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Comparative assessment of anti-radical activity and antioxidant capacity of seven wild mushroom species: Implication of phenolic and flavonoid phytochemicals

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Abstract: Wild mushrooms are important nutritional and medicinal resources, yet their antioxidant potential remains insufficiently documented in Iringa, Tanzania. This study comparatively evaluated the anti-radical activity and antioxidant capacity of the seven wild mushroom species. *Pleurotus ostreatus, Russula alutacea, Russula fragilis, Termitomyces microcarpus, Termitomyces clypeatus, Armillaria mellea and Coprinus comatus.* Phenolic and flavonoid associated anti-radical activity and antioxidant capacity was assessed using the DPPH method and analyzed using one-way ANOVA and paired t-tests. Significant differences among species were observed in phenolic antioxidant capacity ($F=3.4023$, $p=0.0277$). but not in phenolic or flavonoid anti-radical activity or flavonoid antioxidant capacity. *Russula fragilis* (mr2) showed the highest overall performance based on combined ranking, followed by *A. mellea* (ma). The findings indicate promising antioxidant potential, particularly for mr2 and ma. However, high variability and limited biological replication constrained interpretation. Further studies should increase replication, apply complementary assays, conduct post-hoc comparisons, and characterize responsible phytochemicals.

Keywords: Antioxidant potential; wild mushrooms; colorimetric spectrophotometry; antioxidant capacity; anti-radical activity.

Abbreviations: ANOVA, analysis of variance; AOAC, Association of Official Analytical Chemists; CRD, completely randomized design; CV, coefficient of variation; DOI, digital object identifier; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalent; RCBD, randomized complete block design; RUE, rutin equivalent; SD, standard deviation; SE, standard error; TAU, total antioxidant unit; TFC, total flavonoid content; TPC, total phenolic content; UV, ultraviolet. Species codes: mp, *Pleurotus ostreatus*; mt1, *Termitomyces microcarpus*; mt2, *Termitomyces clypeatus*; ma, *Armillaria mellea*; mc, *Coprinus comatus*; mr1, *Russula alutacea*; mr2, *Russula fragilis*.

Introduction

Wild mushrooms are potential nutritional and medicinal foods for human health (Mwanjala et al., 2024), contributing significantly on human immunity and protection in general (Bamidele and Ajayi, 2013). However, their potential is often overlooked due to a lack of awareness regarding their anti-radical abilities in the human body from their phenolic and flavonoid contents (Okhuoya and Afolabi, 2006).

Pleurotus ostreatus, commonly known as the oyster mushroom, is a widespread edible fungus found on decaying wood and other organic matter (McCleary and McLoughlin, 2021). It's renowned for its culinary value and its ability to break down lignocellulosic materials, making it an important organism in bioremediation and sustainable agriculture (Grivetti and Ogle, 2000). *Pleurotus ostreatus* can be found growing naturally in forests of tropical Africa including Tanzania (McCleary, 2023) and preferred by many community members for food (Washa, 2024). *Termitomyces microcarpus* is one of several species in the *Termitomyces* genus, known for its symbiotic relationship with termites (Nyamoga, 2023). Is typically grown in association with termite mounds, where it benefits from the nutrient-rich environment provided by the decomposing organic matter. Is one of the naturally growing mushrooms in forests of tropical Africa, including Tanzania (Osemwegie and Olayiwola, 2015). *Termitomyces clypeatus* is another species within the *Termitomyces* genus. This species also forms a symbiotic relationship with termites and is noted for its unique cap structure and ecological role in nutrient cycling within termite mounds. The species is locally found in tropical African countries, including Tanzania (Samiento, 2006). *Armillaria mellea*, commonly known as honey fungus, is a parasitic and saprotrophic mushroom that can be found on a wide range of woody plants. It is known for its extensive underground mycelial networks and can be a significant pathogen in forestry. Is one of the naturally growing mushrooms in forests of tropical Africa, including Tanzania and is edible with caution that

must be well cooked (Washa, 2022). *Coprinus comatus*, or the shaggy mane, is a distinctive mushroom known for its elongated white cap that resembles a shaggy mane. It is edible and often found in disturbed soils or grassy areas. The cap deliquesces into an inky black liquid as it matures. The species is native in tropical Africa and East Africa countries (Washa, 2023). *Russula alutacea* is a species of the genus *Russula*, known for its diverse colours and habitats. Like many Russulas, it can be found in various forest types and is recognised for its distinct colouration and sporadic fruiting. It is one of the naturally growing mushrooms in forests of tropical Africa, including Tanzania (Verbeken, 2000). *Russula fragilis* is another member of the *Russula* genus, notable for its fragile texture and diverse color variations. It is commonly found in temperate forests and is characterized by its brittle cap and stem. The species is native to tropical Africa, including East Africa (Halling and Nelsen, 2007). The species is not edible unless it is well prepared and well cooked (Buyck, 2001). The study addresses a research gap by focusing on the limited scientific information available on the antioxidant potential of wild mushrooms in Iringa, particularly their phenolic and flavonoid contents and anti-radical activities. Although several wild mushrooms are consumed locally and are recognized for their nutritional and medicinal values, their antioxidant capacities have not been adequately evaluated and compared under local conditions. This study therefore, aimed to determine the total phenolic and flavonoid contents and antioxidant capacities using the DPPH method. It also aimed to compare the scavenging abilities among mushroom species and identify species with significant potential for human dietary and health benefits.

Results

Antioxidant and anti-radical activity analysis

Statistical analysis was conducted using one-way of variance (ANOVA) and t-tests. One-way ANOVA was used to determine whether significant differences existed among the seven species (mp, mt1, mt2, ma, mc, mr1 and mr2), while paired t-tests were used to compare phenolic and flavonoid based measurements obtained from the same species. Statistical significance was assessed at $p = 0.05$. The assumptions for ANOVA included independence of observations, normality, and homogeneity of variance, which were assessed using the Shapiro-Wilk and Levene's tests. For the paired test, normality of the paired differences was assessed using the Shapiro-Wilk test, while boxplots were used to assess potential outliers.

Table 2. Colorimetric results showing antioxidant capacity and anti-radical activity of the studied mushrooms.

Species	Anti-radical activity (TAU)						Antioxidant capacity					
	Phenolic			Flavonoid			Phenolic (mg GAE g^{-1})			Flavonoid (mg RUE g^{-1})		
	1	2	3	1	2	3	1	2	3	1	2	3
mp	50.00	0.644	0.576	0.548	0.500	0.501	179.100	174.544	177.060	175.112	143.100	167.666
mt1	48.00	0.600	0.589	0.500	0.500	0.500	175.989	166.033	179.800	177.991	150.723	170.009
mt2	51.00	0.570	0.500	0.500	0.557	0.553	163.900	162.100	170.894	134.900	162.346	154.547
ma	52.00	0.538	0.567	0.566	3.596	4.842	175.709	177.250	178.769	140.800	156.687	165.345
mc	49.00	0.588	0.598	0.532	4.249	4.573	166.200	160.003	174.098	155.145	177.750	155.453
mr1	42.00	0.776	0.577	0.565	5.767	5.881	174.622	172.389	175.582	154.623	172.000	145.888
mr2	52.00	0.881	0.675	0.506	5.023	5.007	178.776	178.332	170.769	150.200	163.786	172.357

Columns 1–3 show the three reported measurements within each assay. Species codes are defined in the abbreviations and in Fig. 2.

Anti-radical activity

For phenolic based and anti-radical activity (TAU), one-way ANOVA showed no significant differences among species ($F(6, 14) = 0.051$, $p = 1.0000$). This indicates that variation among species was not greater than the within-species variation (Table 4). Similarly, flavonoid based anti-radical activity showed no significant differences among species ($F(6, 14) = 2.0486$, $p = 0.1261$). Thus, although some variation was observed among species, it was not statistically significant at 5% significance level (Table 5). In contrast, the paired comparison between phenolic and flavonoid based anti-radical activity revealed a highly significant difference ($t(5) = 28.1410$, $p < 0.0001$), indicating that the two assays produced significantly different measurements. The three species with the highest mean phenolic ant-radical activity were mr2 (17.8857 TAU), ma (17.7017 TAU), and mt2 (17.3567 TAU) (Table 3). For flavonoid based anti-radical activity, the highest means were recorded for mr1 (4.0710 TAU), mr2 (3.7653 TAU), and mc (3.1180 TAU) (Table 3).

Table 4. Phenolic anti-radical activity (TAU).

Species	Mean	SD	CV (%)
mp	17.073	28.515	167.017
mt1	16.396	27.370	166.925
mt2	17.357	29.136	167.866
ma	17.702	29.703	167.799
mc	16.729	27.948	167.065
mr1	14.451	23.858	165.098
mr2	17.886	29.544	165.182

SD: standard deviation; CV: coefficient of variation. Species codes are defined in the abbreviations.

Table 5. Flavonoid anti-radical activity (TAU).

Species	Mean	SD	CV (%)
mp	0.516	0.027	5.312
mt1	0.500	0.000	0.000
mt2	0.537	0.032	5.929
ma	3.001	2.199	73.272
mc	3.118	2.245	72.014
mr1	4.071	3.037	74.596
mr2	3.765	2.847	75.613

SD: standard deviation; CV: coefficient of variation. Species codes are defined in the abbreviations.

Antioxidant capacity

Phenolic based antioxidant capacity (mg GAE/g) differed significantly among species ($F(6, 14) = 3.4023$, $p = 0.0277$). This indicates that the variation among species were significantly greater than the within-species variation. The highest mean values were observed in ma (177.2427 mg GAE/g), mp (176.9013 mg GAE/g), and mr2 (176.4990 mg GAE/g) (Table 6). In contrast, flavonoid based antioxidant capacity (mg RUE/g) did not differ significantly among species ($F(6, 14) = 0.4825$, $p = 0.8106$), indicating that the observed differences among species were not statistically significant. The highest mean values were recorded for mt1 (166.2410 mg RUE/g), mc (162.7827 mgRUE/g), and mr2 (162.1143 mg RUE/g) (Table 7). The paired comparison between phenolic and flavonoid based antioxidant capacity showed a statistically significant difference ($t(5) = 4.8812$, $p = 0.0045$), demonstrating that the two measurement approaches yielded significantly different results (Table 6 and 7).

Table 6. Phenolic antioxidant capacity (mg GAE g^{-1}).

Species	Mean	SD	CV (%)
mp	176.901	2.282	1.290
mt1	173.941	7.108	4.087
mt2	165.631	4.646	2.805
ma	177.243	1.530	0.863
mc	166.767	7.065	4.236
mr1	174.198	1.638	0.940
mr2	176.499	3.566	2.021

SD: standard deviation; CV: coefficient of variation. Species codes are defined in the abbreviations.

Table 7. Flavonoid antioxidant capacity (mg RUE g^{-1}).

Species	Mean	SD	CV (%)
mp	161.959	16.752	10.343
mt1	166.241	14.019	8.433
mt2	150.598	14.143	9.391
ma	154.277	12.449	8.069
mc	162.783	12.963	7.963
mr1	157.504	13.292	8.439
mr2	162.114	11.173	6.892

SD: standard deviation; CV: coefficient of variation. Species codes are defined in the abbreviations.

Overall statistical interpretation

Overall, species differences were statistically significant only for phenolic based antioxidant capacity, whereas neither phenolic nor flavonoid measurements showed significant species differences in anti-radical activity, nor flavonoid based antioxidant capacity also showed no significant species effect. Nevertheless, the paired comparisons demonstrated significant differences between phenolic and flavonoid based measurements for both anti-radical activity and antioxidant capacity. These findings indicate that the two assay types capture different aspects of the antioxidant related properties of the samples and should therefore be interpreted separately. Table 3 – 7 summarises the statistical results of the analysis while collected data and information of studied species were summarized on Tables 1 and 2 and Fig. 2. The tables and figures are presented alongside the relevant text.

Table 3. Combined ranking of the mushroom species (lower rank is more favourable).

Species	Phenolic anti-radical activity (TAU)	Flavonoid anti-radical activity (TAU)	Phenolic antioxidant capacity (mg GAE g^{-1})	Flavonoid antioxidant capacity (mg RUE g^{-1})	Average rank
mr2	17.886	3.765	176.499	162.114	2.25
ma	17.702	3.001	177.243	154.277	3.25
mc	16.729	3.118	166.767	162.783	4.00
mp	17.073	0.516	176.901	161.959	4.00
mr1	14.451	4.071	174.198	157.504	4.25
mt1	16.396	0.500	173.941	166.241	4.75
mt2	17.357	0.537	165.631	150.598	5.50

The average rank is the combined descriptive ranking reported by the author. Species codes are defined in the abbreviations.

Discussion

The present study evaluated the anti-radical activity and antioxidant capacity of seven mushroom species using phenolic and flavonoid associated measurements. The results demonstrate that the antioxidant properties of the investigated species were dependent on both the biological characteristics being measured and the analytical approach used. Significant differences were observed among species for phenolic associated antioxidant capacity. Whereas no statistically significant differences were detected for phenolic anti-radical activity, flavonoid anti-radical activity or flavonoid antioxidant capacity. These findings indicate that the seven mushroom species cannot be assumed to possess identical antioxidant profiles and that the choice of antioxidant assay can substantially influence the interpretation of their biological potential (Table 3-7, supplementary Fig. 3-10 in attachment)). For phenolic associated anti-radical activity, the one-way ANOVA produced an F statistic of 0.0051 with p = 1.0000, indicating no statistically significant difference among the seven species. Although mr2 had the highest descriptive mean value (17.886), followed by ma (17.702) and mt2 (17.357), these numerical differences were not statistically supported. Therefore, the observed ranking should be interpreted only as a descriptive pattern rather than evidence that mr2 is significantly more active than the other species. The very high coefficients of variation reported for this variable, ranging from 165% to 168%, indicate substantial variability relative to the means. This high variation warrants careful examination of the raw measurements, analytical precision and possible outliers before firm biological conclusions are drawn. A similar pattern was observed for flavonoid associated anti-radical activity. The one-way ANOVA yielded F = 2.0486 and p = 0.1261, showing that differences among species were not statistically significant at the 5% significance level. Nevertheless, the descriptive results showed considerable differences in mean value, mr1 recorded the highest mean anti-radical activity (4.071), followed by mr2 (3.765) and mc (3.118), while mt1, mp and mt2 recorded much

lower values. The relatively high CV values for ma, mr1 and mr2, ranging from 72% to 76%, suggest considerable within-species variability. This variability may reflect differences in phytochemical composition, biological material, extraction efficiency or analytical response. Consequently, additional biological replicates and tighter control of sample preparation would be useful for confirming whether the observed differences represent true species-level variation (Table 3-7). All scavenging ability revealed in the studied samples was found to be within scientifically accepted range and can be classified as moderate to high moderate as reported by scientific publications including that of Nyamoga (2023) and Mgalula et al. (2025) as recent findings from Tanzania. In contrast, phenolic associated antioxidant capacity showed a statistically significant difference among species ($F = 3.4023$, $p = 0.0277$). This is the strongest species-level finding in the present study. The descriptive ranking showed ma as the highest performing species, with a mean value of 177.243 mg GAE/g, followed by mp (176.901 mg GAE/g) and mr2 (176.499 mg GAE/g). mt2 had the lowest mean value at 165.631 mg GAE/g. The relatively low CVs for most species indicate substantially greater analytical consistency for this measurement than for the anti-radical phenolic measurements. However, because the one-way ANOVA only establishes that at least one group differs, a post-hoc test such as Tukeys HSD is required to establish which species are significantly different (Table 3-7, supplementary Fig. 3-10 in attachment).

For flavonoid associated antioxidant capacity no significant species effect was detected ($F = 0.4825$, $p = 0.8106$). mt1 had the highest descriptive mean at 166.241mg RUE/g, followed by mc (162.783) mg RUE/g and mr2 (162.114 mg RUE/g), whereas mt2 had the lowest value (152.598 mg RUE/g). Despite these numerical differences, the large p-value indicates that the observed variation is insufficient to that species differ significantly under the experimental conditions. The CV value of 6.9-10.3%, were comparatively moderate, suggesting relatively good consistency in this assay. The pared comparison between phenolic and flavonoid associated measurements produced statistically significant differences for both anti-radical activity and antioxidant capacity. For anti-radical activity, $t = 28.1410$ and $p < 0.0001$, while for antioxidant capacity, $t = 4.8812$ and $p = 0.0045$. These findings demonstrate that the phenolic and flavonoid associated measurements generated different numerical responses. However, these results should not automatically be interpreted as evidence that phenolic compounds are biologically superior to flavonoids. These two measurements may represent different chemical constituents, assay responses and standard-equivalent scales. In antioxidant capacity espressed as mg GAE/g and mg RUE/g involves different reference standards, so direct numerical comparison between these units is not necessarily biological meaningful. The overall descriptive ranking identified mr2 as the best performing species, with an average rank of 2.25 followed by ma at 3.25 and mc at 4.00. mr2 performed consistently well across several measurements especially in phenolic anti-radical activity and both antioxidant capacity measures. However, the combined ranking should be regarded as an exploratory method for identifying promising species rather than a formal statistical demonstration of superiority. The results nevertheless suggest that mr2 and ma warrant further phytochemical investigation because they demonstrated comparatively strong performance across multiple antioxidant related parameters (Table 3-7). All scavenging ability revealed in the studied samples was found to be within scientifically accepted range and can be classified as moderate to high moderate as reported by scientific publications including that of Nyamoga (2023) and Mgalula et al. (2025) as recent findings from Tanzania. Overall, the findings support the conclusion that antioxidant activity is complex and cannot adequately be characterized using a single assay. The significant species effect observed for phenolic antioxidant capacity, together with the absence of significant effects in several other assay, suggests that different groups of phytochemicals and different mechanisms may contribute to the antioxidant properties of the investigated species. Further studies should therefore combine several validated antioxidant assay with quantitative phytochemical profiling and where possible identification of individual bioactive compounds (Table 3-7, supplementary Fig. 3-10 in attachment)). All scavenging ability revealed in the studied samples was found to be within scientifically accepted range and can be classified as moderate to high moderate as reported by scientific publications including that of Nyamoga (2023) and Mgalula et al. (2025) as recent findings from Tanzania

Limitations of the study

Funding was the major limitation because with enough funds the study could be extended to several regions and global, several seasons with a number of rain and different environmental conditions. This could obtain results which can be compared regional and season wise. With enough funds the study could use more than DPPH assay in assessing antioxidant properties and could use more than ethanol solvent to extract bioactive compounds. This could enable obtaining of other contents which are important to human body.

Materials and methods

Description of the study area

Mushroom materials were collected in Kiwere Ward (7°37'17.3"S, 35°37'48.1"E) in Iringa Rural District, Tanzania, during the rainy season in 2024 (Fig. 1). Local names, cap structure and cap colour of the selected mushroom species were collected from findings of ethnobotany studied by Washa (2022). Cap structure and cap colour are used for the identification of mushrooms. Iringa is renowned for its diverse landscapes, including highlands, valleys, and river basins, which contribute to its rich agricultural potential. Iringa features a mix of mountainous terrain and fertile plains. Elevation in the region varies significantly; higher areas, at around 2000 metres, experience cooler temperatures, while lower regions, at approximately 1300 metres, have warmer climates (Shelukindo et al., 2014). This variation in altitude influences the local climate, which ranges from temperate in the highlands to subtropical in the lower areas. The region receives moderate-to-high rainfall, with a mean annual precipitation of 578 mm and a mean annual temperature of 22.7 °C. Soils in the area range from basement complex to colluvial soils (Shelukindo et al., 2014). Agriculture is the backbone of Iringa's economy, with the

region being a significant producer of crops such as maize, beans, potatoes, tea, coffee, and various fruits and vegetables. The fertile soils and favourable climatic conditions make it an ideal location for both subsistence and commercial farming. Indigenous fruits, vegetables, and mushrooms also play a role in improving the livelihood, health, and economy of the community (Washa, 2022; Washa, 2023; Washa, 2024). Iringa is home to diverse flora and fauna, with several protected areas and national parks nearby, including Ruaha National Park (Shelukindo et al., 2014). These areas contribute to the conservation of wildlife and natural habitats, providing opportunities for eco-tourism and research. The region's biodiversity is also reflected in its agricultural practices, with farmers cultivating a wide range of crops to promote ecological balance and sustainability (Shelukindo et al., 2014). The study area is accessible via a network of roads connecting Iringa to other major towns and cities in Tanzania.

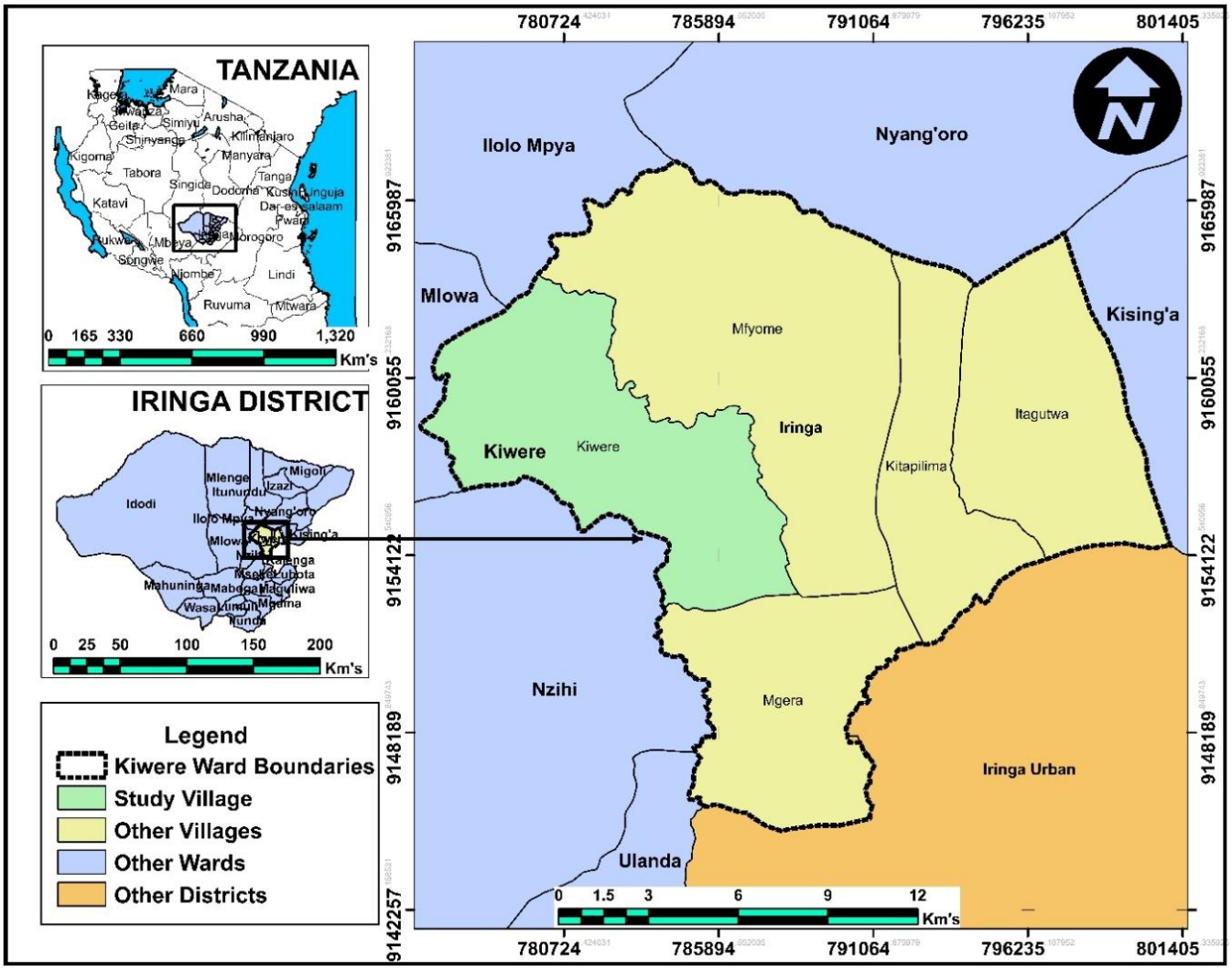


Fig. 1. Map of the study area in Iringa Region, Tanzania.

Study design

The study employed laboratory based comparative experimental design to evaluate the anti-radical activity and antioxidant capacity of extracts obtained from the seven mushroom species coded as mp, mt1, mt2, ma, mc, mr1 and mr2. The study compared the performance of the species using phenolic and flavonoid antioxidant assay DPPH. Since the samples were collected from several villages of different conditions of the ward, a stratified sampling approach was used in the field before a completely randomized design (CRD) was used in the laboratory experiment in which the samples were randomly assigned to analytical runs under relatively homogeneous laboratory conditions (Howell et al., 2020).

Sample preparation

After collection of mushroom samples from identified representative populations, samples were cleaned to remove soil and foreign materials, dried under controlled conditions away from direct sunlight and grinded into uniform powder. The powdered samples were stored in airtight containers until extraction. Standard methods were used to prepare crude extract from mushroom powder (Ahmed et al., 2017), in which ethanol was used because it produces quality extract, is a cost-effective solvent and is easy to use. One kilogram of powder was extracted in ethanol at a ratio of 1:3 (powder to solvent) in a conical flask (5000 mL) to ensure complete soaking of powder in the solvent. The mixture was left for 72 hours with frequent shaking. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator, followed by complete evaporation of the remaining solvent in a water bath at 50 °C to obtain the crude extract.

The extract was stored in tightly covered bottles and stored in a refrigerator at −4 °C till use for assessing phenolics and flavonoids.

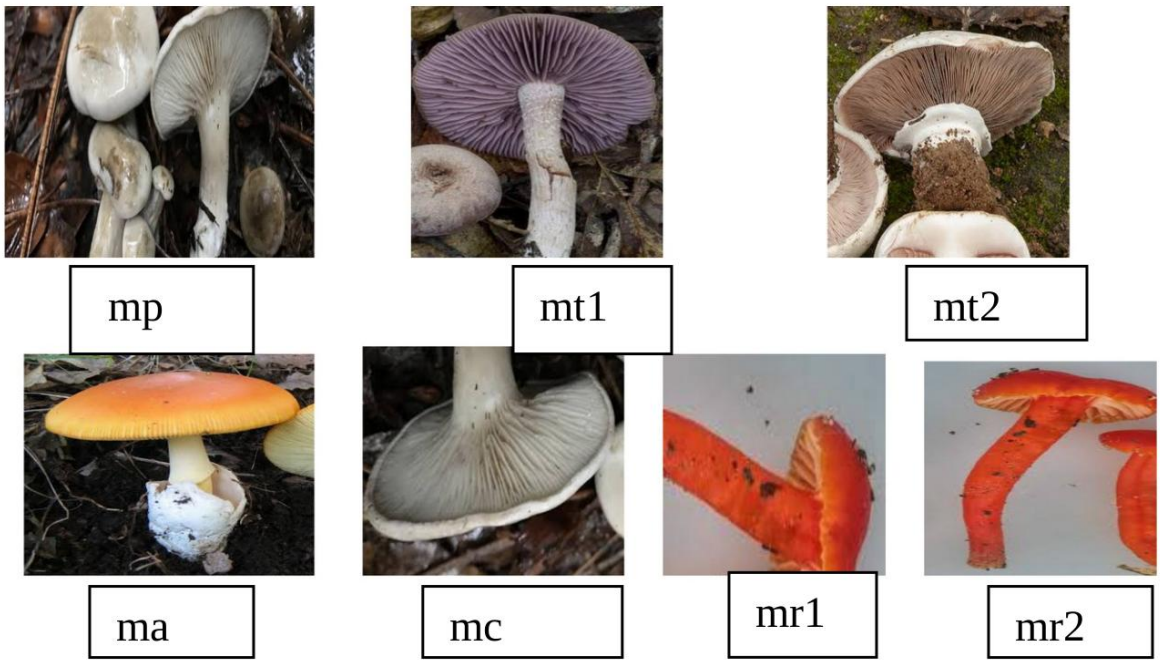


Fig. 2. Studied mushroom species. From top left to right: *Pleurotus ostreatus* (mp), *Termitomyces microcarpus* (mt1), *Termitomyces clypeatus* (mt2); bottom left to right: *Armillaria mellea* (ma), *Coprinus comatus* (mc), *Russula alutacea* (mr1) and *Russula fragilis* (mr2).

Table 1. Studied wild mushrooms from Iringa, Tanzania.

Species name	Cap structure and colour
Pleurotus ostreatus (mp)	Fan-shaped or oyster-shaped, typically with a smooth surface. Pale crem
Termitomyces microcarpus (mt1)	Convex to flat, with a smooth surface..white or pale cream,
Termitomyces clypeatus (mt2)	Convex to flattened, with a smooth to slightly wavy surface Pale to light brown or tan
Armillaria mellea (ma)	Convex to flatten. yellowish to honey-brown
Coprinus comatus (mc)	Cylindrical and covered in white. White when young, turning to black as it decomposes
Russula alutacea (mr1)	Convex to flat with a smooth surface. Brownish or russet, sometimes with a reddish or orange
Russula fragilis (mr2)	Convex to flat, typically smooth or slightly wrinkled. Pale pink to salmon or reddish,

Phytochemical analysis

Antioxidant extraction

Antioxidant extraction from mushroom samples was performed using the methods described by Ahmed et al. (2017).

Determination of total phenolic content by the Folin–Ciocalteu method

The total phenolic content of the mushroom samples was measured using the Folin-Ciocalteu reagent as outlined by Ahmed et al. (2017). Reagents in this determination included Folin-Ciocalteu (diluted 1:10), sodium carbonate (7.5%) and standard gallic acid. A 0.5 mL of crude extract was pipetted into a test tube, and 2.5 mL of diluted Folin-Ciocalteu was added to it and left for 5 minutes to dissolve and react. A 2.0 mL of sodium carbonate was added and mixed thoroughly and incubated for 30 minutes at room temperature. The absorbance speed was measured at 765 nm using a spectrophotometer before plotting the calibration curve of concentration against absorbance (X-axis = concentration, Y-axis = Absorbance), where results of Total Phenolic Contents (TPC) are expressed in mg GAE/g.

$$TPC = \frac{C \times V}{m} \quad (1)$$

Where, C = Concentration from calibration curve (mg/mL), V = Volume of extract (mL), m = mass of sample (g). All results for TPC for the sampled species were presented on Table 2 and 3).

Determination of total flavonoid content by the aluminium chloride colorimetric method

The total flavonoid content was determined using the method of Ahmed et al. (2017). In this determination the reagents included: aluminium chloride (10%), potassium acetate (1 M), methanol and standard Rutin. A 0.5 mL of crude extract was added into test tube followed by 1.5 mL of methanol, 0.1 ml of potassium acetate and 2.8 ml of diluted water. The mixture was shaken well to mix and incubated for 30 minutes at room temperature. Finally, the absorbance speed was measured at 415 nm using a spectrophotometer before plotting a calibration curve of 'absorbance' vs 'concentration' to obtain a linear equation as above. Results are expressed in mg RUE/g.

$$TFC = \frac{C \times V}{m} \quad (2)$$

Where: C = Concentration from calibration curve (mg/mL), V = Volume of extract (mL), m = mass of sample (g). All results for TFC for sampled species were presented on Table 2 and 3.

DPPH free radical scavenging activity

DPPH free radical scavenging activity was assessed following the method of Ahmed et al. (2017). Generally, measuring scavenging (antioxidant activity) using the DPPH assay is a standard, simple method of evaluating how well the mushroom crude extract can neutralise free radicals. DPPH is a stable free radical with a violet colour (deep purple). When react with an antioxidant is reduced, the colour changes from purple to yellow. The more colour it changes the stronger the antioxidant. Materials needed for measuring scavenging activity using DPPH included DPPH solution (0.1 mM in ethanol), mushroom crude extract dissolved in ethanol, spectrophotometer, ethanol (blank/control), standard antioxidant (ascorbic acid). The DPPH solution was left in the dark at room temperature, and different concentrations of mushroom crude extract (µg/mL) was prepared for test conduction. To each test,1 mL of DPPH was mixed with 1 mL of crude extract and to each of control test 1 mL of DPPH was added with 1 mL of ethanol. The mixture was incubated in the dark for 30 minutes, and absorbance speed was measured at 517 nm using a spectrophotometer.

Calculations

$$DPPH\ scavenging\ activity\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (3)$$

Where: Acontrol is the absorbance of DPPH + solvent, and Asample is the absorbance of DPPH + extract.

The determination of scavenging activity is interpreted that the higher the (%) the stronger the antioxidant capacity.

Statistical analysis

All statistical analyses and visualizations were conducted using Python 3.14. Data wrangling, manipulation and statistical testing were performed using Pandas 1.5.3, NumPy 1.24.3, and SciPy 1.10.1 modules. Figs. were generated using Matplotlib 3.7.1 and Seaborn 0.12.2.

The Descriptive statistics included the mean, standard deviation, coefficient of variation (CV%), minimum and maximum values and 95% confidence intervals. The seven mushroom samples were compared using one-way ANOVA. A paired t-test was used in paired measurements from the same experimental unit (anti-radical activity and antioxidant capacity). An overall ranking was presented as supplementary descriptive analysis. Obtained data were presented on Table 3-7 and the supplementary materials attachment Fig. 3-10). Photographs of studied mushrooms were presented on Fig. 2, names and cap structures of the studied mushrooms were presented on Table 1 while colorimetric results were presented on Table 2.

Conclusion

The seven mushroom species exhibit measurable antioxidant capacity and anti-radical activity, but their performance varies according to the parameter and analytical methods used. A statistically significant difference among species was observed for phenolic antioxidant capacity ($F = 3.4023$, $p = 0.0277$), while no significant differences were observed for phenolic anti-radical activity, flavonoid anti-radical activity and flavonoid antioxidant capacity. Descriptively, mr2 showed the strongest overall performance based on the combined ranking, followed by ma and mc. The finding suggest that mr2 and ma are promising candidates for further phytochemical and antioxidant investigation. It is recommended that future research increase the number of independent biological replicates and distinguish clearly biological and technical replication. Post-hoc tests should be conducted following significant ANOVA results to identify specific species differences. Future studies should also employ complementary antioxidant assays, quantify total phenolic and flavonoid contents and use chromatography techniques to identify compounds responsible for the observed activities.

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Ethical issues

Specimens were collected and fieldwork conducted under permits issued by the Mkwawa University College of Education (University of Dar es Salaam). Fungal fruiting bodies were collected only to the extent necessary for their scientific identification. Also, the specimens were transported in mesh collection baskets to allow air flow to assist in the natural dispersal of spores and to help in continued reproduction.

Declaration of interest

There were no competing interests regarding the submission and publication of this manuscript.

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