited the highest ethnomedicinal importance, with the greatest frequency of citation and relative frequency of citation (RFC = 0.90). The species was consistently associated with all recorded ailment categories, particularly digestive disorders, respiratory infections, pain-related conditions, kidney complications, parasitic infections, and skin diseases. Its broad therapeutic coverage highlights its dominant role in traditional medicinal practices. *G applanatum* ranked second in importance (RFC = 0.80) and was frequently cited for wound healing, dermatological disorders, microbial infections, neurological conditions, and pain relief. Moderate citation frequencies were observed for

Table 1. Ethnomedicinal uses of *Ganoderma* species across ailment categories showing number of use reports (CF), and species-level relative citation frequency (RCF)

Category of ailment	Ailment	Use reports (CF)	Species (RCF)
Boils, abscesses and wounds	Abscesses, cuts, wounds, swellings	12	*G. applanatum* (0.80), *G. australe* (0.70)
Dermatological disorders	Eczema, ringworm, dandruff, vitiligo, itch	34	*G. lucidum* (0.90), *G. applanatum* (0.80), *G. resinaceum* (0.20)
Digestive system disorders	Gastritis, diarrhea, ulcers, constipation, indigestion, flatulence, piles, anthelmintic	179	*G. lucidum* (0.90), *G. applanatum* (0.80), *G. australe* (0.70)
Fever and cough	Febrile illness, cough	42	*G. lucidum* (0.90), *G. leucocontextum* (0.60)
General disorders	Tonic, general weakness, vomiting, external injuries	3	*G. lucidum* (0.90)
Kidney disorders	Kidney and bladder stones, urinary problems, diuretic	146	*G. lucidum* (0.90), *G. gibbosum* (0.30), *G. resinaceum* (0.20)
Microbial infection	Cholera, dysentery, fungal infection, measles	49	*G. lucidum* (0.90), *G. applanatum* (0.80), *G. resinaceum* (0.20)
Neurological and psychological disorders	Insanity, analgesic, psychological disorders	72	*G. lucidum* (0.90), *G. applanatum* (0.80)
Pain	Abdominal pain, toothache, headache, migraine, chest pain, gout	188	*G. lucidum* (0.90), *G. applanatum* (0.80), *G. mbrekobenum* (0.20)
Parasitic infection	Malaria, liver cyst, scabies	72	*G. lucidum* (0.90), *G. leucocontextum* (0.60), *G. gibbosum* (0.30)
Respiratory disorders	Asthma, bronchitis, pneumonia, tonsillitis	162	*G. lucidum* (0.90), *G. leucocontextum* (0.60), *G. gibbosum* (0.30)
Rheumatism and fracture	Rheumatism, bone fracture, paralysis	2	*G. lucidum* (0.90), *G. australe* (0.70)
Sexual and reproductive disorders	Infertility, dysmenorrhea, impotence, uterine disorders	112	*G. lucidum* (0.90), *G. leucocontextum* (0.60), *G. mbrekobenum* (0.20)
Snake, dog and insect bites	Envenomation and stings	79	*G. lucidum* (0.90), *G. applanatum* (0.80), *G. australe* (0.70)

G. australe (RFC = 0.70) and *G. leucocontextum* (RFC = 0.60), indicating more specialized medicinal applications. In contrast, *G. gibbosum* (RFC = 0.30), *G. resinaceum*, and *G. mbrekobenum* (RFC = 0.20) were infrequently reported, reflecting localized ethnomedicinal knowledge and narrower therapeutic use. Ailment-specific analysis further emphasized the importance of *Ganoderma* species in managing pain-related conditions (RFC = 0.163), digestive disorders (RFC = 0.155), and respiratory ailments (RFC = 0.141). Food-related utilization was limited, as only 4.3% of respondents reported occasional use of *G. lucidum* and *G. applanatum* as dried flavour additives. Most respondents considered *Ganoderma* species unsuitable for consumption because of their bitter taste and hard texture. The findings indicate that *Ganoderma* species are primarily perceived as medicinal rather than edible resources within Miombo woodland communities.

Mineral and vitamin composition

Table 2 presents the mineral and vitamin composition of the eight investigated *Ganoderma* species, revealing significant interspecific variation in nutritional profiles (*F* = 28.021, *p* = 0.000). The results indicate differences in both macronutrient and micronutrient content among species. Among the macronutrients, *G. arbiforme* contained the highest Ca content (133 mg 100 g^{-1}), followed by *G. applanatum* (91 mg 100 g^{-1}) and *G. lucidum* (88 mg 100 g^{-1}), whereas *G. gibbosum* and *G. resinaceum* recorded comparatively lower Ca levels. Mg content was also highest in *G. arbiforme* (70 mg 100 g^{-1}), with high contributions from *G. applanatum* and *G. australe*, while *G. resinaceum* exhibited the lowest Mg content (23 mg 100 g^{-1}). K levels varied among species, with *G. lucidum* recording the highest content (456 mg 100 g^{-1}), followed by *G. applanatum* and *G. arbiforme*. In contrast, *G. resinaceum* contained the lowest potassium content. Na content was greatest in *G. applanatum* (27 mg 100 g^{-1} whereas *G. mbrekobenum* exhibited the lowest value (8 mg 100 g^{-1}). Trace element composition also differed significantly among species. Fe content was highest in *G. lucidum* (6.5 mg 100 g^{-1}), followed closely by *G. leucocontextum* and *G. australe*, while *G. mbrekobenum* recorded the lowest Fe content. Zn was predominantly accumulated in *G. lucidum* (2.6 mg 100 g^{-1}), *G. arbiforme* (2.1 mg 100 g^{-1}), and *G. australe* (1.8 mg 100 g^{-1}). Cu and Mn content peaked in *G. australe*, with values of 1.25 and 1.02 mg 100 g^{-1}, respectively.

Vitamin composition showed comparable species-level variation. *G. lucidum* exhibited the highest vitamin C content (9.6 mg 100 g^{-1}), followed by *G. mbrekobenum* and *G. australe*, whereas *G. leucocontextum* and *G. gibbosum* recorded the lowest values. Vitamin B_1 was most abundant in *G. applanatum* (0.38 mg 100 g^{-1}), while *G. arbiforme* contained the highest content of vitamin B_2 (0.33 mg 100 g^{-1}). Vitamin B_3 levels were greatest in *G. lucidum* (7.23 mg 100 g^{-1}), followed closely by *G. arbiforme* and *G. australe*. The findings demonstrate distinct nutritional profiles

Table 2. Mineral element and vitamin composition of the investigated *Ganoderma* species across Miombo FRs in northern and central Tanzania, expressed as mean values (± SD) in mg per 100 g (*n* = 3). Values with different lower-case superscript letters in a raws are significantly different at $p < 0.05$. *** indicate statistically significant differences across species at the 95% confidence level

Parameter	**Ganoderma species**								**p value**
	G. applanatum	***G. leucocontextum***	***G. lucidum***	***G. australe***	***G. arbiforme***	***G. mbrekobenum***	***G. gibbosum***	***G. resinaceum***	
Ca	91.33 ± 2.31 a	73.01 ± 1.89 a	88.43 ± 2.22 a	76.67 ± 2.76 a	133.09 ± 1.67b	73.01 ± 1.76 a	42.07 ± 1.13 c	64.43 ± 1.44 c	***
Mg	56.73 ± 2.13 c	33.75 ± 1.43 e	34.61 ± 1.01 e	43.23 ± 1.42 c	69.78 ± 1.33 c	38.67 ± 1.45 e	32.81 ± 1.54 e	23.11 ± 2.11 f	***
K	359.11 ± 1.33 g	231.31 ± 1.31 h	456.44 ± 1.36 g	207.36 ± 1.65 h	335.88 ± 1.31 g	297.21 ± 1.45 h	204.11 ± 1.22 h	189.13 ± 2.71 h	***
Na	26.7 ± 0.98 f	16.21 ± 1.33 f	10.21 ± 0.72 i	14.23 ± 2.44 i	14.58 ± 2.17 i	7.48 ± 1.76 j	13.08 ± 1.12 i	10.35 ± 1.81 i	***
Fe	3.11 ± 0.21 k	6.14 ± 0.22 j	6.46 ± 1.01 j	4.11 ± 1.12 k	2.13 ± 0.14 k	0.19 ± 0.01 m	1.21 ± 0.21 m	0.70 ± 0.11 m	***
Zn	1.45 ± 0.81 m	0.64 ± 0.06 m	2.60 ± 0.36 m	1.75 ± 0.28 m	2.10 ± 1.13 k	0.38 ± 0.14 m	0.68 ± 0.23 m	1.43 ± 0.52 m	***
Cu	0.87 ± 0.61 m	0.78 ± 0.69 m	0.89 ± 0.42 m	1.25 ± 0.09 p	0.67 ± 0.98 m	0.23 ± 0.88 m	0.32 ± 0.67 m	0.18 ± 0.61 m	***
Mn	0.82 ± 0.72 q	0.18 ± 0.87 q	0.82 ± 0.61 q	1.02 ± 0.42 p	0.98 ± 0.77 q	0.49 ± 0.84 p	0.78 ± 0.07 q	0.32 ± 0.03 p	***
Vitamin C	6.79 ± 0.87 j	3.76 ± 0.92 k	9.56 ± 1.69 i	8.94 ± 1.39 i	6.45 ± 1.78 j	9.17 ± 1.32 i	3.67 ± 1.45 k	5.79 ± 1.32 j	***
Vitamin B1	0.38 ± 0.98 m	0.06 ± 0.61 r	0.07 ± 0.66 r	0.06 ± 0.02 r	0.28 ± 0.04 m	0.05 ± 0.01 r	0.26 ± 0.31 m	0.03 ± 0.14 r	***
Vitamin B2	0.19 ± 0.63 m	0.08 ± 0.06 r	0.07 ± 0.02 r	0.09 ± 0.00 r	0.33 ± 0.07 p	0.19 ± 0.06 m	0.04 ± 0.02 r	0.02 ± 0.01 r	***
Vitamin B3	4.67 ± 1.23 k	5.88 ± 1.19 k	7.23 ± 1.13 j	6.83 ± 1.71 j	6.97 ± 1.56 j	3.21 ± 1.83 k	1.96 ± 0.87 m	2.11 ± 0.72 y	***

among the investigated *Ganoderma* species, suggesting potential differences in their dietary and ethnomedicinal relevance.

Proximate composition

The proximate composition of the eight investigated *Ganoderma* species varied significantly (F = 282.35; p = 0.000), demonstrating substantial interspecific differences in nutritional characteristics and potential functional applications (Table 3). Moisture content ranged from 8.46% in *G. arbiforme* to 17.53% in *G. australe*. The relatively low moisture content observed in *G. arbiforme* suggests improved storage stability and reduced susceptibility to microbial spoilage, whereas the higher moisture level in *G. australe* may contribute to its suitability for fresh therapeutic preparations and functional food formulations.

Crude fibre content was highest in *G. applanatum* (7.94%), followed by *G. lucidum* (6.98%), suggesting their potential importance in dietary applications associated with digestive regulation and gastrointestinal health. The comparatively lower fibre content in *G. arbiforme* (2.34%) suggests reduced roughage characteristics. Ash content, which reflects total mineral composition, was notably high in *G. applanatum* (18.91%) and *G. mbrekobenum* (17.43%), indicating that these species are relatively rich in mineral constituents.

Considerable variation was also observed in crude fat content. *G. mbrekobenum* (7.32%) and *G. australe* (6.01%) exhibited the highest fat content, whereas *G. applanatum* (1.76%) and *G. arbiforme* (2.87%) contained relatively low levels. Carbohydrate content, an important contributor to energy value, was greatest in *G. mbrekobenum* (57.11%) and *G. leucocontextum* (54.99%), while *G. australe* (29.67%) and *G. resinaceum* (27.93%) recorded comparatively lower content.

Protein content also differed among species. *G. australe* contained the highest crude protein level (34.82%), followed by *G. leucocontextum* (28.41%), suggesting their comparatively high nutritional value as protein-rich fungal resources. Lower protein content was observed in *G. gibbosum* (10.97%) and *G. mbrekobenum* (14.56%).

Calorific values generally reflected carbohydrate and protein composition, with *G. leucocontextum* (375 kcal 100 g^{-1}) and *G. mbrekobenum* (353 kcal 100 g^{-1}) providing the highest energy values. In contrast, *G. resinaceum* (205 kcal 100 g^{-1}) and G. gibbosum (216 kcal 100 g^{-1}) exhibited comparatively lower calorific content. The results demonstrate distinct proximate composition profiles among Ganoderma species, suggesting their potential nutritional supplementation and ethnomedicinal utilization.

Phytochemical constituents

The phytochemical analysis of the eight investigated Ganoderma species revealed considerable interspecific variation in total phenolics, flavonoids, and tannins (Fig. 3), suggesting differences in their bioactive composition and potential medicinal value.

Total phenolic content ranged from 235 mg GAE 100 g^{-1} in *G. applanatum* to 567 mg GAE 100 g^{-1} in *G. arbiforme*, with *G. arbiforme* emerging as the major contributor of phenolic compounds. Elevated phenolic levels suggest enhanced antioxidant capacity and possible relevance in traditional medicinal applications associated with oxidative stress reduction and immune support. Flavonoid content also differed among species. *G. applanatum* recorded the highest flavonoid content (14.9 mg QE 100 g^{-1}), whereas *G. lucidum* exhibited the lowest content (2.7 mg QE 100 g^{-1}). Since flavonoids are associated with antioxidant and anti-inflammatory activities, the high flavonoid level observed in *G. applanatum* may suggest potential suitability for nutraceutical and functional food applications.

Tannin content ranged from 0.33 mg TAE 100 g^{-1} in *G. australe* to 5.72 mg TAE 100 g^{-1} in *G. mbrekobenum*. The comparatively high tannin content in *G. mbrekobenum* suggests possible antimicrobial and preservative-related properties. The findings demonstrate distinct phytochemical

Table 3. Proximate composition of the investigated *Ganoderma* species across Miombo FRs in northern and central Tanzania. Values with different lower-case superscript letters in a column are significantly different at $p < 0.05$

Species	Moisture (%)	Crude fibre (%)	Ash content (%)	Crude fat contents(%)	Carbohydrate content (%)	Crude protein content (%)	Calorific value (kcal 100 g^{-1})
G. applanatum	13.67 a	7.94 c	18.91 e	1.76 g	43.22 k	21.76 f	275.76 a
G. leucocontextum	15.71 b	6.87 c	11.07 c	4.56 h	54.99 j	28.41 h	374.64 f
G. lucidum	9.43 c	6.98 c	14.89 b	3.72 h	33.9 l	23.06 f	261.32 a
G. australe	17.53 b	5.89 c	11.32 d	6.01 j	29.67 l	34.82 k	312.05 g
G. arbiforme	8.46 c	2.34 d	6.89 f	2.87 g	53.33 j	21.61 f	325.59 g
G. mbrekobenum	16.72 b	3.41 d	17.43 e	7.32 j	57.11 j	14.56 c	352.56 f
G. gibbosum	9.87 c	5.32 c	6.22 f	4.98 h	31.76 l	10.97 d	215.74 m
G. resinaceum	12.56 a	4.74 d	13.54 b	3.63 h	27.93 l	15.21 c	205.23 m

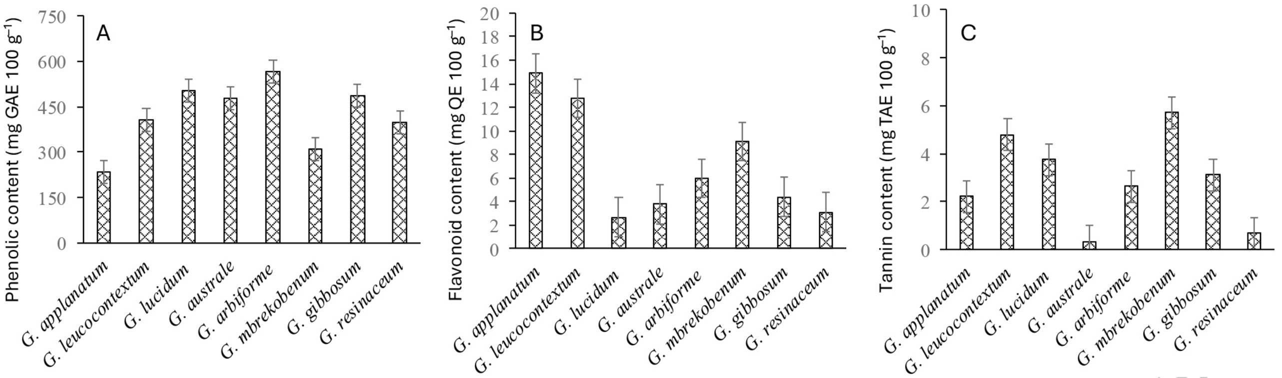

Fig. 3. Phytochemical composition of the investigated *Ganoderma* species, showing the quantitative distribution of major secondary metabolites: (A) total phenolic content expressed as gallic acid equivalents, (B) total flavonoid content expressed as quercetin equivalents, and (C) total tannin content expressed as tannic acid equivalents.

profiles among *Ganoderma* species, with *G. arbiforme, G. applanatum*, and *G. mbrekobenum* representing important sources of phenolics, flavonoids, and tannins, respectively.

Anti-nutritional composition

The qualitative analysis of anti-nutritional compounds among the eight investigated *Ganoderma* species revealed interspecific variation in the occurrence and relative content of phytochemical constituents (Table S3). High alkaloid content was detected (+++) in *G. applanatum, G. leucocontextum, G. lucidum, G. australe*, and *G. arbiforme*, whereas moderate levels (++) occurred in *G. mbrekobenum, G. gibbosum*, and *G. resinaceum*. Although alkaloids are associated with antimicrobial and pharmacological activities, elevated content may limit direct dietary utilization of some species. Phlobatannins generally occurred at low content (+) across most species, while *G. arbiforme* and *G. mbrekobenum* lacked detectable levels. Saponins showed marked variation, with high content (+++) observed in *G. leucocontextum* and *G. australe*, moderate levels (++) in *G. applanatum* and *G. lucidum*, and low content (+) in the remaining species. These compounds are often associated with cholesterol-lowering and immunomodulatory properties, although high levels may reduce palatability because of bitterness.

Flavonoids were absent in *G. applanatum, G. arbiforme*, and *G. mbrekobenum*, but occurred at low (+) to moderate (++) content in other species, indicating potential antioxidant relevance. Anthraquinones were highly represented in *G. applanatum* (++) but absent in *G. leucocontextum* and *G. lucidum*. Steroidal compounds occurred predominantly in *G. leucocontextum* (+++), with moderate content in *G. lucidum* and *G. gibbosum*. Terpenes were especially abundant in *G. gibbosum* (+++), followed by moderate levels in *G. resinaceum*. These compounds are commonly associated with antimicrobial and therapeutic activities, although strong terpene content may influence flavour acceptability. Cardenolides and cardiac glycosides were present at low to moderate u in most species, although some taxa lacked detectable cardenolides. Phytates were absent in *G. leucocontextum* and *G. lucidum* but occurred at low content in the remaining species. Oxalates were absent in *G. gibbosum* and occurred only at low levels in other species, suggesting minimal risk of mineral interference. Lectins were absent in *G. australe* and *G. mbrekobenum* but detected at low content in the remaining taxa. Betacyanins and quinones also varied considerably among species, while goitrogens were absent in several taxa, including *G. leucocontextum, G. australe, G. arbiforme,* and *G. gibbosum*. Species-specific variation in anti-nutritional composition among *Ganoderma* taxa, suggest varying implications for dietary utilization and medicinal applications.

Pearson correlation analysis

The correlation analysis revealed clear patterns of association among minerals, vitamins, proximate components, energy value, and phytochemicals, indicating coordinated nutrient accumulation and compositional trade-offs (Table S4). Strong and significant positive correlations were observed among several minerals and vitamins, notably between Ca and Mg ($r = 0.84$, $p < 0.01$), Ca and vitamin B_2 ($r = 0.84$, $p < 0.01$), and Mg and vitamin B_2 ($r = 0.92$, $p < 0.01$), suggesting shared metabolic regulation pathways. Fe exhibited significant positive relationships with copper ($r = 0.76$, $p < 0.05$) and vitamin B3 ($r = 0.77$, $p < 0.05$), while Cu was strongly correlated with vitamin B_3 ($r = 0.86$, $p < 0.01$), reflecting their complementary roles enzymatic functions. Zn and Mn showed moderate positive associations with several macronutrients and vitamins, indicating mineral synergy rather than isolated accumulation. Vitamins also displayed notable mineral linkages, including a significant association between vitamin B_1 and Na ($r = 0.74$, $p < 0.05$) and positive correlations of vitamin C with K, Zn, Cu, and Mn, consistent with its involvement in antioxidant activity.

Moisture content was generally negatively correlated with minerals, fibre, ash, and phytochemicals, indicating a dilution effect, while showing a positive relationship with crude fat. Crude fat exhibited negative correlations with most minerals and vitamins, whereas crude protein was positively associated with vitamin B_2 and caloric value (*r*

= 0.83, $p < 0.05$), underscoring its contribution to energy density. Carbohydrate content showed strong positive correlations with iron, copper ($r = 0.91$, $p < 0.01$), vitamin B_3 ($r = 0.81$, $p < 0.05$), and caloric value, confirming its dominant role in energy provision. Phytochemicals displayed more variable relationships; phenolic content was negatively associated with ash and moisture, while flavonoids were positively correlated with crude protein, ash, and caloric value but negatively associated with total phenolics ($r = -0.72$, $p < 0.05$). Tannins showed a strong positive correlation with crude protein ($r = 0.74$, $p < 0.05$) and negative associations with several minerals and flavonoids, suggesting potential mineral-binding effects that may influence nutrient bioavailability.

Principal component analysis

The principal component analysis explained 33.36% and 25.50% of the total variance along PC1 and PC2, respectively, cumulatively accounting for 58.86% of the total variability among the studied *Ganoderma* species (Fig. 4). The ordination revealed clear biochemical differentiation associated with nutritional composition, phytochemical constituents, and ethnomedicinal relevance. PC1 was strongly influenced by phenolic compounds, K, vitamin C, and Ca, whereas PC2 was mainly associated with flavonoids, tannins, Mg, and micronutrient variability. Species positioned on the positive side of PC1, particularly *G. lucidum, G. aribiforme,* and *G. gibbosum*, exhibited elevated phenolic content, together with relatively high K content. These species clustered closely with the phenolic and vitamin vectors, indicating strong positive biochemical associations linked to antioxidant and ethnomedicinal potential. Among these, *G. lucidum* showed the strongest association with K and vitamin C, while *G. aribiforme* was strongly associated with Ca, Mg, and total phenolics. Similarly, *G. gibbosum* grouped closely with phenolic-rich species despite having comparatively lower mineral content, suggesting that phenolic accumulation contributed substantially to its ordination position. Species located closer to flavonoid and tannin vectors included *G. applanatum, G. leucocontextum*, and *G. mbrekobenum. G. applanatum* and *G. leucocontextum* contained the highest flavonoid content, while *G. mbrekobenum* exhibited the highest tannin content. Their proximity to these phytochemical trajectories suggests possible specialization in antimicrobial and therapeutic applications associated with tannin- and flavonoid-mediated bioactivity.

Cluster analysis

Hierarchical cluster analysis classified the studied *Ganoderma* species into distinct biochemical groups according to similarities in mineral composition, phytochemical constituents, vitamin profiles, and ethnomedicinal relevance (Fig. 5). The clustering pattern suggests that species with similar nutritional and phytochemical composition also share comparable ethnomedicinal utilization profiles within Miombo woodland communities. The dendrogram revealed three major clusters, reflecting compositional affinities associated with traditional medicinal utilization patterns. The first cluster comprised *G. lucidum, G. aribiforme, G. australe,* and *G. gibbosum*, which grouped together at relatively low Euclidean distances, indicating strong biochemical similarity. This grouping was mainly influenced by

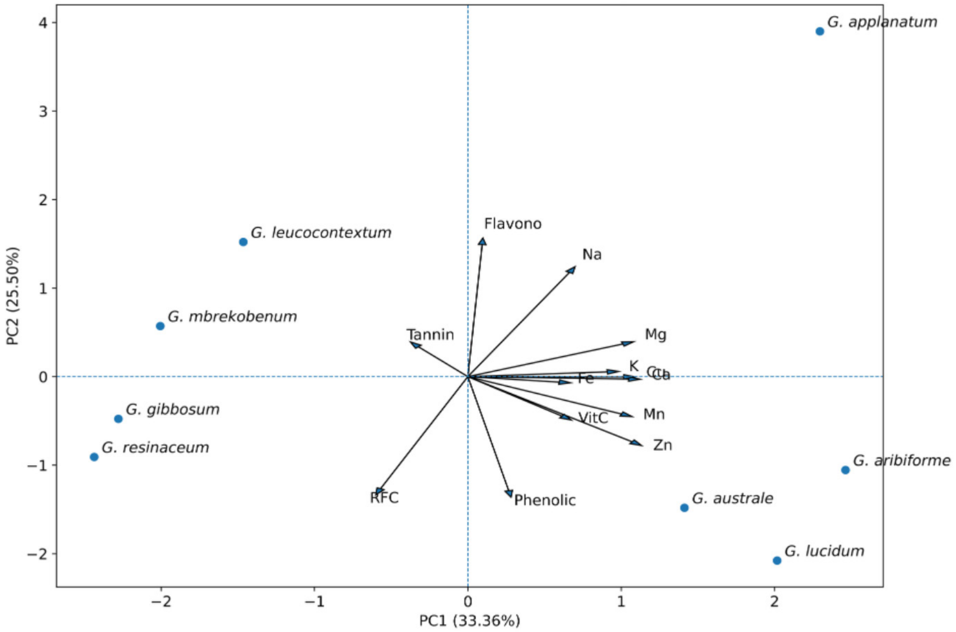

Fig. 4. Principal component analysis illustrating the relationship between nutritional composition, phytochemical constituents, and ethnomedicinal relevance among studied *Ganoderma* species.

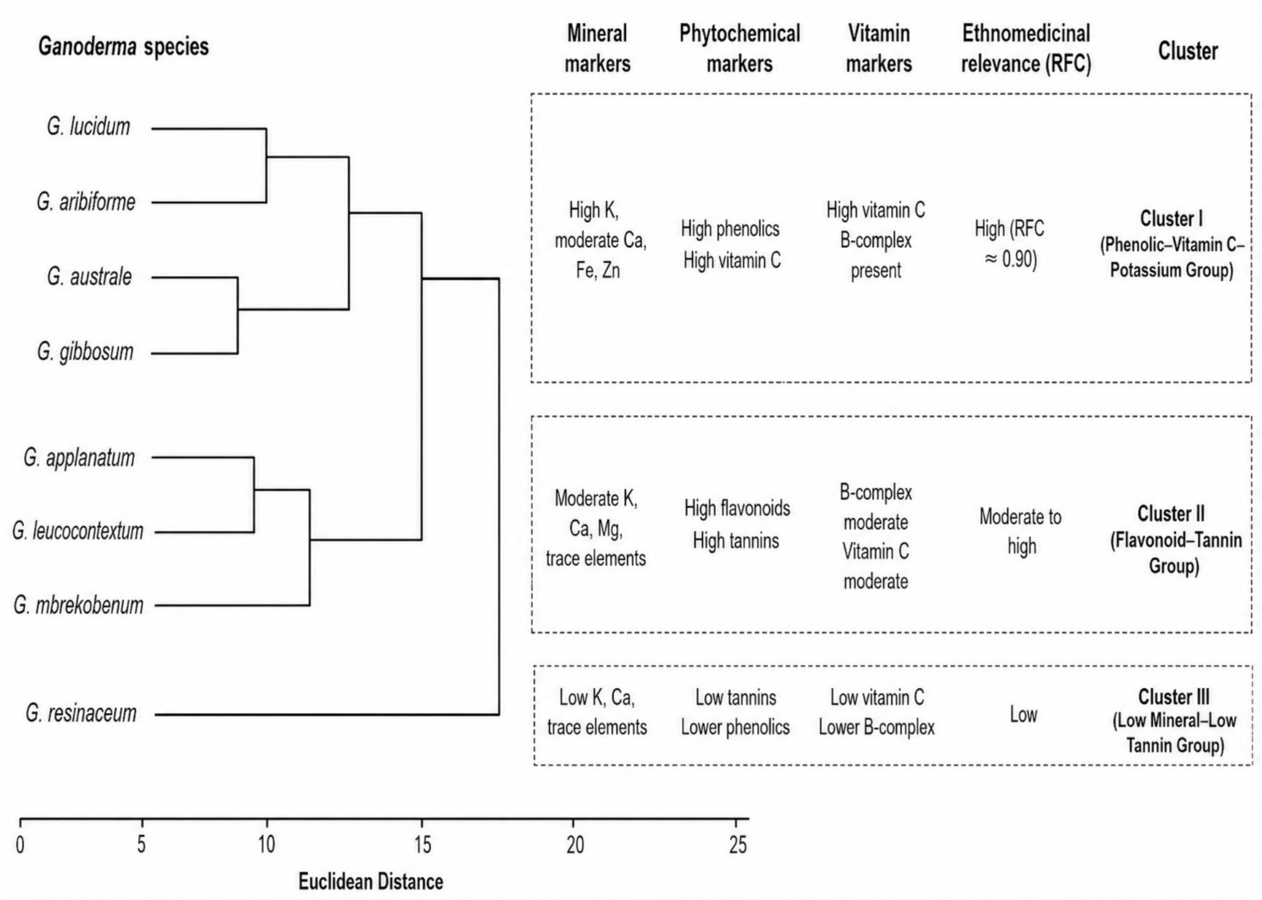

Fig. 5. Hierarchical cluster dendrogram illustrating the biochemical and ethnomedicinal relationships among the *Ganoderma* species based on mineral composition, phytochemical constituents, vitamin profiles, and ethnomedicinal relevance.

elevated phenolic content, vitamin C levels, and potassium content. *G. aribiforme* and *G. lucidum* exhibited the closest association due to their high phenolic content, elevated potassium content, and similar ethnomedicinal citation values. Likewise, *G. australe* and *G. gibbosum* clustered together because of comparable phenolic content and related phytochemical characteristics, suggesting similar antioxidant and therapeutic potential.

The second cluster included *G. applanatum, G. leucocontextum*, and *G. mbrekobenum*, characterized by relatively higher flavonoid and tannin content. *G. applanatum* and *G. leucocontextum* formed a close subgroup due to elevated flavonoid content, while *G. mbrekobenum* grouped nearby because of its highest tannin content. These associations suggest possible antimicrobial and anti-inflammatory potential linked to flavonoid- and tannin-rich profiles. *G. resinaceum* occupied a relatively isolated position within the dendrogram, associated with lower tannin, potassium, and calcium content. The clustering pattern demonstrated that species with high ethnomedicinal relevance shared similar phytochemical signatures, supporting the biochemical basis underlying traditional medicinal utilization of *Ganoderma* species.

Discussion

Diversity of Ganoderma *species*

The present study recorded eight Ganoderma species across the surveyed forest reserves. Mdabulo FR is likely to harbour higher species diversity due to the availability of abundant deadwood, mature host trees, and relatively low anthropogenic disturbance, conditions that are widely recognized to enhance saprotrophic fungal colonization (Brady 2024). Conversely, Lualage FR exhibited reduced abundance and species richness, indicating habitat degradation and limited substrate availability, consistent with disturbance-induced simplification of fungal communities (Solé et al. 2004; Dobson et al. 2006; Fischer, Lindenmayer 2007). The great distribution of *G. lucidum* across all reserves reflects its broad ecological amplitude and strong substrate adaptability, whereas the limited occurrence of *G. mbrekobenum, G. gibbosum*, and *G. resinaceum* suggests narrower ecological niches and possible dependence on specific microclimatic conditions. These abundance patterns further reflect functional ecological differentiation of *Ganoderma* species within Miombo woodland ecosystems, consistent with evidence that wood-decaying fungal assemblages are strongly influenced by host tree composition, coarse woody debris, and microhabitat structure (Solé et al. 2004; Dobson et al. 2006; Fischer, Lindenmayer 2007; Lonsdale, Pautasso 2008; Flibert et al. 2025).

The dominance of *G. lucidum* and *G. applanatum* across multiple reserves suggests their key role in decomposition processes, nutrient mineralization, and carbon cycling through enzymatic breakdown of cellulose, hemicellulose, and lignin (Sileshi et al. 2023; Konara et al. 2025). In contrast, the restricted distribution of species such as *G. mbrekobenum* indicates ecological specialization potentially driven by host specificity and sensitivity to microenvironmental variation, underscoring the importance of habitat integrity for sustaining fungal diversity and ecosystem functioning. Shannon–Wiener diversity indices (Fig. 2) further confirmed spatial heterogeneity among reserves, reflecting gradients in disturbance intensity and resource availability. Mdabulo FR emerged as a key diversity hotspot, supported by favourable ecological conditions, including mature host trees, abundant coarse woody debris observed during field surveys, and minimal anthropogenic disturbance. In contrast, Lualage FR consistently exhibited lower species richness and abundance, likely as a consequence of habitat degradation as also reported by Solé et al. (2004), Dobson et al. (2006), Fischer and Lindenmayer (2007), and Konara et al. (2025). From a conservation perspective, these findings emphasize the importance of maintaining habitat integrity in high-diversity reserves such as Mdabulo FR to safeguard both common and rare species and their ecological functions. In degraded areas such as Lualage FR, targeted restoration strategies, including enrichment of deadwood resources and reduction of anthropogenic pressures, may enhance species richness and improve ecosystem resilience.

Habitat and ecological drivers of Ganoderma *diversity*

The observed variation in *Ganoderma* distribution across the studied forest reserves demonstrates the strong influence of habitat structure and ecological drivers on macrofungal community assembly. The strong ecological associations detected between *G. lucidum, G. applanatum*, and variables such as soil moisture, canopy cover, deadwood abundance, and soil organic matter (Fig. S1), confirm that fungal occurrence is largely regulated by microclimatic stability and substrate availability. Forest ecosystems with dense canopy cover likely maintained favourable humidity and moderated temperature fluctuations, thereby enhancing mycelial establishment, sporocarp formation, and lignocellulosic decomposition processes. Similar ecological relationships have been widely reported in forest fungal communities, where moisture-retaining habitats and woody debris significantly determine fungal diversity and persistence (Fischer, Lindenmayer 2007; Lonsdale, Pautasso 2008). The predominance of *G. lucidum* in Mdabulo and Itulu FRs further suggests high ecological adaptability and broad substrate utilization capacity, traits commonly associated with successful saprotrophic and weakly parasitic fungi (Dobson et al. 2006). In contrast, the restricted occurrence of *G. mbrekobenum* and *G. resinaceum* indicates narrower ecological amplitudes and possible dependence on specific host substrates or localized environmental conditions. Such habitat specialization has previously been associated with variations in vegetation composition, deadwood heterogeneity, and forest disturbance gradients (Solé et al. 2004; Sileshi et al. 2023).

The strong positive association between *Ganoderma* abundance and deadwood availability emphasizes the ecological importance of decaying woody substrates in sustaining white-rot fungal communities. Deadwood serves not only as a nutritional resource but also as a critical ecological niche supporting fungal-mediated nutrient cycling and carbon turnover within forest ecosystems (Flibert et al. 2025; Konara et al. 2025). Such variation reflects ecological differentiation consistent with studies showing that wood-decaying fungi respond strongly to coarse woody debris, host composition, and microhabitat conditions rather than climate alone (Solé et al. 2004; Dobson et al. 2006; Fischer, Lindenmayer 2007; Lonsdale, Pautasso 2008; Flibert et al. 2025). Moreover, forests with high soil organic matter likely enhance moisture retention and support continuous decomposition dynamics, thereby promoting fungal productivity and diversity.

The diversity and distribution of *Ganoderma* species observed across the studied forest reserves appear to be strongly influenced by ecological and environmental conditions. Variations in substrate availability, vegetation structure, canopy cover, humidity, and decomposition intensity likely contributed to differences in species abundance and occurrence among sampling sites. Most *Ganoderma* taxa were predominantly associated with decaying hardwood substrates and mature forest stands characterized by relatively stable microclimatic conditions. These findings support the ecological role of *Ganoderma* species as important saprotrophic decomposers involved in lignocellulosic degradation and nutrient cycling within forest ecosystems. Furthermore, the observed habitat specificity suggests that environmental disturbance and changes in forest composition may significantly affect macrofungal diversity and ecological functioning. This study extends understanding of *Ganoderma* ecology in Miombo woodlands, linking species distribution patterns with habitat structure, while providing a baseline for biodiversity monitoring. Future research should integrate molecular tools and long-term monitoring to refine species detection and ecological inference, improving conservation planning outcomes under changing climatic conditions.

Linking ethnomycological utility and biochemical composition

As illustrated in Fig. 4 and 5, the ethnomycological relevance of *Ganoderma* species recorded in this study is strongly associated with their underlying phytochemical profiles and nutritional composition. Species frequently cited for managing pain, respiratory disorders, digestive complications, kidney-related ailments, and inflammation-associated conditions consistently exhibited rich profiles of phenolics, flavonoids, tannins, terpenoids, vitamins, and essential minerals known to contribute to antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory functions. However, despite these strong compositional associations, the present findings should not be interpreted as direct evidence of therapeutic efficacy because pharmacological validation, toxicity evaluation, and bioactivity-guided investigations were beyond the scope of this study. Consequently, the medicinal relevance of these species is best understood as ethnomycological knowledge supported by preliminary biochemical evidence rather than experimentally confirmed clinical effectiveness.

The widespread recognition of *Ganoderma* species within Miombo woodland communities reflects the persistence of indigenous healthcare systems and aligns with the broader global resurgence of interest in traditional medicinal fungi and wild bioresources (Fongnzossie et al. 2020; Kamalebo, De Kesel 2020). Similar to observations elsewhere, respondents associated these fungi with the treatment of chronic and inflammation-related conditions, suggesting that *Ganoderma* species function as accessible therapeutic alternatives integrated into local healthcare practices (Vicente-Pérez et al. 2024; Flibert et al. 2025). The broad range of ailments treated further indicates a long-standing cultural familiarity with the genus and sustained intergenerational transmission of ethnomycological knowledge.

Among the investigated taxa, *G. lucidum* emerged as the most culturally salient species, consistent with its global reputation as a medicinal macrofungus widely utilized in traditional medicine systems (Lee et al. 2012; Money 2016; Orywal et al. 2022; Flibert et al. 2025). Its prominence may partly derive from its abundance of antioxidant-associated compounds and micronutrients previously linked to immune regulation and metabolic support (Iyingiala et al. 2024; Plosca et al. 2025). Likewise, the frequent use of *G. applanatum* for wound healing and dermatological disorders corresponds with earlier reports highlighting its flavonoid-rich composition and associated antioxidant properties (Tibuhwa et al. 2013; Chelela et al. 2014). Other species, including *G. australe* and *G. leucocontextum*, demonstrated more specialized ethnomedicinal roles, suggesting species-specific therapeutic knowledge shaped by perceived efficacy and ecological availability.

The low reporting of culinary applications reinforces the perception of *Ganoderma* species as primarily medicinal rather than edible fungi within the study area. This pattern is consistent with previous studies attributing limited food use to their woody texture, bitter taste, and restricted culinary traditions (Vicente-Pérez et al. 2024; Karunarathna et al. 2025). Occasional use as dried flavour additives likely represents emerging adaptation rather than established dietary practice (El Sheikha 2022). Collectively, the strong correspondence between citation frequency, therapeutic versatility, and biochemical richness supports the utility of ethnomycological indices such as FC and RFC in identifying culturally significant species with potential pharmacological relevance (Faruque et al. 2018; Swallah et al. 2023; Shah et al. 2025). RFC and FC indices provided quantitative measures of ethnomycological importance; however, their interpretation should be approached cautiously because shared traditional knowledge among informants may influence citation frequency patterns. Consequently, high RFC values, particularly for culturally prominent species such as *G. lucidum*, may reflect strong cultural transmission in addition to perceived medicinal significance.

Linking mineral, vitamin composition and nutraceutical potential

The investigated *Ganoderma* species exhibited considerable interspecific variation in mineral and vitamin composition, highlighting their potential importance as supplementary nutraceutical resources. Essential macro- and micronutrients identified across species are widely recognized for their roles in metabolic regulation, enzymatic activity, immune function, electrolyte balance, and oxidative stress protection. Nevertheless, although these compositional attributes suggest potential health-promoting value, nutritional composition alone cannot be equated with physiological efficacy because bioavailability, digestibility, extraction efficiency, dosage, and compound stability substantially influence functional outcomes in humans. Therefore, further nutritional bioavailability studies and controlled pharmacological investigations remain necessary before therapeutic or commercial nutraceutical claims can be substantiated.

Species enriched with Ca and Mg may contribute to bone metabolism, neuromuscular regulation, and enzymatic processes, functions commonly associated with these minerals in human nutrition (Taskozhina et al. 2024; da Silva et al. 2025; Razzaque, Wimalawansa 2025;). Although wild mushrooms are often considered relatively poor sources of these elements (Nakalembe et al. 2015), the comparatively elevated content observed in some species indicate their possible value as complementary dietary supplements in mineral-deficient populations. Similarly, the predominance of K across the studied taxa aligns with broader reports demonstrating that potassium and phosphorus constitute major components of mushroom mineral profiles (Nakalembe et al. 2015). Such mineral composition may support cardiovascular regulation and electrolyte balance, reinforcing the nutritional significance of these fungi. The medicinal relevance of trace elements was further supported by the occurrence of Fe, Zn, Cu, and Mn, all of which function as cofactors in antioxidant enzymes, haematopoietic pathways, immune regulation, and tissue repair. Comparable observations have been reported in medicinal and edible macrofungi such as *Xerocomus badius* and *Boletus edulis* (Orywal et al. 2022), suggesting that *Ganoderma* species may similarly contribute to micronutrient supplementation. The coexistence of vitamin C with these trace elements may additionally enhance antioxidant protection and mineral bioavailability, particularly non-haem Fe absorption (Effiong et al. 2024; Vieira et al. 2025). This relationship corresponds with traditional uses of these species in strengthening immunity and reducing fatigue-associated conditions (Yang et al. 2025).

B-complex vitamins further strengthen the nutraceutical relevance of the investigated species. Vitamins such as thiamine, riboflavin, and niacin are central to energy metabolism, nervous system regulation, enzymatic reactions, and cellular maintenance (Mikkelsen, Apostolopoulos 2019; Hrubša et al. 2022). Their occurrence within Ganoderma species aligns with previous findings demonstrating that mushrooms can serve as valuable dietary sources of both water- and fat-soluble vitamins without substantial toxicity risks (Gharib et al. 2022; Effiong et al. 2024; Rijia et al. 2024). Consequently, the observed mineral–vitamin profiles provide mechanistic support for the ethnomedicinal relevance of these species while reinforcing their potential as functional nutraceutical resources.

Linking proximate, phytochemical composition and nutraceutical potential

The proximate and phytochemical composition of the investigated *Ganoderma* species provides important insight into their potential nutraceutical and medicinal significance. The occurrence of proteins, dietary fibre, carbohydrates, minerals, phenolics, flavonoids, polysaccharides, tannins, terpenoids, and related secondary metabolites suggests that these fungi may possess nutritional and health-promoting properties. However, the present findings should be interpreted cautiously because compositional evidence alone does not confirm pharmacological activity. Biological functions such as antioxidant, antimicrobial, anti-inflammatory, or anticancer effects require validation through targeted bioassays, toxicological studies, and clinical investigations. Consequently, the current study primarily establishes biochemical potential rather than confirmed therapeutic efficacy.

Substantial interspecific variation in phytochemical composition provides mechanistic explanation for the differential ethnomedicinal applications of *Ganoderma* species within local communities. Species rich in phenolics and flavonoids are likely to possess stronger antioxidant and anti-inflammatory properties through modulation of oxidative stress pathways and free radical scavenging activity. Similarly, tannin-rich taxa may exhibit enhanced antimicrobial and preservative functions due to protein-binding and membrane-disrupting mechanisms. Such biochemical distinctions may partly explain why particular species are selectively used in treating inflammation-associated disorders, respiratory conditions, microbial infections, and wound-related ailments.

Comparable observations have been reported in other medicinal macrofungi where phenolic metabolites contribute significantly to antioxidant and antimicrobial activity (Atri et al. 2019; Mafe et al. 2025). The proximate composition further supports the nutraceutical relevance of these species. Moisture variation likely influences shelf stability, storage capacity, and processing suitability, consistent with trends reported in wild mushrooms globally (Bhattu et al. 2024; Ablegue et al. 2025). High dietary fibre content strengthens their functional food value because mushroom fibre has been associated with gastrointestinal regulation and metabolic health improvement (Wickramasinghe et al. 2023; El-Maradny et al. 2025). Likewise, elevated ash content reflects substantial mineral accumulation, reinforcing their importance in nutraceutical formulations targeting enzymatic and cardiovascular functions (Nakalembe et al. 2015; Malunguja et al. 2025; Shah et al. 2025). The generally low-fat content observed across species is nutritionally advantageous for low-fat dietary applications, whereas the coexistence of proteins and carbohydrates suggests dual roles in energy provision and dietary supplementation.

Mushroom polysaccharides, including glucans and mannans, are also widely recognized for immunomodulatory and prebiotic functions (Barcan et al. 2024; Ma et al. 2025), supporting the ethnomedicinal relevance of Ganoderma species. At the same time, the presence of compounds commonly regarded as antinutritional, including tannins, phytates, oxalates, saponins, steroids, and terpenoids, highlights potential trade-offs between medicinal value and nutrient bioavailability (Vainio-Mattila 2000; Kaale et al. 2023; Mbwana, Bundala 2023; Mgalula 2024; Olesen et al. 2024; Swai et al. 2025). Nevertheless, many of these metabolites also possess recognized pharmacological functions, including anti-inflammatory and immune-modulating activities (Asad et al. 2025; Konara et al. 2025). Collectively, these findings reinforce the potential of *Ganoderma* species as multifunctional medicinal bioresources with promising nutraceutical applications.

Correlational analysis

The correlation analysis revealed strong biochemical interrelationships among minerals, vitamins, proximate constituents, and phytochemicals, providing important insights into the medicinal and nutritional functionality of the studied *Ganoderma* species. Significant positive associations among Ca, Mg, and vitamin B_2 indicated coordinated accumulation of structural minerals and energy-related vitamins, suggesting shared physiological pathways linked to enzymatic regulation and cellular metabolism. Such mineral–vitamin interactions are nutritionally important because they support bone development, neuromuscular coordination, and metabolic efficiency (Lyamuya et al. 2023; Mwamatope et al. 2023). Similarly, Fe showed strong positive correlation with Cu and vitamin B_3, while Cu was also closely associated with vitamin B_3, indicating an integrated redox-metabolic system. Iron and copper function as cofactors in oxidative enzymes and electron transport pathways, whereas vitamin B_3 serves as a precursor for NAD and NADP required in cellular respiration and redox homeostasis (Doroftei et al. 2020). These interactions provide biochemical support for the haematinic, immunomodulatory, and anti-fatigue properties widely reported for medicinal fungi and nutrient-rich functional foods (Mafe et al. 2025).

Additional mineral-vitamin interactions highlighted functional complementarity within the studied species. Positive associations between vitamin B_1 and Na emphasized the role of ionic balance in carbohydrate metabolism and nerve signalling, while vitamin C positively correlated with Zn, Cu, and Mn, suggesting improved mineral bioavailability and antioxidant protection. However, some weak correlations may reflect reduced bioavailability caused by matrix composition and anti-nutritional compounds (Nakalembe et al. 2015). Proximate analysis further demonstrated nutritional trade-offs. Moisture content negatively correlated with minerals, fibre, ash, and phytochemicals, indicating dilution effects that reduce nutrient density in high-moisture samples (Atri et al. 2019). Consequently, low-moisture matrices may favor preservation, extraction efficiency, and stability of bioactive compounds (Srivastava et al. 2024). Crude fat showed inverse relationships with several minerals and vitamins, whereas crude protein positively correlated with vitamin B2 and caloric value, emphasizing its role in tissue repair, immune regulation, and metabolic function (Konara et al. 2025). Carbohydrates were strongly associated with Fe, Cu, vitamin B_3, and energy value, reflecting their importance in metabolic activity and energy provision.

Phytochemical interactions further clarified the medicinal significance of *Ganoderma* species. Phenolic compounds were concentrated in nutrient-dense, low-moisture samples and contributed substantially to antioxidant capacity, whereas flavonoids positively correlated with protein, ash, and caloric value but negatively with total phenolics, indicating differentiation among antioxidant classes. Tannins positively associated with protein but negatively correlated with several minerals and flavonoids due to their chelating and protein-binding properties. These nutrient-phytochemical interactions suggest coordinated biochemical accumulation that may enhance therapeutic effectiveness through synergistic physiological effects.

The multivariate analyses reinforced these findings by linking biochemical composition with ethnomedicinal importance. PCA identified phenolics, minerals, vitamin C, and tannins as the principal variables driving species differentiation, while cluster analysis grouped species with comparable nutritional and phytochemical profiles. Species with higher ethnomedicinal citation frequency (RFC) were positively associated with phenolic content, whereas RFC negatively correlated with flavonoids, indicating stronger traditional preference for phenolic-rich species. The close ordination of *G. lucidum* and *G. aribiforme* reflected similarities in phenolic richness and mineral composition, while *G. australe* grouped with *G. gibbosum* because of comparable phenolic and vitamin C profiles. In contrast, *G. resinaceum* occupied a distinct position associated with lower tannin, potassium, and calcium content. These ecological, nutritional, and ethnomycological relationships demonstrate that *Ganoderma* species within Miombo woodlands represent important medicinal bioresources with significant nutraceutical and pharmacological potential.

Limitations of the study

Although the present study provides important ecological, ethnomycological, nutritional, and phytochemical insights into *Ganoderma* species occurring within Miombo woodlands, several limitations should be acknowledged when interpreting the findings.

A major limitation relates to species identification, which relied primarily on macro-morphological characteristics and ethnomycological knowledge without molecular confirmation. Because morphologically similar Ganoderma taxa may include cryptic species that cannot be reliably distinguished using morphology alone, the absence of DNA-based analyses limits taxonomic resolution and may influence species delimitation. Future studies should therefore integrate molecular approaches such as internal transcribed spacer sequencing, multilocus phylogenetic analyses, and metabolomic-assisted taxonomy to improve identification accuracy and validate species boundaries.

Another important limitation concerns the phytochemical and medicinal interpretation of the investigated species. Although the study established strong associations between ethnomedicinal importance and biochemical composition, experimental validation of biological activity was beyond the scope of this work. The observed relationships should be interpreted as preliminary compositional evidence rather than direct confirmation of therapeutic efficacy. Therefore, controlled laboratory investigations involving antioxidant, antimicrobial, anti-inflammatory, cytotoxicity, and ligninolytic bioassays are necessary to clarify the mechanistic biological functions underlying the traditional uses of these fungi.

Furthermore, phytochemical characterization relied primarily on semi-quantitative screening methods, which may not fully capture the structural complexity of individual metabolites. The application of advanced analytical techniques such as high-performance liquid chromatography, liquid chromatography–mass spectrometry, and gas chromatography–mass spectrometry would provide improved biochemical resolution through accurate identification and quantification of pharmacologically important compounds. Integrating these advanced metabolomic approaches would further strengthen understanding of the nutraceutical and medicinal potential of *Ganoderma* species within Miombo woodland ecosystems.

Conclusions

This study provides baseline ecological, ethnomycological, nutritional, and phytochemical information on *Ganoderma* species occurring within Miombo woodland ecosystems of Tanzania. Ethnomycological findings indicate that *Ganoderma* species are widely recognized within local healthcare systems, particularly in relation to chronic, recurrent, and inflammation-associated conditions. Species exhibiting broader ethnomedicinal recognition, especially *G. lucidum* and *G. applanatum*, also demonstrated comparatively rich nutritional and phytochemical profiles, suggesting possible relationships between traditional utilization patterns and biochemical composition. Nevertheless, these associations should be interpreted cautiously because the present study did not include experimental pharmacological validation.

The nutritional, mineral, vitamin, proximate, and phytochemical analyses demonstrated interspecific variation among the investigated *Ganoderma* species, indicating that biochemical composition is not uniform within the genus. Variability in content of phenolics, flavonoids, tannins, minerals, and other secondary metabolites may partly explain differences in ethnomedicinal applications reported by local communities. Similarly, the occurrence of antinutritional compounds suggests the importance of species-specific evaluation when considering potential dietary and medicinal utilization.

The integration of ecological distribution, ethnomycological knowledge, nutritional composition, and phytochemical characterization provides an important reference dataset for African medicinal macrofungi and may assist in prioritizing species for future molecular, pharmacological, toxicological, and metabolomic investigations. The findings also suggest the potential value of incorporating *Ganoderma* species into sustainable non-timber forest product initiatives aimed at supporting biodiversity conservation, local value addition, and community-based forest management within Miombo woodland ecosystems.

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Table S1. Demographic characteristics of informants from surveyed villages surrounding the studied FRs

Village surveyed	Informants	Age group	No. of persons	Percentage
Itul	Male	18 – 29	3	2.14
		30 – 44	4	2.86
		45 – 59	4	2.86
		60 – 70	4	2.86
	Female	18 – 29	5	3.57
		30 – 44	5	3.57
		45 – 59	5	3.57
		60 – 70	5	3.57
Mdabulo	Male	18 – 29	3	2.14
		30 – 44	4	2.86
		45 – 59	4	2.86
		60 – 70	4	2.86
	Female	18 – 29	5	3.57
		30 – 44	5	3.57
		45 – 59	5	3.57
		60 – 70	5	3.57
Lila	Male	18 – 29	3	2.14
		30 – 44	4	2.86
		45 – 59	4	2.86
		60 – 70	4	2.86
	Female	18 – 29	5	3.57
		30 – 44	5	3.57
		45 – 59	5	3.57
		60 – 70	5	3.57
Lualage	Male	18 – 29	3	2.14
		30 – 44	4	2.86
		45 – 59	4	2.86
		60 – 70	4	2.86
	Female	18 – 29	5	3.57
		30 – 44	5	3.57
		45 – 59	5	3.57
		60 – 70	5	3.57
			140	100.00

Table S2. Species diversity (Shannon–Wiener index), abundance, and density (individuals ha^{-1}) of *Ganoderma* species recorded in the studied Miombo woodland FRs

Species	**Ituli FR**		**Mdabulo FR**		**Lila FR**		**Lualage FR**	
	Abund.	**Density**	**Abund.**	**Density**	**Abund.**	**Density**	**Abund.**	**Density**
Ganoderma applanatum (Pers.) Pat.	69	2197	108	3439	91	2898	33	1051
Ganoderma leucocontextum T.H. Li, W.Q. Deng & Sheng H. Wu	82	2611	75	2389	88	2803	41	1306
Ganoderma lucidum (Curtis) P. Karst.	143	4554	146	4650	89	2834	68	2166
Ganoderma australe (Fr.) Pat.	46	1465	102	3248	71	2261	52	1656
Ganoderma arbiforme A.B. De & Ryvarden	44	1401	113	3599	45	1433	67	2134
Ganoderma mbrekobenum Ryvarden & Mensah	–	–	33	1051	–	–	–	–
Ganoderma gibbosum (Blume & T. Nees) Pat.	24	764	76	2420	26	828	–	–
Ganoderma resinaceum Boud.	37	1178	59	1879	–	–	–	–
Diversity (Shannon–Wiener index)	1.66		2.18		1.58		1.16	

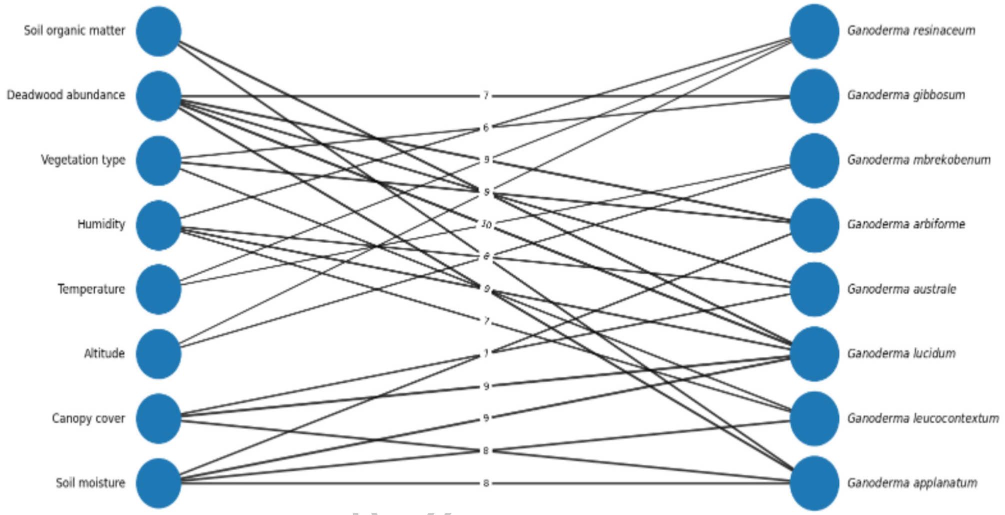

Fig. S1. Bipartite ecological association network illustrating the relationships between habitat variables and the distribution of *Ganoderma* species across the studied FRs. Connecting lines represent ecological associations between habitat factors and species occurrence, while the numerical values positioned along the connecting edges indicate the relative strength of association.

Table S3. Secondary metabolite profiles of the investigated *Ganoderma* species across Miombo forest reserves in northern and central Tanzania. '+++' denoting high concentration, '++'denoting moderate concentration, '+' denoting small concentration, and '–' denoting complete absence

Ant-nutritional factor	***G. applanatum***	***G. leucocontextum***	***G. lucidum***	***G. australe***	***G. arbiforme***	***G. mbrekobenum***	***G. gibbosum***	***G. resinaceum***
Alkaloids	+++	+++	+++	+++	+++	++	++	++
Phlobatannin	+	+	+	+	–	–	+	+
Saponin	++	+++	++	+++	+	+	++	+
Flavonoids	–	+	+	++	–	–	+	+
Anthraquinones	++	–	–	+	+	+	+	+
Steroids	+	+++	++	+	+	+	++	–
Terpenes	+	+	+	+	+	+	+++	++
Cardenolides	–	+	+	–	+	+	–	+
Cardiac glycosides	+	+	+	+	+	–	+	++
Phytates	+	–	–	+	+	+	+	+
Oxalates	+	+	+	+	+	+	–	+
Lectins	+	+	+	–	+	–	+	+
Phlobatannins	+	+	+	–	+	+	+	+
Betacyanins	+	–	+	–	–	+	+	+
Quinones	+	+	–	+	–	–	+	–
Goitrogens	+	–	+	–	–	+	–	+

Table S4. Pearson's correlation matrix showing relationships among minerals, vitamins, proximate components, and phytochemical constituents of the studied *Ganoderma* species

	Ca	Mg	K	Na	Fe	Zn	Cu	Mn	VitC	VitB1	VitB2	VitB3	Moisture	Fibre	Ash	CrudeF	CrudeP	Carbo	Calor	Phen	Flav
Ca	01																				
Mg	0.84**	1																			
K	0.59	0.42	1																		
Na	0.25	0.51	0.14	1																	
Fe	0.19	−0.01	0.40	0.24	1																
Zn	0.61	0.34	0.59	0.07	0.43	1															
Cu	0.37	0.38	0.29	0.46	0.76*	0.53	1														
Mn	0.43	0.63	0.36	0.23	0.04	0.59	0.52	1													
VitC	0.32	0.15	0.54	−0.27	0.10	0.47	0.34	0.42	1												
VitB1	0.33	0.69	0.26	0.738*	−0.16	0.09	0.09	0.50	−0.30	1											
VitB2	0.84**	0.92**	0.41	0.26	−0.20	0.16	0.10	0.40	0.20	0.50	1.00										
VitB3	0.70	0.51	0.53	0.18	0.77*	0.67	0.86**	0.44	0.42	−0.02	0.37	1									
Moisture	−0.31	−0.22	−0.41	0.05	0.02	−0.50	0.21	−0.32	0.22	−0.45	−0.16	−0.04	1								
Fibre	−0.30	−0.26	0.19	0.56	0.66	0.10	0.51	−0.07	−0.06	0.09	−0.50	0.16	0.20	1							
Ash	0.00	−0.10	0.41	0.19	0.04	−0.02	0.04	−0.21	0.54	−0.13	−0.01	−0.06	0.44	0.41	1						
Crude F	−0.47	−0.41	−0.40	−0.66	−0.22	−0.52	−0.18	−0.18	0.26	−0.63	−0.22	−0.20	0.57	−0.32	−0.04	1					
Crude P	0.45	0.45	0.24	0.08	−0.01	−0.37	−0.11	−0.27	−0.05	0.11	0.69	0.20	0.17	−0.32	0.12	0.11	1				
Carboh	0.33	0.24	0.05	0.30	0.723*	0.37	0.91**	0.23	0.27	−0.21	0.05	0.81*	0.44	0.37	0.02	−0.01	0.01	1			
Calor	0.40	0.37	0.11	0.04	0.301	−0.24	0.33	−0.14	0.16	−0.18	0.51	0.51	0.51	−0.14	0.09	0.34	0.825*	0.5	1		
Pheno	0.24	0.10	0.00	−0.42	0.222	0.49	0.18	0.39	−0.06	−0.12	0.01	0.41	−0.55	−0.38	−0.85	0.02	−0.23	0.2	−0.1	1	
Flavo	0.13	0.33	0.11	0.69	0.115	−0.45	0.11	−0.30	−0.25	0.40	0.35	0.01	0.39	0.35	0.43	−0.22	0.66	0.1	0.5	−0.718*	1
Tannin	−0.03	−0.06	0.34	−0.25	0.097	−0.46	−0.30	−0.39	−0.02	−0.11	0.19	−0.02	0.04	−0.09	0.16	0.34	0.735*	−0.3	0.5	−0.19	0.40

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