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Towards medicinal tea from untapped Namibian *Ganoderma*: Phenolics and in vitro antioxidant activity of wild and cultivated mushrooms

Ganoderma is a genus of mushrooms that is prized in developed nations, especially those in Asia, due to its health-promoting properties, which are attributed to bioactive compounds such as phenolics. However, in developing countries, particularly in Africa, *Ganoderma* mushrooms are untapped and are barely identified. In this study, we identified *Ganoderma* species collected from different host trees in the wild in Namibia, cultivated them on one substrate and determined their water absorption and solubility indices. Total phenolics (TP), total flavonoids (TF), condensed tannins (CT) and in vitro antioxidant activity (AA) were determined in hot water infusions made from wild and cultivated *Ganoderma* mushrooms. Folin–Ciocalteu, aluminium chloride, vanillin-HCl, and DPPH assay methods were used to determine TP, TF, CT and AA, respectively. Wild species had 6.12–11.70% moisture, 1.91–5.32% ash, 11.55–24.40 (g of absorbed water/g of dry sample) water absorption index, 3.60–24.10% water solubility index, 18.37–44.78 (mg GAE/g of sample) TP, 0.09–1.67 (mg QE/g of sample) TF, 2.97–6.37 (mg CAE/g of sample) CT and 40.8–49.3% AA. Cultivated species had 9.64–13.45% moisture, 2.34–6.20% ash, 13.55–28.30 water absorption index, 6.40–25.35% water solubility index, 36.70–52.73 (mg GAE/g of sample) TP, 0.41–0.86 (mg QE/g of sample) TF, 11.38–15.29 (mg CAE/g of sample) CT and 53.6–63.7% AA. Infusions prepared from cultivated *Ganoderma* species had higher levels of TP, CT and AA, but lower levels of TF than those prepared from wild *Ganoderma* species, suggesting that they have potential as nutraceuticals.

Significance:

- The identification and confirmation of highly prized Lingzhi 'mushrooms of immortality' in Namibia highlights the presence of this untapped resource in Africa that is potentially worth billions of dollars.
- The cultivation and phenolic content of this high-value medicinal mushroom have been demonstrated.
- Cultivation could lead to sustainable utilisation and employment creation in developing countries which suffer from unemployment rates of at least 30%.

Introduction

Ganoderma is a genus of mushrooms that are used in food[1] and medicinal[2] products, mostly in Asian markets – an industry contributing a total of USD1628.4 million in 1995[1]. *Ganoderma* products exist in various forms which include capsules, tablets, and infusions such as coffee and tea.[3] *Ganoderma* mushrooms are distributed in many Asian, African and European countries, the United States of America and the United Kingdom.[1,4-7]

The diversity of species of *Ganoderma* includes *Ganoderma lucidum* (basal stem rot), *Ganoderma applanatum* (artist's conk), *Ganoderma tsugae* (hemlock varnish shelf), *Ganoderma neo-japonicum* and *Ganoderma australe* (southern bracket).[8,9] Studies on, particularly, Asian *Ganoderma* mushrooms are abundant in the literature. These studies include those on their taxonomy[1,10] and nutrients[11]. The medicinal effects, health-promoting activities such as antibacterial, anticancer[12], antitumour[13], anti-inflammatory[13], antidiabetic[14] and antioxidant[8], and biologically active compounds such as polysaccharides, triterpenoids[15] and polyphenolic contents of, mostly Asian, *Ganoderma* mushrooms are noted in the literature.

While developed nations, especially in Asia, have valorised the edible and medicinal properties of *Ganoderma* – the 'mushroom of immortality', particularly *G. lucidum*, Africa lags. *Ganoderma* mushrooms remain untapped resources in developing nations such as Namibia. In fact, there are only a few studies in Africa on *Ganoderma* species, including a survey on the distribution, genetic diversity and opinions on indigenous uses of *Ganoderma* mushrooms[5,16] and a qualitative study on the mycochemical and antibacterial activities of wild *G. lucidum*[17] in Namibia. To contribute to the understanding and potential value-add of *Ganoderma* species in Africa, we investigated the water solubility and absorption indices, phenolic composition and antioxidant activities of different wild *Ganoderma* species collected from different host trees as well as of cultivated samples in Namibia.

Materials and methods

Sample collection and preparation

Ganoderma fruiting bodies ($n=15$) were collected from six different host tree species in three central northern regions in Namibia (Table 1). The collection was done randomly from any host tree on which a fruiting body was seen. The host tree species were identified by their local names with the help and voluntary permission of the owners of the plots from where the mushrooms were collected. The fruiting bodies were transported to Windhoek a day after collection in khaki/brown paper bags. The following day the fruiting bodies were cleaned using a dry paper towel to remove foreign matter such as soil, grass and dust. The fruiting bodies were then sun-dried for at

least 8 h and packaged in clean khaki paper bags which were stored at room temperature until analyses.

Sample identification

Cetyltrimethylammonium bromide (CTAB) extraction buffer (20 g w/v CTAB, 1 M Tris-HCl pH 8.0, 5 M NaCl, 0.5 M EDTA, 2.5 μL 2-mercaptoethanol, 0.02 g polyvinylpolyrrolidone) was used to obtain DNA from wild *Ganoderma* fruiting bodies following a Soltis laboratory CTAB DNA extraction protocol described by Doyle and Doyle[18]. Polymerase chain reaction (PCR) cycles consisted of an initial denaturation at 94 °C for 4 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 48 °C for 1 min 30 s, and extension at 72 °C for 1 min. The final extension was set to 72 °C for 10 min to complete the reaction and the PCR products were stored at 4 °C. PCR products were visualised using GelGreen® dye under UV light after electrophoresis on agarose gel (1% w/v). Internal transcribed spacer (ITS 1 and 4) primer sequences were compared with those in NCBI GenBank using the BLAST search tool. *Ganoderma* species were identified based on the sequences in GenBank with 98–100% similarity.

Mushroom cultivation

Cultivation was done following the procedures outlined by Ueitele et al.[19] with few modifications. Mushroom cultivation included pure culture preparation, spawn development, substrate inoculation and, lastly, fruiting.[20]

Moisture content and water absorption and solubility indices

The moisture content of the ground fruiting bodies was determined by drying them in an oven (Scientific Series 2000, South Africa) at 135 °C for 2 h following the method of the Association of Official Analytical Chemists21. Ash content was determined by burning in the muffle furnace at 600 °C for 2 h following the Association's[21] method. Water absorption index (WAI) and water solubility index (WSI) of the ground fruiting bodies were determined following the method described by Rweyemamu et al.[22] with modifications. WAI was determined by weighing 0.1 g of sample into a 15 mL centrifuge tube and adding 10 mL distilled water. The tubes were vortex mixed for 30 min and centrifuged at 3000 x g for 20 min. The supernatant was decanted off and the weight of water absorbed after decantation was recorded. WAI was calculated according to Equation 1:

$$WAI = \frac{\text{Weight of the absorbed water (g)}}{\text{Dry weight of sample (g)}} \quad \text{Equation 1}$$

WSI was determined by drying the supernatant of the sample obtained in analysis of WAI at 105 °C for 3 h. WSI was calculated according to Equation 2:

$$WSI = \frac{\text{Weight of dry solids in supernatant (g)}}{\text{Dry weight of sample (g)}} X\ 100 \quad \text{Equation 2}$$

Preparation of infusions (hot water extracts)

Hot water infusions were prepared in duplicate from ground fruiting bodies by steeping 0.1 g of ground sample into 40 mL of boiled tap water for 5 min and filtering through 11-μm Whatman paper following the methods described by Hussein et al.[23] and Herrera et al.[24] with few modifications. After filtration, the infusions were stored in the fridge at -4 °C for 2 days prior to analysis of phenolic composition and in vitro antioxidant activities.

Total phenolics

Total phenolic content was determined using the Folin–Ciocalteu method described in McDonald et al.[25] using a spectrophotometer (Spectro UV-11, MRC Lab, Essex, UK). The total phenolic content is expressed as gallic acid (Sigma-Aldrich, Germany) equivalent (GAE) on dry weight of the sample.

Total flavonoids

Total flavonoid content was determined using the aluminium chloride method described by Chang et al.[26] using a spectrophotometer (Spectro UV-11, MRC Lab). The total flavonoid content is expressed as quercetin (Sigma-Aldrich, Germany) equivalent (QE) on dry weight of the sample.

Condensed tannins

Condensed tannins were determined using the vanillin-HCl method described by Price et al.[27] using a spectrophotometer (, Spectro UV-11, MRC Lab). The condensed tannins were expressed as catechin (Sigma-Aldrich, Germany) equivalent (CAE) on dry weight of the sample.

Antioxidant activity

Spectrophotometric antioxidant activity of infusions was done according to the method of McCune and Johns[28]. A mixture consisting of 1 mL of sample extract, 1 mL of 0.3 mM DPPH (2,2-diphenyl-1-picryl-hydrazyl) solution (Sigma-Aldrich, Germany) and 1 mL of methanol (Merck, Germany) was incubated for 10 min in the dark. The radical scavenging activity was calculated as a percentage inhibition of DPPH discolouration according to Equation 3:

$$\%\ inhibition = \frac{A_0 - As}{A_0} \text{ x } 100, \quad \text{Equation 3}$$

where A_s is the absorbance of the sample extract or standard and A_0 is the absorbance of the negative control, which is the blank. Quercetin was used as the standard.

Statistical analysis

All determinations for physicochemical properties were done in duplicate. Determinations for phenolics and in vitro antioxidant activity were done in triplicate following two independent extractions. The results are reported as mean±standard deviation. Statistical analyses were done using SPSS software version 21. One-way analysis of variance (ANOVA) was done for the comparison of mean values and means that differed significantly ($p<0.05$) were separated using Duncan's post-hoc test.

Results and discussion

Sample identification

The number of identified *Ganoderma* species per host tree studied is given in Table 1. Four of these *Ganoderma* species were cultivated on one substrate.

Cultivated Ganoderma *and yield*

Cultivated *G. enigmaticum* collected from *Sclerocarya birrea* (C-PA-SBGE), cultivated *G. wiireonse* collected from *Mundelea sericea* (C-PA-MSGW$_1$), cultivated *G. wiireonse* collected from *Colophospermum mopane* (C-PA-CMGW) and cultivated *G. lucidum* collected from *Colophospermum mopane* (C-PA-CMGL) yielded fruiting bodies. The weight of the harvested fruiting bodies was recorded to be 3.65 g, 4.01 g, 5.72 g and 2.62 g for samples C-PA-CMGL, C-PA-MSGW$_1$ and C-PA-SBGE, respectively. The yield (0.762 g/kg) and biological efficiency (0.08%) obtained during cultivation of *Ganoderma* species in this study were lower when compared to the yields (210.9–235.2 g/kg) and biological efficiencies (6.8–7.6%) reported by Roy et al.[29] This difference could be due to inadequate nutrients provided by the substrates for mushrooms to sprout, as reported by Kadhila-Muandingi et al.[30]

Moisture

The moisture content of the wild and cultivated *Ganoderma* species ranged between 6.12% and 13.45% (Table 2) and differed significantly ($p<0.05$) in the following order: C-PA-CMGL ≥ C-PA-SBGE ≥ C-PA-MSGW = W-SE-GW ≥ W-CM-GE3 ≥ C-PA-CMGW = W-CM-GL = W-CM-GW = W-SB-GE = W-PL-GE = W-CC-GE4 = W-CC-GE3 = W-MS-GE > W-MS-GW2 = W-MS-GW1 = W-CC-GE2 = W-CC-GE1. All wild species had moisture contents <10%, except W-CM-GE3 and W-PL-GE. All the cultivated species had moisture contents >10% except C-PA-CMGW. Cultivated species had higher moisture

Table 1: Wild *Ganoderma* species collected from different host trees of different species or from different host trees of the same species

Location (region)	Scientific names of host tree species	Local names of host tree species	*Ganoderma* collected from the wild	Cultivated *Ganoderma* that yielded fruiting bodies
Oshana	*Colophospermum mopane*	Omusati	*G. enigmaticum* (3) *G. lucidum* (1) *G. wiireonse* (1)	1 1
Oshikoto	*Mundelea sericea*	Omumbanganyana	*G. enigmaticum* (1) *G. wiireonse* (2)	1
Ohangwena	*Combretum collinum*	Omupupwaheke	*G. enigmaticum* (4)	
Ohangwena	*Pechuel-Loeschea leubuitziae*	Edimba	*G. enigmaticum* (1)	
Ohangwena	*Sclerocarya birrea*	Omwoongo	*G. enigmaticum* (1)	1
Ohangwena	*Senegaria erioloba*	Omwoonde	*G. wiireonse* (1)	
Total			**15**	**4**

content than all wild species except for C-PA-CMGW. The differences in moisture content could be due to variation in environmental conditions such as temperature and humidity during the growing period.[29] The moisture contents of the wild and cultivated species were comparable to the moisture contents reported for wild *G. lucidum,* such as 7.5%[31], 8.10%[11], 10.78% and 11.47%[32].

Ash

The ash content of wild and cultivated *Ganoderma* species ranged between 1.91% and 6.20% (Table 2) and differed in the following significant ($p<0.05$) order: C-PA-CMGL > W-MS-GE ≥ C-PA-SBGE ≥W-CM-GE ≥ W-MS-GW2 = W-CM-GW ≥ W-CC-GE4 ≥ W-CC-GE2 = W-CM-GE2 ≥ W-CC-GE1 ≥ C-PA-CMGW = W-SE-GW = W-CM-GE3= W-CM-GE1 ≥ C-PA-MSGW = W-SB-GE = W-CC-GE3 ≥ W-MS-GW1 ≥ W-PL-GE. For wild species, the highest ash content was observed in W-MS-GE (5.32%) and the lowest in W-PL-GE (1.91%). The differences in their ash contents could be due to the influence of the host trees.[10] Ash content (1.91–5.32%) of wild species was within values (0.88–9.70%) reported for wild *G. lucidum* and other *Ganoderma* species.[2]

For cultivated species, the highest ash content (6.20%) was observed in C-PA-CMGL and the lowest (2.34%) in C-PA-MSGW$_1$. The difference in their ash contents could be due to the influenced of the species type.[33] Ash content (2.34–6.20%) of cultivated species was within the range of that reported for cultivated *G. lucidum* (1.40–10.07%).[34] Although the highest ash content was reported in a cultivated species (C-PA-CMGL), the second highest was reported in a wild species (W-MS-GE) and their ash contents were not statistically different ($p>0.05$). Ash contents of the other three cultivated species (C-PA-SBGE, C-PA-CMGW, C-PA-MSGW$_1$) were also not significantly different ($p>0.05$) from most of those of the wild species. This finding could indicate that both cultivated and wild species are potential sources of minerals.

Water absorption index

The water absorption indices of wild and cultivated *Ganoderma* species ranged between 11.55 g and 28.30 g of absorbed water/g dry sample (Table 2) and differed in the following significant ($p<0.05$) order: W-CM-GE$_2$ = W-PL-GE ≤ W-CC-GE$_1$ W-SB-GE ≤ C-PA-MSGW$_1$ ≤ W-CC-GE$_3$ = C-PA-CMGL < W-CM-GE$_3$ < W-CM-GE$_1$ W-SE-GW ≤ W-MS-GE ≤ C-PA-CMGW ≤ W-CM-GW = W-CM-GL < C-PA-SBGE. The lowest water absorption index for wild species was observed in W-PL-GE (11.55 g of absorbed water/g of dry sample) and the highest was observed in W-CM-GE$_1$ (21.30 g of absorbed water/g of dry sample).

For cultivated species, the lowest water absorption index was observed in C-PA-MSGW$_1$ (13.55 g of absorbed water/g of dry sample) and the highest in C-PA-SBGE (28.30 g of absorbed water/g of dry sample). The water absorption indices of some species (W-CM-GE$_2$, W-CC-GE$_1$, W-PL-GE, W-SB-GE, C-PA-MSGW$_1$) were comparable to those reported by Singh et al.[10], while the rest of both cultivated and wild species had higher water absorption indices. The differences could be due to variation in the amounts of water-soluble constituents of the individual *Ganoderma* mushrooms.[10,35]

A low water absorption index could indicate that the species has more hydrophilic constituents (soluble sugars, organic acids, phenolic compounds).[35] Therefore, cultivated (C-PA-MSGW$_1$, C-PA-CMGL) and wild (W-CM-GE$_2$, W-CM-GE$_3$, W-CC-GE$_1$, W-CC-GE$_3$, W-SB-GE) species that have low water absorption indices could be considered suitable for the formulation of nutraceuticals such as hot water extracts (infusions, tea).

Water solubility index

The water solubility indices of wild and cultivated *Ganoderma* species ranged between 3.60% and 25.35% (Table 2) in the following significant ($p<0.05$) order: C-PA-CMGL = W-SE-GW ≥ C-PA-MSGW = W-CM-GL ≥ W-CM-GE1 > C-PA-CMGW > W-CC-GE3 = C-PA-SBGE = W-CM-GW = W-PL-GE = W-CC-GE1 = W-MS-GE = W-CM-GE2 > W-SB-GE. The highest water solubility index for wild species was observed in W-SE-GW (24.10%) and the lowest in W-SB-GE (3.60%).

For cultivated species, the highest water solubility index was observed in C-PA-CMGL (25.35%) and the lowest in C-PA-SBGE (6.40%). Some species (W-SB-GE, W-PL-GE, W-CMGW, W-CM-GE$_2$, and C-PA-SBGE) had water solubility indices comparable to those (5.35–6.70%) reported for wild *G. lucidum* and *G. brownie.*[36] The rest of the species had higher water solubility indices than that reported by Singh et al.[36]

Significant differences ($p<0.05$) in high solubility indices were observed in both wild (W-SE-GW, W-CM-GE$_1$, W-CM-GE$_3$) and cultivated (C-PA-CMGL, C-PA-MSGW$_1$, C-PA-CMGW) species. This could mean that both wild and cultivated species have high amounts of water-soluble polysaccharides and phenolic compounds.[36,37]

Total phenolics

The total phenolic content of infusions prepared from wild and cultivated *Ganoderma* species ranged between 18.37 mg GAE/g of sample and 52.73 mg GAE/g of sample (Table 3). This was in the following significant ($p<0.05$) order: C-PA-CMGL > W-MS-GE ≥ C-PA-MSGW ≥ C-PA-SBGE > C-PA-CMGW > W-SE-GW > W-MS-GW2 > W-CC-GE4 ≥ W-CC-GE3 ≥ W-SB-GE = W-CC-GE2 ≥ W-MS-GW1 = W-CC-GE1 > W-CM-GL = W-CM-GE2 > W-CM-GW = W- PL GE = W-CM-GE3 = W-CM-GE1.

For wild species, the infusion prepared from W-MS-GE had the highest total phenolic content (44.78 mg GAE/g of sample) and the infusion prepared from W-CM-GW had the lowest total phenolic content (18.89 mg GAE/g of sample). For cultivated species, the infusion prepared from C-PA-CMGL had the highest total phenolic content (52.73 mg GAE/g of sample) and that prepared from C-PA-CMGW had

Table 2: Physicochemical properties of ground fruiting bodies of wild and cultivated *Ganoderma* species

Sample code	Moisture (%)	Ash (%)	WAI	WSI (%)
W-CM-GE$_1$	7.56 ± 0.08^{e}	2.89 ± 0.21^{fghi}	21.30 ± 0.00^{cd}	19.10 ± 0.14^{b}
W-CM-GE$_2$	7.41 ± 0.54^{e}	3.30 ± 0.42^{efg}	12.55 ± 0.35^{g}	6.50 ± 0.42^{f}
W-CM-GE$_3$	9.56 ± 0.08^{cd}	2.51 ± 0.21^{ghi}	18.70 ± 0.92^{e}	16.60 ± 0.92^{c}
W-MS-GE	9.29 ± 0.64^{d}	5.32 ± 0.62 ^{ab}	20.75 ± 0.57^{bcd}	7.00 ± 0.50^{ef}
W-CC-GE$_1$	6.12 ± 0.18^{e}	3.19 ± 0.67^{efgh}	13.70 ± 0.92^{fg}	8.95 ± 1.48^{e}
W-CC-GE$_2$	6.18 ± 0.74^{e}	3.33 ± 0.28^{efg}	–	–
W-CC-GE$_3$	9.70 ± 0.79^{d}	2.40 ± 0.13^{ghi}	15.40 ± 1.84^{f}	11.90 ± 0.35^{d}
W-CC-GE$_4$	9.20 ± 0.31^{d}	3.62 ± 0.14^{def}	–	–
W-PL-GE	10.15 ± 0.01^{d}	1.91 ± 0.03^{i}	11.55 ± 0.85^{g}	6.40 ± 1.70^{f}
W-SB-GE	9.14 ± 0.32^{d}	2.48 ± 0.60^{ghi}	13.15 ± 0.28^{fg}	3.60 ± 0.57^{g}
W-CM-GW	9.07 ± 1.42^{d}	4.07 ± 0.43^{cde}	24.40 ± 0.14^{b}	6.20 ± 0.28^{f}
W-MS-GW$_1$	7.08 ± 0.87^{e}	2.19 ± 0.12^{hi}	–	–
W-MS-GW$_2$	6.56 ± 0.16^{e}	4.21 ± 0.03^{cde}	–	–
W-SE-GW	11.70 ± 0.46^{bc}	2.90 ± 0.34^{fghi}	19.55 ± 0.64^{cd}	24.10 ± 1.70^{a}
W-CM-GL	9.64 ± 0.10^{d}	4.50 ± 0.85^{bcd}	24.25 ± 0.35^{b}	19.20 ± 0.28^{b}
C-PA-SBGE	12.32 ± 0.28^{ab}	4.64 ± 0.01^{bc}	28.30 ± 0.57^{a}	6.40 ± 1.06^{f}
C-PA-CMGW	9.64 ± 0.04^{d}	2.83 ± 0.22^{fghi}	22.65 ± 1.48^{bc}	15.00 ± 2.33^{c}
C-PA-MSGW$_1$	11.75 ± 0.28^{bc}	2.34 ± 0.03^{ghi}	13.55 ± 1.98^{efg}	21.90 ± 0.85^{b}
C-PA-CMGL	13.45 ± 0.62^{a}	6.20 ± 1.66^{a}	15.40 ± 2.12^{f}	25.35 ± 1.91^{a}

WAI, water absorption index (expressed as gram of water absorbed per gram of dry sample); WSI, water solubility index; W, wild; CM, Colophospermum mopane*; GE,* Ganoderma enigmaticum*; MS,* Mundelea sericea*; CC,* Combretum collinum*; PL,* Pechuel-Loeschea leubuitziae*; SB,* Sclerocarya birrea*; GW,* Ganoderma wiireonse*; SE,* Senegaria erioloba*; GL,* Ganoderma lucidum*; C, cultured; PA,* Pterocarpus angolensis

1–4 on a sample code indicate the same Ganoderma *species collected from different host trees of the same species*

Values are mean±s.d. (n=*2). Means with different superscripted letters in the same column differ significantly* (p<*0.05).*

–, not analysed

the lowest total phenolic content (36.70 mg GAE/g of sample). The total phenolic contents of infusions prepared from both wild and cultivated species were comparable to those reported by Cor et al.[38] and Raseta et al.[39] (21.06–46.97 mg GAE/g and 11.55–77.10 mg GAE/g, respectively). On the other hand, the total phenolic contents found in this study were higher than those reported by Rajoriya et al.[40] (8.44–11.60 mg GAE/g) and were lower than those found by Sharif et al.[41] (60.72–360.72 mg GAE/g). Higher total phenolic contents (360.72 mg GAE/g) reported for hot water extracts by Sharif et al.[41] could be influenced by their longer extraction time (overnight) compared to the 5-min extraction time used in this study.

Infusions prepared from cultivated species had significantly ($p<0.05$) higher total phenolic contents than infusions prepared from wild species, except for one prepared from W-MS-GE. The collected wild *Ganoderma* fruiting bodies used appeared to be more mature than the cultivated fruiting bodies, which might explain why the infusions from wild species had lower total phenolic contents than those of cultivated species, because the total phenolic content of a mushroom is influenced by the species, the substrate, and the maturity of the fruiting body.[33] Furthermore, low total phenolic content could be a result of defence mechanisms due to aging.[33] The total phenolic content comprises compounds such as phenolic acids, flavonoids and tannins, and these compounds are known to have health-promoting properties such as antioxidant[8], anticancer[12], antidiabetic[14], anti-inflammatory[13] and antimicrobial[42] properties.

Total flavonoids

The total flavonoid content of infusions prepared from wild and cultivated *Ganoderma* species ranged between 0.09 mg QE/g of sample and 1.67 mg QE/g of sample on dry weight (Table 3) in the following significant ($p<0.05$) order: W-MS-GE > W-CC-GE2 > C-PA-SBGE > W-MS-GW1 > C- PA-CMGL = C-PA-CMGW = W-CC-GE3 > C-PA-MSGW > W-SE-GW = W-PL-GE ≥ W-SB-GE ≥ W-MS-GW2 = W-CM-GE2 = W-CM-GE1 ≥ W-CC-GE4 = W-CC-GE1 = W-CM-GE3 > W-CM-GL = W-CM-GW. For wild species, the infusion prepared from W-MS-GE had the highest total flavonoid content (1.67 mg QE/g of sample) and the infusion from W-CM-GL had the lowest total flavonoid content (0.09 mg QE/g of sample). For cultivated species, the infusion prepared

from C-PA-SBGE had the highest total flavonoid content (0.86 mg QE/g of sample) and the infusion from C-PA-MSGW$_1$ had the lowest total flavonoid content (0.41 mg QE/g of sample).

Infusions prepared from W-MS-GE, W-CC-GE$_2$, W-CC-GE$_3$, W-MS-GW$_1$, C-PA-SBGE, C-PA-CMGW and C-PA-CMGL had total flavonoid contents comparable to those reported by Rajoriya et al.[40] (0.62–2.14 mg QE/g). All other infusions had lower total flavonoid contents than those reported by Rajoriya et al.[40] Low levels of flavonoids could be a result of their involvement in defence mechanisms due to aging of fruiting bodies, which results in decreased contents during extraction as reported by Wandati et al.[33] who found high levels of total flavonoids (1129.75 mg/100g) in young fruiting bodies compared to relatively low levels (890.87 mg/100g) in mature fruiting bodies.

Although apparent total flavonoid content was determined in mushrooms in this study and previous studies[41,43], Gil-Ramírez et al.[44] contended that mushrooms do not contain flavonoids because they lack the main enzymes (chalcone synthase and chalcone isomerase) involved in their metabolic pathway. Apparently, what is determined by the aluminium chloride colourimetric method used for detection of flavonoids by most researchers are other phenolic compounds such as chlorogenic acid, o-diphenols, melanin-precursors or ergosterol, which are not flavonoids.

Condensed tannins

The condensed tannins of infusions prepared from wild and cultivated *Ganoderma* species ranged between 2.97 mg CAE/g of sample and 15.29 mg CAE/g of sample on dry weight (Table 3) in the following significant ($p<0.05$) order: C-PA-CMGL = C-PA-CM-GW > C-PA-CMGE > C-PA-MSGW > W-SE-GW ≥ W-CC-GE4 ≥ W-SB-GE = W-MS-GE ≥ W-MS-GW1 = W-PL-GE ≥ W-MS-GW2 = W-CC-GE3 ≥ W-CM-GE3 ≥ W-CC-GE2 ≥ W-CM-GL ≥ W-CM-GE2 ≥ W-CC-GE1 ≥ W-CM-GW = W-CM-GE1. For wild species, the infusion prepared from W-SE-GW had the highest levels of condensed tannins (6.37 mg CAE/g of sample) and the infusion prepared from W-CM-GW had the lowest levels of condensed tannins (2.97 mg CAE/g of sample). For cultivated species, the infusion prepared from C-PA-CMGL had the highest levels of condensed tannins (15.29 mg CAE/g of sample) and the infusion prepared from C-PA-MSGW$_1$ had the lowest levels of condensed tannins (11.38 mg CAE/g of sample). All infusions prepared from cultivated species had significantly ($p<0.05$) higher levels of condensed tannins than those prepared from wild species. Higher levels of condensed tannins in cultivated species could be a result of the substrate (*Pterocarpus angolensis*) on which they were grown.

Condensed tannin contents of both wild and cultivated species in this study were higher than the condensed tannin contents (1.82–2.43 mg/g of sample) reported for wild *G. lucidum* (2.29 mg/g of sample),

Table 3: Phenolic composition and antioxidant activities of infusions from wild and cultivated *Ganoderma* species

Sample code	TPC (mg GAE/g of sample)	TFC (mg QE/g of sample)	Condensed tannins (mg CAE/g of sample)	% Inhibition (DPPH assay)
W-CM-GE$_1$	19.50 ± 0.95^{k}	0.20 ± 0.02^{jkl}	3.00 ± 0.49^{h}	46.9 ± 0.1^{fg}
W-CM-GE$_2$	23.60 ± 1.48^{j}	0.21 ± 0.02^{jk}	3.56 ± 0.67^{gh}	47.4 ± 0.2^{f}
W-CM-GE$_3$	19.25 ± 0.94^{k}	0.19 ± 0.02^{jkl}	4.69 ± 0.73^{fgh}	43.1 ± 0.1^{j}
W-MS-GE	44.78 ± 2.40^{b}	1.67 ± 0.02^{a}	5.82 ± 2.12^{de}	45.9 ± 0.2^{hi}
W-CC-GE$_1$	23.93 ± 1.46^{j}	0.15 ± 0.02^{lm}	3.47 ± 0.80^{gh}	45.0 ± 0.2^{i}
W-CC-GE$_2$	24.97 ± 0.55^{gh}	1.17 ± 0.02^{b}	4.73 ± 1.09^{efg}	45.3 ± 0.1^{hi}
W-CC-GE$_3$	25.15 ± 1.95^{gh}	0.60 ± 0.13^{ef}	4.85± 1.44^{efg}	40.8 ± 0.2^{k}
W-CC-GE$_4$	26.02 ± 0.31^{g}	0.16 ± 0.04^{jk}	6.09 ± 1.46^{de}	46.2 ± 0.2^{gh}
W-PL-GE	18.37 ± 0.85^{k}	0.31 ± 0.02^{h}	5.11 ± 1.55^{def}	40.9 ± 0.2^{k}
W-SB-GE	24.21 ± 0.17^{hi}	0.25 ± 0.07^{ij}	5.71 ± 1.05^{de}	43.3 ± 0.2^{j}
W-CM-GW	18.89 ± 0.77^{k}	0.10 ± 0.01^{m}	2.97 ± 0.49^{h}	47.2 ± 0.2^{fg}
W-MS-GW$_1$	23.27 ± 0.12^{j}	0.69 ± 0.02^{d}	5.40 ± 0.93^{de}	47.1 ± 0.2^{fg}
W-MS-GW$_2$	28.78 ± 2.74^{f}	0.20 ± 0.01^{jkl}	4.99 ± 1.54^{def}	49.3 ± 0.4^{e}
W-SE-GW	31.53 ± 1.38^{e}	0.30 ± 0.01^{hi}	6.37 ± 0.89^{d}	49.2 ± 0.2^{e}
W-CM-GL	20.86 ± 0.54^{ij}	0.09 ± 0.01^{m}	3.90 ± 0.73^{fgh}	43.4 ± 0.1^{j}
C-PA-SBGE	42.11 ± 2.11^{c}	0.86 ± 0.08^{c}	12.99 ± 1.01^{b}	61.7 ± 2.6^{b}
C-PA-CMGW	36.70 ± 1.34^{d}	0.57 ± 0.08^{f}	14.89 ± 1.71^{a}	53.6 ± 0.8^{d}
C-PA-MSGW$_1$	43.40 ± 0.33^{bc}	0.41 ± 0.03^{g}	11.38 ± 1.33^{c}	63.7 ± 2.5^{a}
C-PA-CMGL	52.73 ± 1.67^{a}	0.63 ± 0.09^{e}	15.29 ± 0.92^{a}	55.1 ± 0.4^{c}
Quercetin				30.7 ± 0.1^{l}

TPC, Total Phenolic Content; GAE, Gallic Acid Equivalent; TFC, Total Flavonoids Content; QE, Quercetin Equivalent; CAE, Catechin Equivalent; W, Wild; CM, Colophospermum mopane*; GE,* Ganoderma enigmaticum*; MS,* Mundelea sericea*; CC,* Combretum collinum*; PL,* Pechuel-Loeschea leubuitziae*; SB,* Sclerocarya birrea*; GW,* Ganoderma wiireonse*; SE,* Senegaria erioloba*; GL,* Ganoderma lucidum*; C, Cultivated; PA,* Pterocarpus angolensis

1–4 on a sample code indicate the same Ganoderma *species collected from different host trees of the same species*

*Values are mean±s.d. (*n=*6). Means with different superscripted letters in the same column differ significantly (*p<*0.05).*

G. applanatum (2.43 mg/g of sample) and *G. tsugae* (1.82 mg/g of sample) by Rajoriya et al.[40] This suggests that Namibian *Ganoderma* mushrooms are a potential source of condensed tannins.

Antioxidant activity

The DPPH scavenging activities of infusions prepared from both wild and cultivated *Ganoderma* species ranged between 40.8% and 63.7% (Table 3) in the following significant ($p<0.05$) order: C-PA-MSGW > C-PA-SBGE > C-PA-CMGL > C-PA-CMGW > W-SE-GW = W-MS-GW2 >W-CM-GE2 ≥ W-MS-GW1 = W-CM-GW = W-CM-GE1 ≥ W-CC-GE4 ≥ W-CC-GE2 = W-MS-GE ≥ W-CC-GE1 > W-CM-GL = W-SB-GE = W-CM-GE3 > W-PL-GE = W-CC-GE3. For wild species, the infusion prepared from W-MS-GW_2 had the highest DPPH scavenging activity (49.3%) and that prepared from W-PL-GE had the lowest DPPH scavenging activity (40.9%). The higher the percentage, the higher the antioxidant activity. For cultivated species, the infusion prepared from C-PA-$MSGW_1$ had the highest DPPH scavenging activity (63.7%) and infusions prepared from C-PA-CMGW had the lowest (53.6%).

All the infusions prepared from cultivated species had significantly higher ($p<0.05$) DPPH scavenging activities than infusions prepared from wild species. This difference could be due to the high total phenolic content of these infusions which is positively correlated with radical scavenging activities.[38] Quercetin had DPPH scavenging activity of 30.6% inhibition at a concentration of 0.2 mg/mL. Infusions of all wild and cultivated species had antioxidant activities higher than that of quercetin at the concentration (0.2 mg/mL) that was used. The DPPH scavenging activities of infusions prepared from both wild and cultivated species were within the range of the DPPH scavenging activities (17.1–93.2% inhibition) reported for wild and cultured *G. lucidum*.[38,40] The high levels of DPPH scavenging activity observed in the infusions prepared from cultivated species indicate that they are a potential source of antioxidants.

Conclusions

The highest ash content and water absorption and solubility indices were found in cultivated species. W-CM-GE_1, W-CM-GE_3, W-SE-GW, W-CM-GL, C-PA-CMGW, C-PA-CMGL, and C-PA-$MSGW_1$ had high water solubility indices, suggesting that they have more water-soluble constituents and thus can be potentially used in formulations of hot water extracts. Infusions prepared from cultivated *Ganoderma* species had higher levels of total phenolics, condensed tannins and antioxidant activity, except for total flavonoids, than those prepared from wild *Ganoderma* species. Although wild species had relatively lower levels of total phenolics, condensed tannins and antioxidant activity than those of cultivated *Ganoderma* species, they still had comparable levels to those reported in the literature, which makes both wild and cultivated species investigated in this study potential candidates for use as nutraceuticals and sources of possibly healthful antioxidants, pending safety and consumer tests. Cultivation of *Ganoderma* once procedures are optimised, can be a way of ensuring sustainable supply for commercialisation of *Ganoderma* mushrooms, especially to reduce the levels of unemployment in Africa.

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Competing interests

We have no competing interests to declare.

Authors' contributions

K.K.N.H.: Data collection; writing – initial draft; data analysis. I.S.E.U.: Conceptualisation; methodology; validation; student supervision; writing – revisions. N.P.K.: Conceptualisation; methodology; student supervision; writing – revisions. W.E.: Methodology; validation; student supervision; writing – revisions. K.K.M.N.: Conceptualisation; student supervision; writing – revisions; project leadership.

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