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Occurrence, use and antioxidant potential of *Termitomyces reticulatus* in Cameroon

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Termitomyces species are among the most utilised mushrooms in tropical Africa and Asia, with some species having putative medicinal properties. However, data on their diversity, distribution, ethnomycology, biological activities and metabolites are still to be completed, especially in central Africa. During field surveys in the Western Highlands of Cameroon, basidiomes of a species of this fungal genus were collected and morphologically described. The extracts were used to screen and measure some antioxidant compounds and their respective activities, including polyphenols, flavonoids, thiols, DPPH radicals scavenging activity, ferric reducing power (FRAP) and the superoxide dismutase-like (SOD-like) activity in comparison to that of vitamin C.

Morphological features allowed identifying this fungal species as *Termitomyces reticulatus*, which is here reported for the first time from Cameroon. This species is used by locals as food. Mycochemical antioxidant analyses revealed polyphenols as the major antioxidant compounds, followed by flavonoids and thiols. Extracts also showed significant DPPH, FRAP and SOD-like activities, although less strongly than those of vitamin C. The global antioxidant potential of this species is comparable to that of many mushroom species, such as *T. heimii* and *T. microcarpus*. Extensive work on other metabolites and biological activities of *T. reticulatus* are needed for a better description of its potential health benefits, especially in the fight against various human oxidative stress-related diseases.

Key words: *Lyophyllaceae*, ethnomycology, antioxidant metabolites, free radicals, Central Africa.

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Druhy rodu *Termitomyces* patří v tropické Africe a Asii mezi nejvíce využívané houby; některým z nich jsou přisuzovány léčivé vlastnosti. Nicméně údaje o jejich diverzitě, rozšíření, etnomykologii, biologických aktivitách a metabolitech mají stále daleko k úplnosti, zejména ve střední Africe. Zástupce tohoto rodu, jehož plodnice byly sebrány a morfologicky popsány během terénního průzkumu Západní Vysočiny v Kamerunu, byl v aktuální studii analyzován na obsah účinných látek. V extraktech byly zjištěny a kvantifikovány antioxidační látky a jejich aktivity, konkrétně polyfenoly, flavonoidy, thioly, antiradikálová aktivita, redukční působení na sloučeniny železa a schopnost přeměny superoxidu ve srovnání s vitamínem C.

Na základě morfologických znaků byl druh určen jako *Termitomyces reticulatus*; i když je místním obyvatelům znám jako jedlá houba, jde o první publikovaný záznam o výskytu tohoto druhu v Kamerunu. Na základě mykochemické analýzy se jako nejsilnější antioxidanty jeví polyfenoly, následované flavonoidy a thioly. Extrakty též ukázaly silnou schopnost odbourávat radikály, superoxidy a působit jako redukční činidla, i když nižší než vitamín C. Celkový antioxidační potenciál *T. reticulatus* je srovnatelný s dalšími druhy, jako jsou *T. heimii* a *T. microcarpus*. Zpracování dalších metabolitů a biologických aktivit tohoto druhu je žádoucí pro poznání příznivých účinků na lidské zdraví, především na léčbu nemocí spojených s oxidativním stresem.

INTRODUCTION

Termitomyces R. Heim species are obligate symbionts living in association with termites of the subfamily Macrotermitinae (Isoptera). This fungal genus belongs to the family *Lyophyllaceae* and is widely distributed in tropical Africa and Asia (Frøslev et al. 2003, Tibuhwa 2012, Mossebo et al. 2017, Koné et al. 2018). In these areas, *Termitomyces* constitutes one of the economically most important wild edible mushroom groups, providing income to local populations, mainly during the rainy seasons (Buyck 1994, Härkönen et al. 1995, Yongabi et al. 2004, Eyi et al. 2011, Koné et al. 2013, Fadeyi et al. 2017, Kinge et al. 2017). They have unique and subtle flavours and are characterised by interesting nutrient contents (Kansci et al. 2003, Thatoi & Singdevsachan 2014). They also have some medicinal potentials, including antimicrobial and immunomodulatory, antioxidant, anti-inflammation and antitumor activities (Loganathan et al. 2010, Atri et al. 2012, Hsieh & Ju 2018, Mahamat et al. 2018a, 2018b).

There are about 30 to 40 species of *Termitomyces* described worldwide (Kirk et al. 2008). However, 92 names including species, subspecies, varieties and forms with many synonyms (www.indexfungorum.org) are available in the literature. Mossebo et al. (2017) mentioned 34 taxa including infraspecific ones (species and forms) in Africa and Asia, with 23 and 17 species in Africa and Cameroon, respectively. The latter country is known as 'Africa in miniature' due to, among others, the diversity of tropical African vegetation, habitats, climate, fauna and

flora concentrated in its territory. Hence, it is possible to find species known elsewhere in Africa or Asia, but not yet known from this country.

During field work and ethnomycological survey in the Western Highlands of Cameroon, a species of *Termitomyces* was found. After morphological description, this species was identified as *Termitomyces reticulatus* van der Westh. & Eicker, locally known as "Puo' ngùgùe" (pwɔ' ngʉngʉə) (Njouonkou et al. 2016) and consumed by locals, especially Bamun people. The aim of this study is to give the first account of morphology, uses and antioxidant potential of this mushroom species in Cameroon.

MATERIAL AND METHODS

Collection and morphological study. Basidiomes of *Termitomyces reticulatus* were collected in the Western Highlands of Cameroon. The macroscopic description was based on observation of fresh material. Colour names and codes follow Watling (1969).

The microscopical study was based on free-hand sections of basidiomes mounted in 5% KOH and stained with ammoniacal Congo Red, according to the usual approach for the study of agarics (Eyi et al. 2011). Observations and drawings were made using a Leitz research microscope equipped with a drawing tube at a magnification of 1000×.

The spore dimensions are given as follows: L_1–L–$L_2 \times l_1$–l–l_2 µm; $Q = Q_1$–Q_m–Q_2 where L_1 represents the minimum length, L the average length, L_2 the maximum length, l_1 the minimum width, l the average width, and l_2 the maximum width; Q the ratio of length and width, Q_1 the lowest ratio, Q_m the average ratio, and Q_2 the highest ratio. A total of 30 spores were measured; for basidia, cheilocystidia, pleurocystidia, at least 10 elements of each were measured. The dimensions of basidia do not include the sterigmata.

The specimens are deposited at the Herbarium of Meise Botanic Garden, Belgium (BR), with duplicates preserved at the laboratory of Biological Sciences of the University of Bamenda, Cameroon.

Ethnomycology. Ethnomycological information was obtained from discussions with locals of the West and North-West Regions of Cameroon; mainly in the areas where the species was collected. This survey concerned mainly the local names and their significations as well as their uses.

The local distribution of the fungal species was compiled from our field observations and the collected ethnomycological data.

Evaluation of antioxidant activities. For the study of antioxidant activities, samples of fresh basidiomes were cleaned with distilled water, minced,

dried at 45 °C using a fruit dryer up to constant weight. The dry material was then powdered using a grinder. The powder obtained was kept at 4 °C until the preparation of the extracts. A 10% aqueous extract was prepared by boiling a portion of 10 g of powder in 100 ml distilled water for 30 min. and was centrifuged after cooling (1620 g, 15 min., 4 °C). The methanol extract was prepared according to Sharma & Atri (2014). Some antioxidant compounds were assessed, including total phenols and flavonoids (both determined following Dhar et al. 2012), thiols (according to Ellman 1959) and total amino acid content (Yemm & Cocking 1955). Also, antioxidant activities were evaluated using vitamin C at the final concentration of 0.05 g/ml as a reference in the following spectrophotometric experiments. Since polyphenols are known to be antioxidants (Mitra et al. 2016), 50 µl of the mushroom extract containing 0.053 mg Quercetin Equivalent was tested. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity was determined according to Zengin et al. (2010) based on the methanolic solution of DPPH changing colour from purple to yellow, measured at 517 nm in a spectrophotometer. The ferric reducing antioxidant power (FRAP) estimation was based on the reduction of potassium ferricyanide $K_3[Fe(CN)_6]$ to potassium ferrocyanide $K_4[Fe(CN)_6]$, with the absorbance read at 700 nm (Sim et al. 2010). Finally, the evaluation of superoxide dismutase inhibition of pyrogallol autoxidation (SOD-like activity) was carried out following Marklund & Marklund (1974) with the absorbance of the mixture read at 420 nm against a negative control.

The experiments were performed in triplicate and the results presented as mean ± standard deviation. Statistical analysis was performed using the GRAPHPAD PRISM 5.03 software. Comparisons between the standard and control groups were performed by means of one-way analysis of variance (ANOVA). For the groups showing a significant difference between the variances, the averages were compared by the Tukey test. Probabilities lower than 0.05 were considered statistically significant.

RESULTS

Termitomyces reticulatus van der Westh. & Eicker, Mycological Research 94: 928, 1990 Figs. 1–2

Description, ecology and distribution

Macroscopic features. Basidiomes angiocarpic, pluteoid. Pileus 1.5–12.7(15) cm diam., at first globose, then convex expanding to plane or sometimes applanate, with a pronounced umbonate perforatorium; surface viscid when moist, entirely olivaceous black to olivaceous grey towards the margin when young, then producing persisting spot-like thin grey olivaceous to clay-buff

Fig. 1. *Termitomyces reticulatus* (BR0000020500580), macroscopic features: **a** – basidiomes of various ages in the field; **b** – young basidiome; **c** – end of pseudorhiza, disc opened; **d** – end of pseudorhiza, disc closed; **e** – very young basidiome in longitudinal section; **f** – longitudinal section of mature basidiome. Photos A.-L. Njouonkou.

concentric reticulations on a whitish to olivaceous background, their density increasing towards the centre where it forms a wide smooth violaceous black to olivaceous black plate covering the perforatorium, with soil particles adhering to the reticulation, margin slightly striate, entire or sometimes incised when mature, inflexed or rarely reflexed in very old individuals; pileipellis very thin and hardly separable from the context. Lamellae free, white then whitish to pinkish, up to 12 mm wide, crowded with 1 or 2 lamellulae in-between; edge crenulated. Stipe 3–12 × 1–3.5 cm in epigeous part, central, gradually attenuating downwards to a pseudorhiza up to 50 cm long, ending with a hemispherical bulbous rounded disc at the attachment to the comb; surface glabrous and white above the annulus, with dark brown to olivaceous snakeskin pattern below the annulus, its density and thickness decreasing towards the base, where it becomes gradually olivaceous to whitish, fistulose even in a young stage, bulbous and closed at the end of the pseudorhiza, but becoming discoid and opened in mature specimens. Partial veil present, giving a persistent, more or less pendant, membranous annulus. Context white, firm, up to 13 mm thick at the centre of the pileus. Taste slightly sweet. Spore print buff to clay-pink. Macrochemical reactions with the chemicals KOH, $FeSO_4$, HCl and phenol none on any part of the basidiomes.

Microscopic features. Basidiospores 6–*7.5*–8(9) × (3.5)4–*4.8*–5.8 µm; Q = 1.4–*1.6*–1.9; ellipsoid, hyaline to pale, inamyloid, smooth, thick-walled (up to 1 µm thick), with one or two refractive guttules. Basidia 20–32 × 8.5–10 µm, clavate, bearing 4 or sometimes 2 sterigmata, generally thin-walled, some slightly thick-walled, mainly in very old basidiomes. Lamellar edge sterile. Cheilocystidia crowded, 20–47 × 9–24 µm, clavate, thin-walled. Pleurocystidia present, but scarce especially in old basidiomes, 30–55 × 11–24 µm, not exceeding for more than 15 µm above the basidia, clavate, sometimes fusiform to lageniform or cylindrical, slightly thick-walled, especially in very old basidiomes, and generally containing refractive guttules. Hymenophoral trama bilateral (divergent) to regular, made up of thin-walled, inflated hyphae reaching 25 µm in diameter and lacking clamp connections. Subhymenial layer pseudo-parenchymatous, reaching 10 µm in width. Pileipellis a cutis made up of brownish to greyish repent, radial inflated, slightly thick-walled hyphae, 3–12(22) µm at the margin, darker towards the centre around the perforatorium with ramified, sometimes inflated, thin-walled hyphae ending in cystidioid elements of various shapes (clavate, cylindrical, globose or fusiform to utriform).

Habitat. Growing in large clusters on the ground, associated with termite nests.

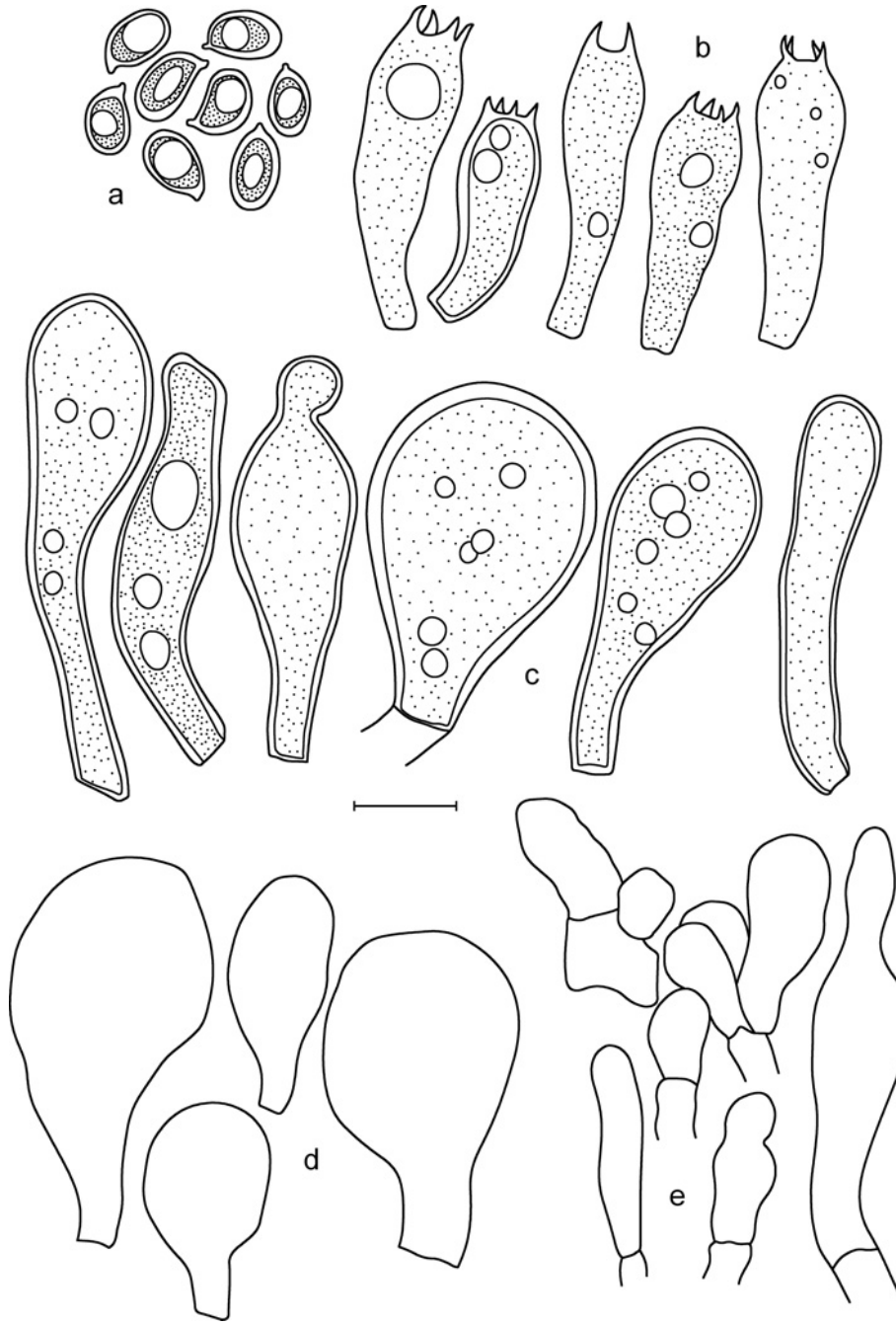

Fig. 2. *Termitomyces reticulatus* (BR0000020500580), microscopic features: **a** – basidiospores; **b** – basidia; **c** – pleurocystidia; **d** – cheilocystidia; **e** – pileipellis elements. Scale bar = 10 µm. Drawings A.-L. Njouonkou.

Distribution. In Cameroon, according to field observations and information, this species occurs in the Western Highlands including the West Region, where voucher specimens were collected, and the North-West Region, where some specimens were found at the village of Banja near Bamenda. The species has also been reported in the South-West Region, especially in the Lebialem Division. It is known from Ghana, Togo, Benin, Democratic Republic of Congo (DRC), Zambia and Zimbabwe, while it has also been recorded from the west coast and south-eastern part of India.

Specimens examined

Cameroon. West Region. Noun Division, Bangourain Subdivision, village of Koumemgba (05°46'41.28" N, 10°38'00.21" E, 1189 m altitude), 19 August 2015, leg. & det. A.-L. Njouonkou (BR0000020500580, NAL622). – Nde Division, Bamena Subdivision (bought on Bamena market by A.-L. Njouonkou), 12 August 2017 (BR0000020500573, NAL745).

Ethnomycology

In the Noun Division, *Termitomyces reticulatus* is named "Puo' ngùgùe (pwɔ' ngʉngʉə)" in reference to the reticulated structure of the pileus surface, which makes it look like the skin of a panther locally called "gùe (ngʉə)". It is used as food by many ethnic groups of the West and North-West region of Cameroon, where it is occasionally even sold on some markets. Unlike other species of *Termitomyces*, it is not popular and is mostly known by elders and not largely appreciated because it is slimy when cooked fresh. It needs to be boiled and washed several times in order to remove the slime and soil before preparation, or to be fried in oil.

Antioxidant activities

Regarding antioxidant compounds, polyphenols had the highest content, followed by flavonoids and thiols (Tab. 1). Three standard methods were used to estimate the antioxidant activities of *T. reticulatus* extracts: DPPH radical scavenging activity, superoxide dismutase inhibition of pyrogallol autoxidation (SOD-like activity), and ferric reducing antioxidant power (FRAP). All demonstrated positive antioxidant activities were lower than those of vitamin C, taken as a standard (Fig. 3). The scavenging efficacy of extracts against DPPH radical was 77.22% against 95.82% for vitamin C. SOD-like activity was relatively low (32.77% of inhibition) compared to that of vitamin C (59.60%). The ferric reducing antioxidant power (FRAP) was 0.88 and 1.07 for the extract and vitamin C, respectively.

Tab. 1. Antioxidant compound contents of *Termitomyces reticulatus*.
Values are expressed as means ± SD by three independent experiments.
Abbreviations: GAE – Gallic acid equivalent, QE – Quercetin equivalent, CysE – Cysteine equivalent, GlyE – Glycine equivalent.

Antioxidants	**Flavonoids** (mg GAE/g extract)	**Polyphenols** (mg QE/g extract)	**Thiols** (mg CysE/g extract)	**Total amino acids** (mg GlyE/g extract)
Content	1.071 ± 0.000	10.606 ± 1.071	0.583 ± 0.038	6.124 ± 0.121

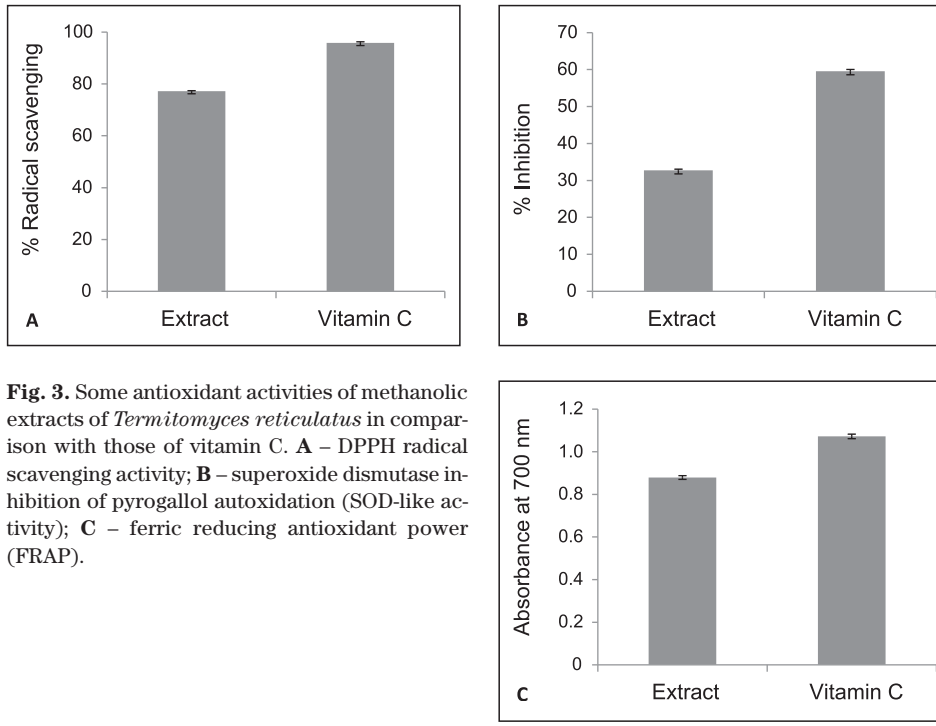

Fig. 3. Some antioxidant activities of methanolic extracts of *Termitomyces reticulatus* in comparison with those of vitamin C. **A** – DPPH radical scavenging activity; **B** – superoxide dismutase inhibition of pyrogallol autoxidation (SOD-like activity); **C** – ferric reducing antioxidant power (FRAP).

DISCUSSION

Species variability, ecology, phenology and distribution

The macro- and microscopic characters of the studied materials agree with the description of *Termitomyces reticulatus* by van der Westhuizen & Eicker (1990). However, our samples had a fistulose stipe, even in a very young stage, and also produced bisporic basidia, which were not mentioned by van der Westhuizen & Eicker (1990) nor De Kesel et al. (2017). Moreover, the Cameroonian samples are closer to those from South Africa in the fact that both have a greyish reticulate pileipellis, unlike Congolese samples, which are characterised by a brownish

orange reticulation. In addition, none of the previous authors mentioned the slimy nature of the species when boiled, which makes it less appreciated by locals of the Noun Division in contrast to the population of DRC and other African countries, where according to De Kesel et al. (2017), it is highly relished. No morphological description of Indian samples is available. However, according to the key provided by Karun & Sridhar (2013), the pileus is creamy white with appressed or upturned brown scales and with an unpronounced to undeveloped umbo. A molecular study of this species including samples from various countries is needed to verify its infraspecific variability.

Termitomyces reticulatus was described as a new species from the Republic of South Africa (RSA) where it grows in association with *Odontotermes badius* Haviland and *O. transvaalensis* Sjöstedt (van der Westhuizen & Eicker 1990). It seems to have a paleotropical distribution and is an edible species locally consumed in tropical Africa and Asia (Härkönen et al. 1995, Loganathan et al. 2010, Sharp 2011, Karun & Sridhar 2013, De Kesel et al. 2017). In the Western Highlands of Cameroon, this species fructifies in July and August, when rains are heavy. This is also the case in DRC, where it grows in the full rainy season (De Kesel et al. 2017). The association with *Odontotermes* termites, a widespread termite genus in tropical areas, can justify its wide distribution in the Western Highlands of Cameroon and predicts its presence in countries where tropical savannah and rainforest biomes are present.

Antioxidant activities

The antioxidant capacity of mushrooms originates from different groups of compounds such as phenolics, flavonoids, glycosides, polysaccharides, tocopherols, ergothioneine, carotenoids, and ascorbic acid (Kozarski et al. 2015). The present study shows that phenolic compounds are the major antioxidants in *T. reticulatus*, followed by flavonoids. This is in agreement with what is observed in other mushrooms, especially *Termitomyces* species (Atri et al. 2012, Johnsy & Kaviyarasan 2014, Hsieh & Ju 2018). The quantities of antioxidants obtained in the present study are higher than those found by Loganathan et al. (2010) from the same species in India. This difference could be due to the variation in composition of the substrate (termite combs) of the collection areas. *Termitomyces reticulatus* seems to have the lowest polyphenol and flavonoid contents compared to other *Termitomyces* species tested (Atri et al. 2012, Johnsy & Kaviyarasan 2014). Thiols include many metabolites like coenzyme A, glutathione, cysteine and ergothioneine. In this study, thiols were found at low levels (0.583 CysE/g extract), but quantitative data on thiols in mushrooms is scarce. Mohamed & Farghaly (2014) detected 7 thiol metabolites in dried and fresh cultivated *Pleurotus ostreatus*.

The evaluated antioxidant potential of *T. reticulatus* is in accordance with the presence of antioxidant compounds in its extracts. The antiradical activity shows that extracts had the capacity to rapidly discolour DPPH with 77.22% radical scavenging. This capacity is higher than that found by Loganathan et al. (2010), who obtained 56.12% in the same species from India. It is also higher than those of *T. heimii*, *T. microcarpus* and *Agaricus bisporus* according to the results of studies by Johnsy & Kaviyarasan (2014) and Keles et al. (2011). However, it is lower than those of *Boletus edulis*, *Suillus luteus* and *Pleurotus ostreatus* according to Keles et al. (2011). Inhibition of pyrogallol by extracts of this mushroom species was effective, showing the presence of compounds mimicking the activity of the SOD enzyme. This has been observed with many species including *T. heimii*, even though its values for the SOD radical scavenging assay was as low as 0.19 mg/ml (Mitra et al. 2016). The extract exhibited ferric reducing antioxidant power, demonstrating that the presence of antioxidants induced the reduction of Fe^{3+} to Fe^{2+}. Loganathan et al. (2010) obtained an optical density of 0.420 at 3.2 mg/ml of extract for *T. reticulatus* from India, while Johnsy & Kaviyarasan (2014) obtained 0.388 and 0.362 for extract of *T. heimii* and *T. microcarpus* tested at 200 µg/ml, respectively.

CONCLUSION

This study provides additional data on the diversity of the genus *Termitomyces* in Cameroon thanks to this first report of *T. reticulatus* in this country. However, molecular data are still needed to determine its phylogenetic position among other species. It also reveals that this species is used as food even though less known by locals than other *Termitomyces* species. *Termitomyces reticulatus* possesses antioxidant potential, although a dose-response study is needed, and could be beneficial in the prevention of oxidative stress-related diseases.

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