Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
12(1): 125–135 (2022) ISSN 2229-2225
www.creamjournal.org **Article**
Doi 10.5943/cream/12/1/10

In vitro screening of the anti-diabetic activity of six species of edible termite associated mushrooms (*Termitomyces* spp.) from the Western Highlands of Cameroon

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Kanjo WR, Njouonkou A-L, Yongabi AK, Manfo TFP, Tume C, Nantia AE 2022 – *In vitro* screening of the anti-diabetic activity of six species of edible termite associated mushrooms (*Termitomyces* spp.) from the Western Highlands of Cameroon. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 12(1), 125–135, Doi 10.5943/cream/12/1/10

Abstract

Diabetes mellitus is a chronic metabolic disorder characterized by impaired glucose homeostasis with disturbances in carbohydrate, fat and protein metabolism. The present study aimed at evaluating the anti-diabetic potential of six different *Termitomyces* mushrooms (*T. letestui, T. microcarpus, T. schimperi, T. aurantiacus, T. clypeatus,* and *T. umkowaan*) by testing their ability to inhibit the enzymes *α*-amylase, *β*-glucosidase, invertase and lipase that are involved in carbohydrate and lipid digestion within the gastrointestinal tract. Different inhibitory assays were carried out using aqueous extracts of the mushroom species at five different concentrations (0.5–2.5 mg/mL). Results showed inhibition enzyme specific variability with *T. umkowaan*, *T. clypeatus*, *T. microcarpus*, and *T. aurantiacus* showing the highest inhibitory activity on *α*-amylase (78.8%, IC_{50} = 1.355 ± 0.001mg/mL), *β*-glucosidase (77.0%, IC_{50} = 1.387 ± 0.006mg/mL), invertase (85.8%, IC_{50} = 1.171 ± 0.002 mg/mL) and lipase (61.6%, IC_{50} = 1.834 ± 0.007mg/mL), respectively. In general, the studied *Termitomyces* possess anti-diabetic potentials with some specificity according to the targeted enzyme.

Keywords – Anti-diabetic activity – diabetes mellitus – invertase – lipase – *Termitomyces* – *α*-amylase – *β*-glucosidase

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by impaired glucose homeostasis with disturbances in carbohydrate, fat, and protein metabolism, resulting from defects in insulin secretion (type I diabetes mellitus), insulin action (type II diabetes mellitus) or both. The incidence of diabetes mellitus is increasing at an alarming rate, affecting more than 5% of the world's population (Kazeem et al. 2013). The number of people with diabetes rose from 108 million in 1980 to 422 million in 2014 (WHO 2021). Risk factors for diabetes include excess body weight, high blood pressure, old age, lack of exercise and unhealthy dieting. Early symptoms of diabetes mellitus include polyuria, polyphagia and polydipsia (WHO 2019).

Submitted 15 April 2022, Accepted 7 August 2022, Published 19 August 2022
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Diabetes mellitus is considered to be a serious health issue in many countries including Cameroon which recorded 680,300 cases of diabetes in 2017 with a prevalence rate of 5.9% (IDF 2017). Clinical diabetes is managed with drugs such asacarbose, miglitol and voglibose (Chandrasekar et al. 2012). However, these hypoglycaemic agents have limitations because of their non-specificity and are known to produce serious side effects such as gastrointestinal bloating, abdominal discomfort, diarrhoea and flatulence (Gondokesumo et al. 2017). Natural products are generally considered to be safer, accessible and cost effective with regard to pharmaceutical drugs. Traditional medicines with anti-diabetic potential have been found to have different modes of action – mimic insulin, act on insulin secreting beta cells, and modify glucose utilization (Chandrasekar et al. 2012), and also inhibit different carbohydrate digesting enzymes (Kazeem et al. 2013).

Degradation of dietary carbohydrates proceeds rapidly and leads to elevated post-prandial hyperglycaemia (Bhatia et al. 2019). It has been shown that activity of *α*-amylase, *β*-glucosidase and invertase in the digestive system correlates to an increase in post-prandial glucose levels, the control of which is therefore an important aspect in treatment of diabetes mellitus (Gondokesumo et al. 2017). Hence, control of carbohydrate digestion by inhibiting these enzymes can lead to reduction in the rate of glucose absorption and lower post-prandial glucose levels (Rhabasa-Lhoret et al. 2004). Obesity is considered as a risk factor and a cause of type 2 diabetes mellitus (Golay & Ybarra 2005). Obesity and type 2 diabetes mellitus frequently occur together, and statistics show that 60-90% of patients with type 2 diabetes mellitus are or have been obese (Stumvoll et al. 2005).

Termitomyces R. Heim includes a group of termites associated mushroom generally found in Africa. The name *Termitomyces* was coined by Heim (1942), referring to a group of agarics associated with termite nests in central Africa (Sangvichien & Taylor-Howksworth 2001). The nutritional and medicinal properties of *Termitomyces* mushrooms have been documented (Mizuno et al. 1995). Mushrooms contain about twice the protein of most fresh vegetables, apart from beans, peas and lentils (Buyck 1994). In addition to being highly nutritious, *Termitomyces* mushrooms also have potential medicinal properties. *Termitomyces* have been demonstrated to possess potential antimicrobial, antitumor, and antioxidant properties, as well as potentials for treating neurodegenerative disorders (Elkhateeb & Daba 2020, Thu et al. 2020). Their unique chemical properties make them promising for research for novel therapeutic agents. This study aimed at assessing the *in vitro* anti-diabetic effects of six *Termitomyces* mushrooms (*T. letestui* (Pat.) R. Heim, *T. microcarpus* (Berk. & Broome) R. Heim, *T. schimperi* (Pat.) R. Heim, *T. aurantiacus* (R. Heim) R. Heim, *T. clypeatus* R. Heim, and *T. umkowaan* (Cooke & Massee) D.A. Reid) on the inhibition of enzymes (*α*-amylase, *β*-glucosidase, invertase and lipase) activities.

Materials & methods

Reagents

P-nitrophenyl *β*-D-glucopyranoside, P-nitrophenyl butyrate and 3, 5 dinitrosalicylic acid (DNS) were gotten from Sigma-Aldrich Co. (St Louis, USA), while starch was purchased from J.T. Baker Inc. (Phillipsburg, USA). Other reagents were of analytical grade.

Collection of mushrooms and preparation of aqueous extracts

Termitomyces mushroom species namely *T. letestui, T. microcarpus, T. schimperi, T. aurantiacus, T. clypeatus,* and *T. umkowaan* (Fig. 1) were collected from the North West and West regions of Cameroon between May and November 2019. The different species were identified in the field according to their morphological characteristics by a mycologist - Prof. Njouonkou André-Ledoux. The mushrooms were well cleaned from soil and other dirt, chopped into small pieces and dried using an electric dryer between 35-40^{o}C until a constant dry weight was obtained. The dried mushrooms were then ground and reduced to fine powder using a blender. The powder from each species was stored in the freezer at -18^{o}C until extraction.

For each mushroom, thirty grams of powder were introduced in 300 mL boiled distilled water, the mixture allowed to stand for 6 hours, and then filtered using Whatman filter paper grade No. 1. The filtrate was concentrated by evaporating to dryness in an oven at 40°C, and the resulting dried residue stored at 4°C until biochemical analyses.

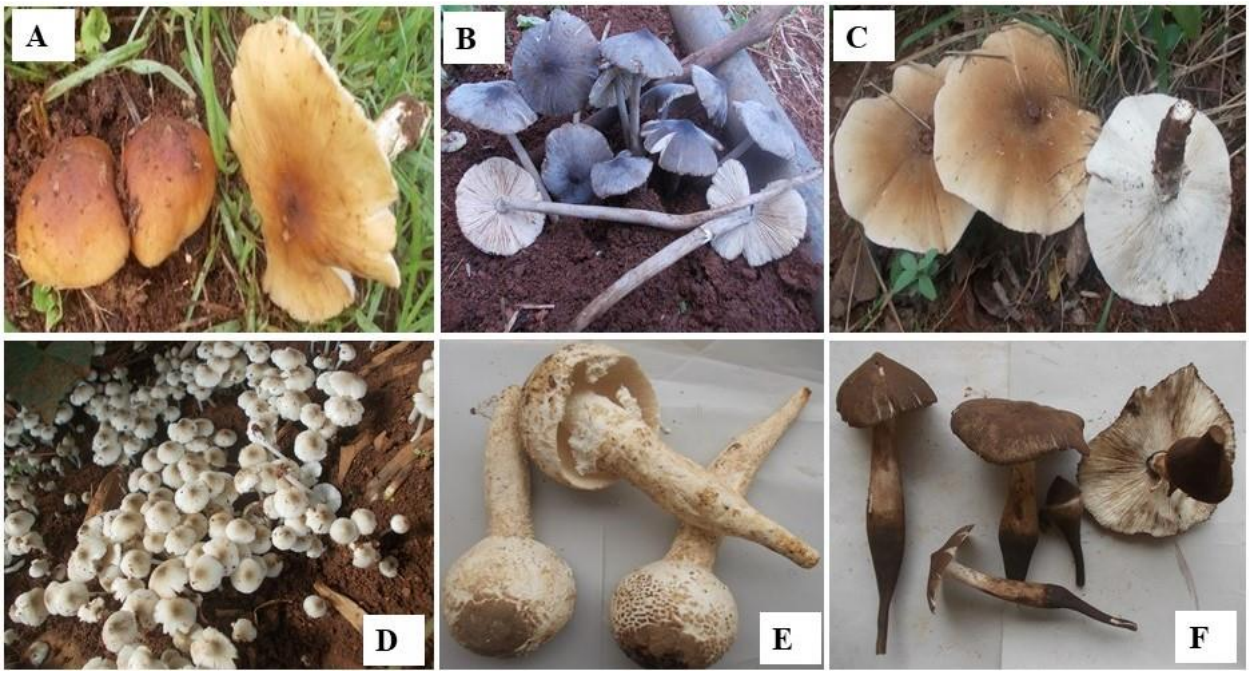

Fig. 1 – *Termitomyces* mushrooms species used in the study. A *T. aurantiacus*. B *T. clypeatus*. C *T. letestui*. D *T. microcarpus*. E *T. schimperi*. F *T. umkowaan*.

Preparation of the enzyme extracts from rat small intestine

Homogenates containing enzymes of interest were prepared using organs from adult albino Wistar rats. Rats were handled according to ethical guidelines of the Cameroon National Veterinary Laboratory. Rats obtained from the animal house of the Department of Biochemistry of the University of Bamenda, Cameroon, were anesthetized using diazepam, sacrificed, and small intestine dissected out. The intestine was homogenized in phosphate buffer (pH 7.1, 0.01 M), the homogenate centrifuged (3000 rpm, 15 min, 4°C) and the supernatant used as a source of digestive enzymes α-amylase, β-glucosidase, invertase and lipase.

α-amylase inhibitory assay

The α-amylase inhibitory assay was performed using the starch-iodine method as described by Anuradha et al. (2017), with slight modifications. Assay mixture containing different concentrations of 500 μL mushroom aqueous extract (0, 0.5, 1, 1.5, 2 and 2.5 mg/mL), 500 μL of phosphate buffer (pH 6.9, 0.02 M) and 500 μL of enzyme extract were prepared in assay tubes. The mixtures were pre-incubated for 10 min at 37°C in a water bath, and then 500μL of 1% starch was added. The resulting mixture was incubated at 37°C for 15 min and the enzymatic reaction was stopped with 20 μL of 1 M HCl, and of 100 μL of iodine reagent (5 mmol I_2 and 5 mmol KI) were added. The control representing 100% enzyme activity was prepared accordingly without the mushroom extract. The absorbance of the solution was then read at 620 nm, and inhibition of enzyme activity calculated as:

$$\%\text{Inhibition} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}}\right] \times 100.$$

β-glucosidase inhibitory assay

The β-glucosidase inhibitory assay was performed using the method reported by Gondokesumo et al. (2017) with some modifications. Briefly, an assay mixture containing 250 μL of phosphate buffer (pH 6.9, 0.02 M), 200 μL of p-nitrophenyl β-D-glucopyranoside (20 mM), and 250 μL of mushroom aqueous extract (0, 0.5, 1, 1.5, 2 and 2.5 mg/mL) was pre-incubated at 37^{o}C for 5 minutes. The enzyme extract (250 μL homogenate was added, the mixture incubated for 15 minutes and the reaction stopped by adding 1000 μL of sodium carbonate (100 mM). The control was prepared accordingly but without mushroom extracts. Absorbance of the solution was then measured at 400 nm and percentage inhibition of β-glucosidase calculated using the formula:

$$\%\text{Inhibition} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}}\right] \times 100.$$

Invertase inhibitory assay

The invertase inhibitory assay was carried out as described by Honda & Hara (1993), with slight modifications. The enzyme extract (10 μL) and mushroom aqueous extract (10 μL; 0, 0.5, 1, 1.5, 2 and 2.5 mg/mL) were incubated at 37^{o}C in a water bath for 10 minutes, then 180 μL of phosphate buffer (pH 6.9, 0.1 M) were added. The enzyme reaction was started by adding sucrose solution (100 μL, 60 mM), and the reaction terminated after 30 minutes by adding 200 µL of 3, 5 dinitrosalysilic acid reagent. The colour was developed by boiling the mixture in a water bath for 5 minutes, the absorbance of the solution measured at 540 nm and the percentage inhibition of invertase calculated using the formula:

$$\%\text{Inhibition} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}}\right] \times 100.$$

The control was prepared accordingly without the mushroom extracts.

Lipase inhibitory assay

The lipase inhibitory assay was carried out as described by Bustanji et al. (2011) with slight modifications. Summarily, 100 μL of enzyme extract were introduced into the test tube and 200 μL and 700 μL of mushroom aqueous extract (0, 0.5, 1, 1.5, 2 and 2.5 mg/mL) and Tris-HCl buffer (pH 7.8, 0.01 M), respectively were added. The mixture was pre-incubated at 37^{o}C for 15 minutes, and then 100 μL of p-nitrophenyl butyrate solution (10 mg/mL) were added the mixture incubated once more for 30 minutes at 37^{o}C. The control was prepared accordingly without the mushroom extracts. The absorbance of the solution was measured at 405 nm and percentage inhibition of lipase calculated using the formula:

$$\%\text{Inhibition} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}}\right] \times 100.$$

Statistical analyses

Data were analysed using SPSS version 20.0. Data were expressed as mean ± standard deviations (SD) of duplicate measurements. The concentration of mushroom extract at which the enzyme activity is reduced by 50% (IC_{50}) was calculated using linear regression analysis with Microsoft Excel 2010. The significance of the difference was evaluated using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. Values of $p<0.05$ were considered as significant.

Results

α-amylase activity

The percentage inhibition of *α*-amylase activity by different *Termitomyces* spp. aqueous extracts is shown in Fig. 2. The aqueous extract from *T. umkowaan* exhibited highest inhibition on *α*-amylase assay with 78.8% inhibition (at the highest concentration investigated, 2.5 mg/mL) with the lowest and significantly different ($P< 0.05$) IC_{50} value (1.355 ± 0.001 mg/mL). This was followed by *T. aurantiacus* (70.1% inhibition, IC_{50} = 1.455 ± 0.005 mg/mL) and *T. letestui* (65.2% inhibition, IC_{50} = 1.597 ± 0.002 mg/mL) aqueous extracts. *T. microcarpus* aqueous extract showed the lowest inhibitory activity with 61.8% maximum inhibition, and IC_{50} of 1.793 ± 0.004 mg/mL (Table 1). In general, the IC_{50} values showed significant difference ($P< 0.05$) among mushroom extracts on the inhibition of the *α*-amylase activity (Table 1).

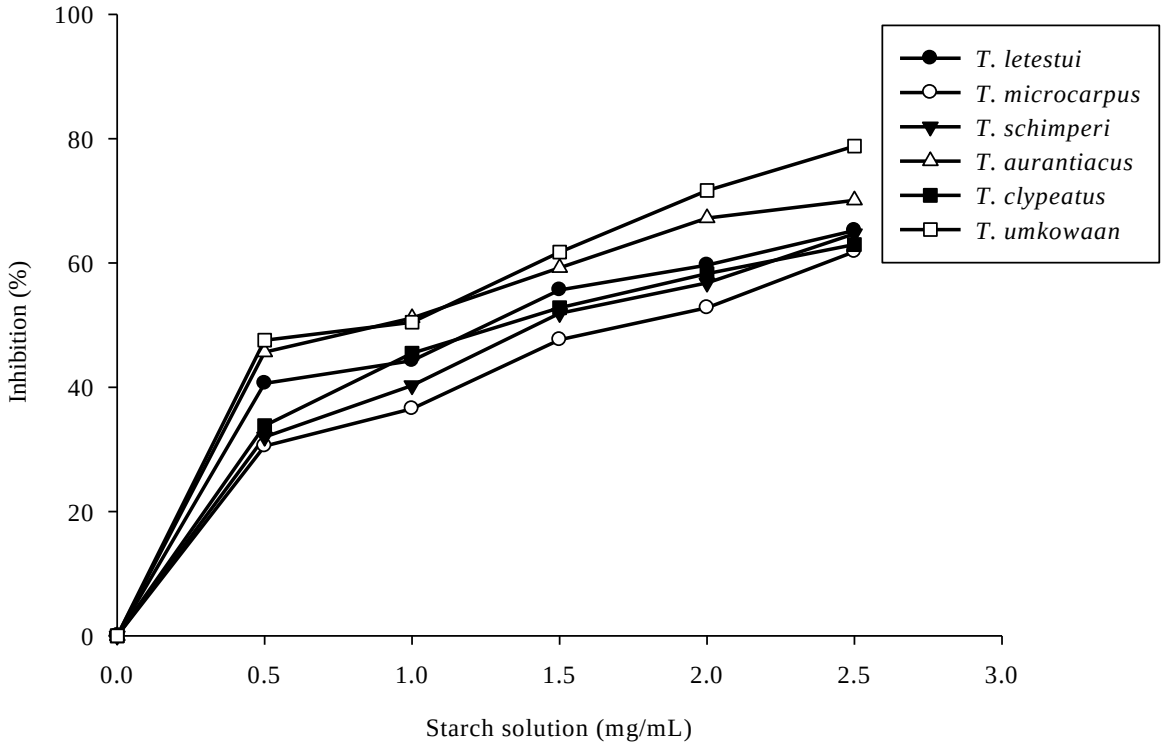

Fig. 2 – Percentage inhibition of Termitomyces mushroom aqueous extracts on α-amylase activity.

β-glucosidase activity

On *β*-glucosidase inhibitory assay (Fig. 3), *T. clypeatus* aqueous extract exhibited the highest activity with 77.0% maximum inhibition (at the highest concentration, 2.5 mg/mL) and IC_{50} of 1.387 ± 0.006 mg/mL (Table 1). *T. microcarpus* (73.3% inhibition, IC_{50} = 1.438 ± 0.009 mg/mL) and *T. aurantiacus* (68.0% inhibition, IC_{50} = 1.666 ± 0.000 mg/mL) aqueous extracts showed moderate inhibitory activity on the enzyme, while *T. schimperi* extract displayed the lowest activity (45.6% inhibition at highest concentration, IC_{50} = 2.429 ± 0.006 mg/mL). Comparatively, mushroom extracts showed significant difference ($P< 0.05$) in inhibiting the *β*-glucosidase activity with *T. clypeatus* extract having the highest activity (Table 1).

Invertase activity

The aqueous extract from *T. microcarpus* exhibited the highest inhibitory effect on invertase activity (Fig. 4) with 85.8% inhibition (at the highest concentration, 2.5 mg/mL) with an IC_{50} of 1.172 ± 0.002 mg/mL (Table 1), while *T. letestui* (82.6% inhibition, IC_{50} = 1.265 ± 0.001 mg/mL) and *T. aurentiacus* (82.6% inhibition, IC_{50} = 1.326 ± 0.001 mg/mL) aqueous extracts also presented appreciable enzyme inhibition. *T. umkowaan* aqueous extract showed the lowest inhibitory effect

on enzyme activity with 51.8% maximum inhibition (IC_{50} = 2.189 ± 0.027 mg/mL). Comparatively, mushroom extracts showed statistically (P< 0.05) different inhibitory effect on invertase activity with the most significant being *T. microcarpus* (Table 1).

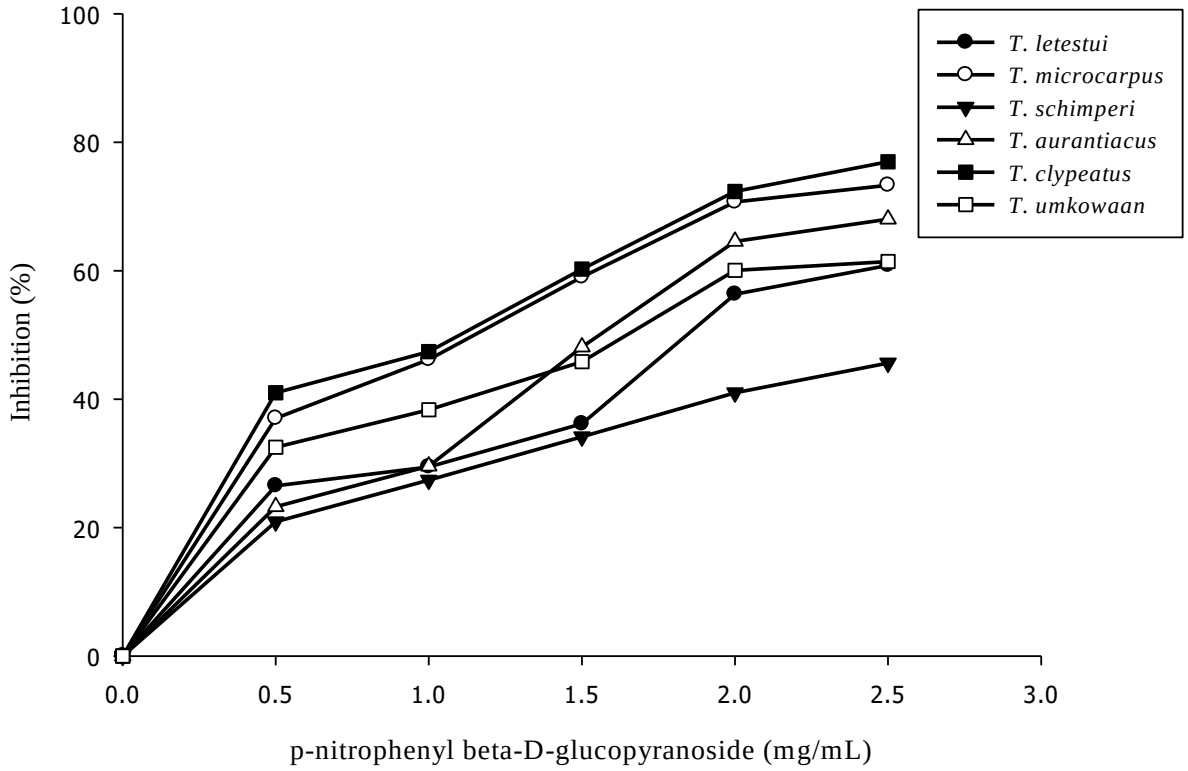

Fig. 3 – Percentage inhibition of Termitomyces mushroom aqueous extracts on β-glucosidase activity.

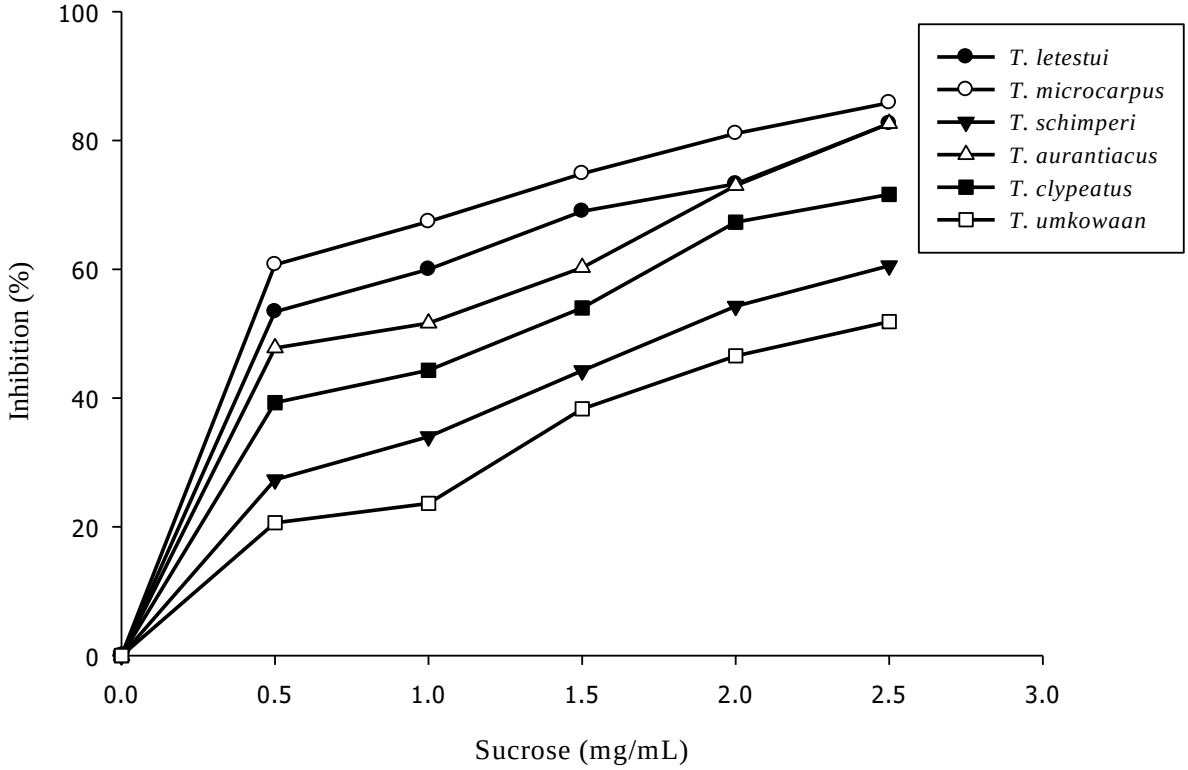

Fig. 4 – Percentage inhibition of Termitomyces mushroom aqueous extracts on invertase activity.

***Lipase* activity**

The aqueous extract from the mushroom *T. aurantiacus* showed the highest inhibition on lipase activity (Fig. 5) with 61.6% inhibition (at the highest concentration, 2.5 mg/mL) and an IC_{50} of 1.834± 0.007 mg/mL (Table 1). Intermediary inhibitory activities were induced by *T. clypeatus* (56.9% inhibition, IC_{50} = 2.008 ± 0.004 mg/mL) and *T. umkowaan* (45.8% inhibition, IC_{50} = 2.559 ± 0.031 mg/mL) aqueous extracts. *T. schimperi* aqueous extract showed the lowest degree of inhibition with a maximum percentage inhibition of 30.2% and an IC_{50} of 4.263 ± 0.004 mg/mL. As function of the IC_{50}, mushroom showed significant ($P< 0.05$) inhibition of the lipase activity with the highest activity obtained with *T. aurantiacus* (Table 1).

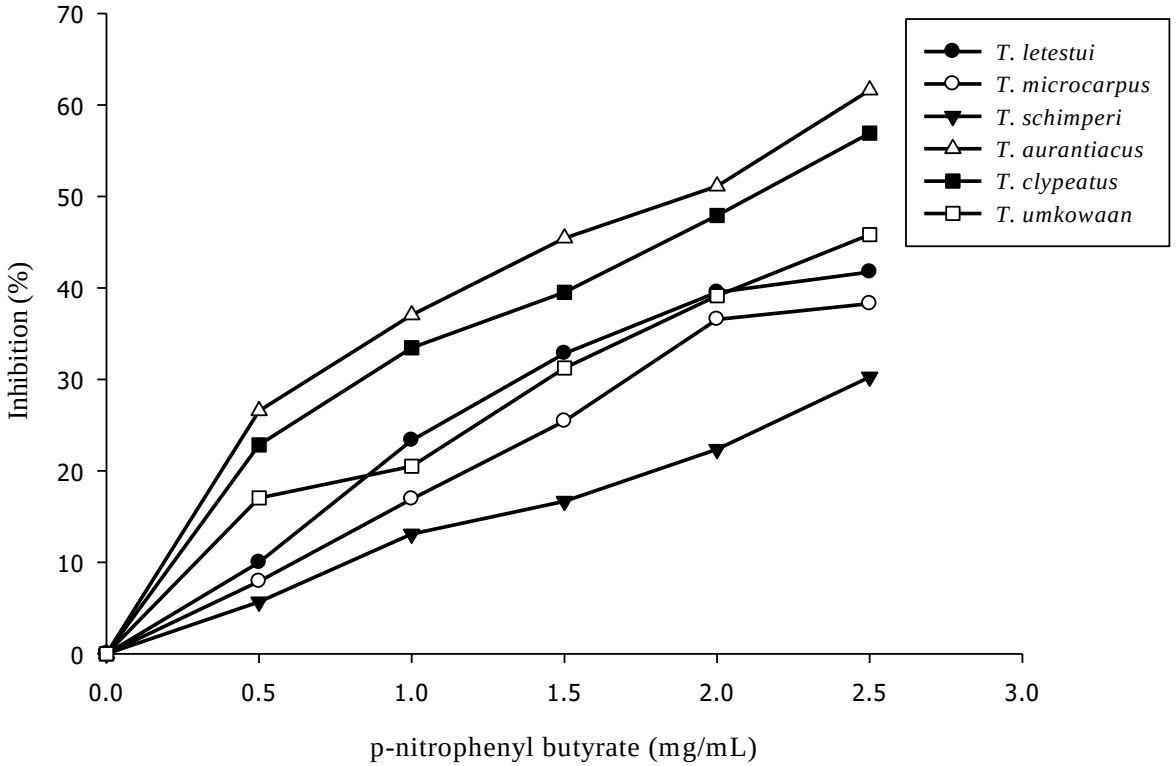

Fig. 5 – Percentage inhibition of *Termitomyces* aqueous mushroom extracts on lipase activity.

Table 1 IC_{50} values of *Termitomyces* mushroom aqueous extracts in *in vitro* *α*-amylase, *β*-glucosidase, sucrose and lipase assays.

Mushroom species	IC_{50} values (mg/mL)			
	***α*-amylase**	***β*-glucosidase**	**Invertase**	**Lipase**
T. letestui	1.597 ± 0.002^{c}	1.900 ± 0.005^{e}	1.265 ± 0.001^{b}	2.647 ± 0.048^{b}
T. microcarpus	1.793 ± 0.004^{f}	1.438 ± 0.009^{b}	1.171 ± 0.002^{a}	3.018 ± 0.025^{d}
T. schimperi	1.679 ± 0.000^{e}	2.429 ± 0.006^{f}	1.839 ± 0.004^{e}	4.263 ± 0.004^{e}
T. aurantiacus	1.455 ± 0.005^{b}	1.666 ± 0.000^{c}	1.326 ± 0.001^{c}	1.834 ± 0.007^{a}
T. clypeatus	1.654 ± 0.003^{d}	1.387 ± 0.006^{a}	1.499 ± 0.002^{d}	2.008 ± 0.004^{c}
T. umkowaan	1.355 ± 0.001^{a}	1.731 ± 0.009^{d}	2.189 ± 0.027^{f}	2.559 ± 0.031^{b}

The data are presented as mean ± SD of duplicate independent measurements (N = 2). Different superscript letters (a, b, c, d, e, f) indicate significant difference within the same column between mushroom species (Dunnett's post hoc test, $P<0.05$).

Discussion

Diabetes mellitus is a common endocrine disorder resulting from defects in insulin secretion, insulin action, or both. Hyperglycaemia is a common feature of all types of diabetes mellitus, but aetiology, underlying pathogenic mechanisms, natural history and treatment for the different types

of diabetes differ (WHO 2019). Recent advances in understanding the role of carbohydrate digesting enzymes in diabetes have led to the development of new pharmacological agents that play an important role in the control or management of the disease. The activity of α-amylase, β-glucosidase and invertase in the digestive system correlates to an increase in post-prandial glucose levels. Hence, control of carbohydrate digestion by inhibiting the activity of these enzymes can lead to reduction in the rate of glucose absorption and thus lowers post-prandial glucose levels thereby contributing in treatment and control of diabetes mellitus (Rhabasa-Lhoret et al. 2004, Gondokesumo et al. 2017).

In vitro tests play an important role in the evaluation of anti-diabetic activity of drugs as initial screening tools, where the screening of a large number of potential therapeutic candidates may be necessary (Sabitha et al. 2012). In the present study, the ability of the aqueous extracts of different *Termitomyces* mushrooms to inhibit different enzymes involved in carbohydrate and lipid digestion present in brush border of the small intestine was investigated. The *in vitro* inhibitory studies on the carbohydrate and lipid digesting enzymes demonstrated that the different *Termitomyces* species studied exhibit inhibitory effect on α-amylase, β-glucosidase, invertase (sucrase) and lipase. In all the inhibitory assays, the percentage inhibition increased in a concentration-dependent manner. The concentration dependent activity of the mushroom extracts is an important point that could be exploited in further evaluation of the pharmacological action of these mushrooms.

Inhibition of α-amylase promotes retardation of starch hydrolysis, reducing the digestion and absorption rate of carbohydrates, and consequently a decrease in post-prandial hyperglycaemia (Soeng et al. 2015). It has also been suggested that α-amylase inhibition may preserve β-cell integrity and function by removing free radicals, resulting in enhancement of the protection against the progression of insulin resistance into type 2 diabetes mellitus (Kwon et al. 2007). The maximum percentage inhibitions of the studied *Termitomyces* on α-amylase activity are slightly lower than that of the milky mushroom (*Calocybe indica* Purkay. & A. Chandra) with percentage inhibition of 89.49 ± 3.54% (Prabu & Kumuthakalavalli 2017), but similar to that of *Agaricus bisporus* (78.85%) (Periyan et al. 2018). However, the inhibitory effect of the mushrooms in the current study was higher than that of the white oyster mushroom (*Pleurotus florida* (Mont.) Singer) with maximum inhibitory activity of 26.5%, on the activity of α-amylase (Sumathy et al. 2015). *Termitonyces* have been shown to contain phenolic and flavonoid, compounds (Aryal & Budhathoki 2016, Mitra et al. 2016, Njouonkou et al. 2020, Choumessi et al. 2021), which play an important role in the inhibition of α-amylase activity (Gondokesumo et al. 2017, Gudise et al. 2019).

The enzyme β-glucosidase also plays a significant role in digestion of dietary carbohydrates and is associated with metabolic disorders such as diabetes mellitus. β-glucosidase catalyzes hydrolysis of glycosidic bonds to terminal non-reducing residues in β-D-glucosides and oligosaccharides, with the release of glucose. In humans, the enzyme breaks down glucosylceramide into ceramide and glucose (Singh et al. 2016). It is therefore targeted in the therapeutic approach to alleviate hyperglycaemia. The inhibitory effect of *Termitomyces* mushroom extracts observed in the current study could be due their richness in phenolic and flavonoid compounds, which have been shown to inhibit the enzyme of β-glucosidase (Parizadeh & Garampalli 2016, Gondokesumo et al. 2017, Njouonkou et al. 2020, Choumessi et al. 2021).

Enterocyte of the small intestine can absorb monosaccharides such as glucose and fructose thanks to the presence of intestinal sucrase or invertase which is an important enzyme that breaks down dietary sucrose to glucose and fructose (Bracho & Whitaker 1990). This greatly increases post-prandial blood glucose levels. A previous study reported that the mushroom *Phellinus linteus* (Berk. & M.A. Curtis) Teng exert anti-diabetic potentials by inhibiting invertase with an IC_{50} value of 1.37 mg/mL (Hwang-Young et al. 2012), which is lower than the IC_{50} values for the mushrooms *T. microcarpus* and *T. letestui* obtained herein. However, *T. aurantiacus* in the current study exhibit similar activity as the mushroom *Phellinus linteus*. Inhibition of invertase activity by *Termitomyces*

mushrooms could be attributed to phytonutrients including flavonoid glycosides, hydrolysable tannins, rutin and quercetin (Ahmed et al. 2009, Abdelhady et al. 2015).

Diabetes and obesity are closely linked, with obesity regarded as a major risk factor in the development of type 2 diabetes mellitus (Golay & Ybarra 2005). Therefore, the inhibition of the enzyme lipase which breaks down dietary lipids and makes absorption possible is an important step towards control of obesity and consequently development of type 2 diabetes mellitus. The present study demonstrated that *Termitomyces* extracts have inhibitory potential on lipase activity. The maximum inhibitory effect of the studied *Termitomyces* species on lipase activity is relatively greater than that of methanol extracts of the mushrooms *Pleurotus ostreatus* (Jacq.) P. Kumm. (18.2%) and *Cordyceps militaris* (L.) Fr. (10.8%) but lower than that of *Ganoderma lucidum* (Curtis) P. Karst. (66.9%) and *Ganoderma oregonense* Murrill (67.4%) as reported in a study by Lee et al. (2010). The inhibition of lipase activity by the *Termitomyces* extracts could be attributed to phytonutrients such as saponins and polyphenols (phenolic acids such as gallic, coumaric, caffeic, chlorogenic acids and flavonoids), whose anti-lipase activity has been reported. Indeed, chemical analysis of the investigated mushrooms have been shown to contain most of the latter substances (Garza et al. 2011, Aryal & Budhathoki 2016, Jaradat et al. 2017).

Several other mechanisms have been proposed for the hypoglycaemic effects of phytochemicals, such as manipulation of glucose transporters, *β*-cell regeneration and enhancing insulin releasing activity and sensitivity (Tiwari & Rao 2002). These mechanisms could be explored in future evaluation of the anti-diabetic properties of the present *Termitomyces* mushrooms.

Conclusion

Globally, this study suggested inhibition enzyme specific variability, with *T. umkowaan*, *T. clypeatus*, *T. microcarpus*, and *T. aurantiacus* displaying the highest inhibitory activity on *α*-amylase, *β*-glucosidase, invertase and lipase, respectively. The findings demonstrated that *Termitomyces* species possess noticeable but species-specific capacity to reduce glycaemia; thus, these *Termitomyces* mushrooms could be further explored as source of anti-diabetic drugs or part of the diet of people suffering from diabetes.

Acknowledgments

The authors express their gratitude to the Ministry of Higher Education of Cameroon for the support in term of Research Modernization to lecturers. We also acknowledge the assistance of Dr. Sagu Sorel for donating some chemicals of this study.

Author contributions

KWR took part in designing of the work and carried out the experiments; NAL contributed in designing, selection and identification of mushroom samples, follow-up of the experiments and drafting of the manuscript; YAK, MFTP and TC were involved in discussion of data and reviewing the manuscript; NAE contributed in the design and follow up of the experiments, preparation and revision of the manuscript.

Declaration of conflicting interests

The author(s) declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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