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Bioactive Potential of *Lentinus squarrosulus* and *Termitomyces clypeatus* from the Southwestern region of India

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The antioxidants derived from biological sources are generally regarded as safe than synthetic ones owing to their less or no adverse effects. Wild mushrooms being a potential source of nutrients as well as antioxidants, bioactive properties of uncooked and cooked wood-inhabiting *Lentinus squarrosulus* and termite mound-inhabiting *Termitomyces clypeatus* have been compared. Both mushrooms showed appreciable antioxidant potential, which is influenced by their bioactive principles (total phenolics, tannins, flavonoids and vitamin C) and proximal properties (crude protein, crude lipid, crude fibre and carbohydrates). These mushrooms were devoid of trypsin inhibition and hemagglutinin activity. Uncooked as well as cooked mushrooms with adequate nutritional components serve as sources of potential antioxidant to combat many human ailments. The present study and recent perspectives on nutraceuticals of macrofungi denote *L. squarrosulus* and *T. clypeatus* as the high-value nutraceutical sources in Southwestern India.

Keywords: Antioxidants, Bioactive principles, Macrofungi, Mushrooms, Nutraceuticals, Proximate properties, Wild mushrooms.

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Introduction

Exploration of natural and organic products which impart significant nutritional and health benefits has become an utmost priority. Macrofungi have been considered as part of the human diet since several centuries owing to their aroma, taste, texture and nutritional value. In the recent past, they have been gaining importance as functional foods and health-promoting products based on their active principles[1,2]. Besides nutritional attributes, many mushrooms are known for their antioxidant and biological potential (e.g. antibacterial, antiviral, anti-inflammatory, antitumor and cytotoxic activities) thereby serving as nutraceutical sources[3-6]. Mushroom-derived secondary metabolites like phenolics, polyketides, terpenes, steroids and vitamins are of immense value in human diet[7-9]. In addition, immuno-modulating, hypoglycemic, anti-hypercholesterolemic, blood pressure preventing and atherosclerosis controlling principles and products have been derived from mushrooms[10]. Mushroom-derived biopolymers also serve as model compounds in the development of suitable food and pharmaceutical products[11].

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Different biomes of the Western Ghats and west coast of India are known for high diversity of macrofungi and many of them serve as traditional nutritional source[12-27]. Although several wild macrofungi are traditionally known for their nutritional and medicinal values, a precise picture on their importance needs experimentation. Therefore: i) the first objective of the present study was to explore the bioactive potential of uncooked and cooked wild edible mushrooms of Southwest India (*Lentinus squarrosulus* Mont. and *Termitomyces clypeatus* R. Heim); ii) the second objective was to link their bioactive potential with proximal qualities and antioxidant properties to gain knowledge on their nutraceutical potential.

Materials and Methods

Mushrooms and processing

Wood-inhabiting *Lentinus squarrosulus* and termite mound-inhabiting *Termitomyces clypeatus* were collected from the coastal sand dunes and coconut plantations of Southwest India during monsoon season, respectively (Plate 1). Freshly collected mushrooms from five different locations of coastal sand dunes and coconut plantations as replicates were separately collected and each sample was grouped into two portions. The first set was oven dried on aluminum

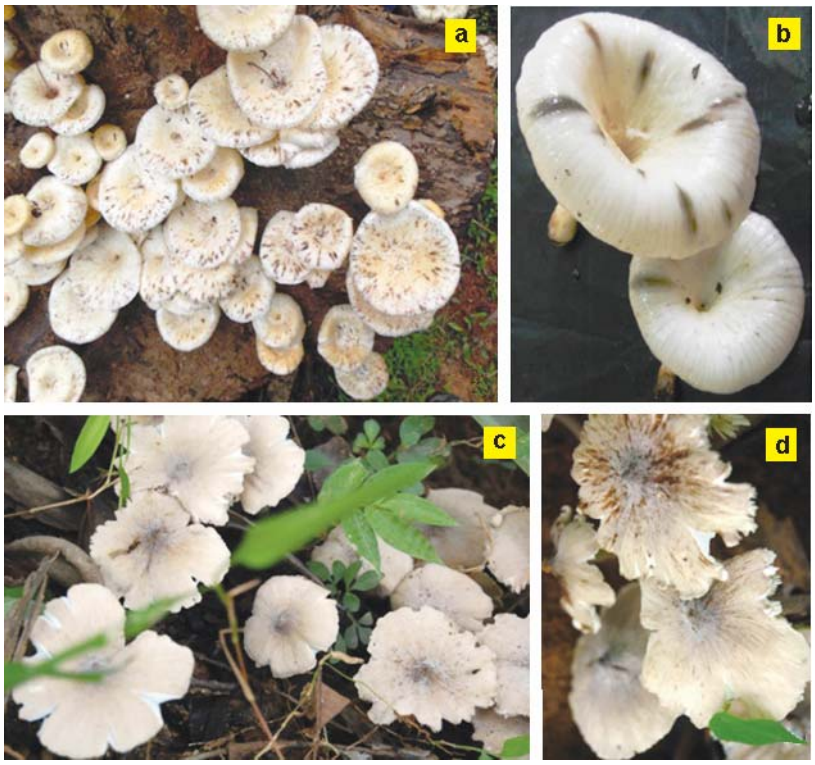

Plate 1 — Mushroom fruiting bodies assessed for nutritional analysis: a and b, *Lentinus squarrosulus* grown on wood; c and d, *Termitomyces clypeatus* grown on termite mound.

foils (50–55 °C) and the second set was cooked in household pressure-cooker with finite water (6.5 L, Deluxe stainless steel, TTK Prestige™, Prestige Ltd., Hyderabad, India) followed by oven drying. Dried samples were milled (Wiley Mill, mesh # 30), transferred to airtight containers and refrigerated (4 °C).

Bioactive components

Total phenolics

Total phenolic content of uncooked and cooked mushroom flours was determined by Rosset *et al.*[28]. Briefly, mushroom flour (50 mg) was extracted in 50 % methanol (5 mL) in a water bath (95±1 °C) up to 10 min and centrifuged (1500 rpm) to collect the supernatant. The extraction was repeated for the pellet in a centrifuge tube, on pooling supernatant was made up to 10 mL. An aliquot of extract (0.5 mL) was mixed with distilled water (0.5 mL) and treated against Na_2CO_3 (0.1 N NaOH, 5 mL). Upon incubation (10 min), Folin-Ciocalteu's reagent (diluted with distilled water, 1:2 v/v; 0.5 mL) was added and the absorbance was measured at 725 nm (UV-VIS Spectrophotometer-118, SYSTRONICS, Ahmadabad, Gujarat, India). The standard curve was prepared using tannic acid and the result was expressed as mg tannic acid equivalents (TAEs/g).

Tannins

The tannin of uncooked and cooked mushroom flour was assayed by Vanillin-HCl method[29]. Mushroom flour (1 g) was extracted with methanol (50 mL at 28 °C, 24 h) and centrifuged to collect the supernatant. The extract (1 mL) was treated with vanillin hydrochloride reagent mixture (4 % vanillin in methanol and 8 % concentrated HCl in methanol, 1:1; 5 mL). The color developed was read after 20 min at 500 nm with 50–250 µg catechin as standard (Sigma Aldrich, 98 % HPLC grade, USA). The tannin content was expressed as catechin equivalents (CEs/g).

Flavonoids

The flavonoids content of mushroom flour was estimated based on the procedure by chang *et al.*[30]. After methanol extraction (1 mg/mL), aliquot (0.5 mL) was mixed with methanol (1.5 mL), aluminium chloride (10 %, 0.1 mL), potassium acetate (1 M, 0.1 mL) and distilled water (2.8 mL). On incubation for 30 min at laboratory temperature, the absorbance was measured (415 nm). Quercetin was used as standard

and the flavonoids content was expressed in mg quercetin equivalents (mg QEs/g).

Vitamin C

To determine vitamin C content of mushroom flour, method outlined by Roe[31] was employed with a slight modification. Mushroom flour (1 g) was extracted in trichloroacetic acid (TCA, 10 mL; 5 %). An aliquot (0.2 mL) was made up to 1 mL (by 5 % TCA) and 2,4-dinitrophenylhydrazine (DNPH, 1 mL) was added. The mixture was boiled (10 min), cooled to laboratory temperature, sulphuric acid was added (65 %, 4 mL), incubated (30 min) at laboratory temperature and the absorbance was measured (540 nm). To prepare standard curve, ascorbic acid was used (Sisco Research Laboratories, Mumbai, India; purity, 99.8 %). The vitamin C content was expressed as ascorbic acid equivalents (mg AAEs/g).

Trypsin inhibition activity

The trypsin inhibition activity of mushroom flours was assayed by Kakade *et al*.[32]. One gram mushroom flour was extracted with NaOH (0.01 N, 50 mL), 1 mL extract was made up to 2 mL with distilled water. Trypsin (2 mL) solution (4 mg in 200 mL 0.001M HCl) was added, incubated up to 10 min in a water bath (37 °C) followed by addition of 5 mL of BAPNA [(40 mg N-a-Benzoyl-DL-Arginine p-nitroanilide hydrochloride in 1 mL dimethyl sulfoxide diluted to 100 mL with tris-buffer at 37 °C)]. On further incubation up to 10 min at laboratory temperature, the reaction was terminated by addition of 1 mL acetic acid (30 %) followed by thorough mixing and filtration. The absorbance was measured (410 nm) against reagent blank (30 % acetic acid containing 2 mL each of trypsin and distilled water + 5 mL BAPNA, 1 mL). One unit of trypsin inhibition (TIu/mg) is defined as release of 1 μM of p-nitroanilide per min by the enzyme reaction.

Hemagglutinin activity

The hemagglutinin activity of mushroom flours was assessed based on Occenã *et al*.[33]. Flour (500 mg) was suspended in 0.9 % NaCl (10 mL) by vigorous mixing and allowed to stand for 1 h followed by centrifugation (2,000 g, 10 min). The upper clear solution was filtered and the crude filtrate was used for agglutinin activity. The RBCs (A, B, AB and O) were separated from 5 mL human blood by centrifugation (2,000 g, 10 min) followed by dilution in cold saline (0.9 %, 1:4), centrifuged (2,000 g, 10 min) and the supernatant was discarded. The pellet was washed in saline until the supernatant became colorless. Four mL of washed erythrocytes were suspended in 100 mL phosphate buffer (0.0006 M, pH 8.4). Trypsin solution (2 %, 1mL) was added to 10 mL washed erythrocytes, mixed and incubated (37 °C for 1 h). The trypsinized erythrocytes were washed 4–5 times in saline to eliminate traces of trypsin. The erythrocyte suspension (1.2–1.5 mL) was re-suspended in 100 mL saline and used to dispense into microtitre plates. To the first well of microtitre plate (8 rows of 12 wells), the crude agglutinin extract was added and the 12th well served as control, which was devoid of extract. Aliquot of saline (0.3 mL) was dispensed to well 2–12. Serial dilution was followed from the well 2–11. Trypsinized RBC in saline (2 %, 0.3 mL) was dispensed to wells 1–12. The contents were mixed and incubated at laboratory temperature (4 h). The pattern of hemagglutination in each well was observed and the hemagglutinating unit (Hu) was determined ($Hu/g = (D_a \times D_b \times S) / V$). Where D_a, dilution factor of extract in well 1 (the crude agglutinin extract remains as 1 if the original extract is not diluted); D_b, dilution factor of well containing 1 Hu is the well in which hemagglutination was first seen; S, mL original extract/g flour; V, volume of extract in well 1.

Antioxidant assays

The extract of mushroom flour was prepared by extracting 0.5 g flour in 30 mL methanol (shaken at 150 rpm, 48 h). The extract was centrifuged and supernatant was collected in a pre-weighed Petri dish and allowed to dry at laboratory temperature. The weight of the extract was determined gravimetrically followed by dissolving in methanol to get the desired concentration (1 mg/mL) to carry out antioxidant assay.

The study consists of five assays of evaluation of antioxidant properties of mushroom flours: i) reduction of Mo(VI) to Mo(V) by antioxidant compounds (total antioxidant activity, TAA); ii) reduction of Fe(III) to Fe(II) ions (Fe^{2+} chelating capacity); iii) the DPPH radical absorption on exposure to radical scavengers (radical-scavenging activity); iv) conversion of peroxide (hydrogen peroxide) to water (peroxide-scavenging activity); v) conversion of Fe^{3+}/ferricyanide complex into the ferrous form (reducing capacity).

Total antioxidant activity

The total antioxidant activity (TAA) was assessed based on Prieto *et al*.[34] by mixing mushroom extract (0.1 mL) with reagent mixture (sulfuric acid, 0.6 M + sodium phosphate, 28 mM + ammonium molybdate, 4

mM; 1 mL). The mixture was incubated (95 °C for 90 min), cooled to ambient temperature and absorbance of phosphomolybdenum complex formed was measured (695 nm) using methanol as blank. The TAA was expressed in µM equivalent of ascorbic acid per gram of the mushroom flour (µM AAEs/g).

Ferrous ion-chelating capacity

Antioxidants convert ferric ions which can react with lipid hydro-peroxidases and form free radicals into ferrous which chelates with chromogenic ligand (ferrozine) to give a coloured compound which can be measured[35,36]. The ferrous ion-chelating capacity was evaluated based on Hsu *et al*.[37]. To the extract (1 mL), $FeCl_2$ (2 mM, 0.1 mL) and ferrozine (5 mM, 0.2 mL) were added and final volume was made up to 5 mL by methanol. The mixture was incubated (10 min) at ambient temperature and Fe^{2+}-ferrozine complex formed was determined at absorbance 562 nm. The sample devoid of extract has been considered as control to calculate ferrous ion-chelating capacity: Ferrous ion-chelating capacity (%) = $[1 - (A_{s562} \div A_{c562})] \times 100$. Where, Ac is absorbance of the control and As is absorbance of sample.

DPPH radical-scavenging activity

The DPPH radical-scavenging activity is one of the widely used methods in assessing antioxidant activity in a short duration, where antioxidants donate their free hydrogen to DPPH which becomes a stable diamagnetic molecule. This causes a change in the colour of the solution from purple to yellow which can be easily measured[1]. Free radical-scavenging activity of mushroom extracts was evaluated based on Singh *et al*.[38] and the effective concentration (EC_{50}; sample required to scavenge 50 % of DPPH radicals) (µg/mL) was obtained by plotting per cent radical-scavenging activity against concentration of the extracts. Various concentrations (0.2–1 mL: 200–1,000 µg) of the test samples were made up to 1 mL using methanol. Four mL [(0.01 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH)] was dispensed and allowed to react at room temperature (20 min). The reagents devoid of extract served as control and absorbance of the mixture was measured at 517 nm and radical-scavenging activity was calculated: Free radical-scavenging activity (%) = $[(A_{c517} - A_{s517})] \times 100$. Where, Ac is absorbance of the control and As is absorbance of sample.

The effective concentration (EC_{50}; concentration of sample necessary to scavenge 50 % of the DPPH radicals) (µg extract/mL) was prepared by plotting per cent radical-scavenging activity vs. concentration of the extracts.

Hydrogen peroxide-scavenging activity

The hydrogen peroxide-scavenging assay was performed according to Ruch *et al*.[39]. The scavenging activity of mushroom extract was determined by addition of H_2O_2 (0.1 mM, 1 mL) and equal volume (1 mg/mL) of mushroom extract and mixed. Two drops of ammonium molybdate (3 %), sulphuric acid (2 M, 10 mL) and potassium iodide (1.8 M, 7.0 mL) were added to the reaction mixture. The mixture was titrated against NaS_2O_3 (5.09 mM) until appearance of yellow colour as end point. The reaction mixture devoid of sample served as control to calculate hydrogen peroxide-scavenging activity: Hydrogen peroxide-scavenging activity (%) = $(V_0 - V_1/V_0) \times 100$. Where, V_0, volume of NaS_2O_3 solution used to titrate the control and V_1, volume of NaS_2O_3 solution used to titrate the test sample.

Reducing capacity

The reducing capacity is one of the robust ways of testing antioxidant activity of a compound across a broad concentration gradient. It was determined according to Pulido *et al*.[40] with a slight modification. Different concentrations (0.2–1 mg: 0.2–1 mL) of mushroom flour extracted in methanol were taken and phosphate buffer was added (0.2 M, pH 6.6; 2.5 mL) followed by potassium ferricyanide (1 %, 2.5 mL). Contents were vortexed and incubated (50 °C, 20 min), TCA was added (10 %, 2.5 mL), centrifuged (3000 rpm, 10 min) and the supernatant (2.5 mL) was mixed with distilled water (2.5 mL). To this, ferric chloride (0.1 %, 5 mL) was added and the absorbance was measured at 700 nm.

Data analysis

Difference in bioactive components between uncooked and cooked mushroom flours was assessed by *t*-test using Statistica (version 8.0)[41]. The principal component analysis (PCA) was performed to find out relationship of bioactive principles (total phenolics, tannins, flavonoids and vitamin C) and proximal properties (crude protein, crude lipid, crude fibre and total carbohydrates) of uncooked and cooked mushroom flours against antioxidant activities (total antioxidant activity, ferrous ion-chelating capacity, DPPH radical-scavenging activity, hydrogen peroxide-scavenging activity and reducing power) (SPSS 16.0: www.spss.com). The PCA score plot was used to

determine whether bioactive principles vs. antioxidant activities and proximal properties could be grouped in to different classes.

Results and Discussion

Bioactive principles

Total phenolics

Mushrooms gained importance in human diet owing to their phenolic contents as potent natural antioxidants and in turn combat many diseases[42]. Total phenolics were lower in uncooked *L. squarrosulus* than uncooked *T. clypeatus*, but although significantly decreased upon cooking. However, total phenols were not as low as in cooked *T. clypeatus* (Fig. 1a). Total phenolics content of uncooked samples of *L. squarrosulus* was comparable with *L. squarrosulus* studied in Malaysia[43] and it was higher than many *Lentinus* spp. of Northern India [(*L. cladopus* Lév., *L. connatus* Berk., *L. sajor-caju* (Fr.) Fr. and *L. torulosus* (Pers.) Lloyd] (29.7 vs. 6.4–20.1 mg/g)[44]. Interestingly, cooking has increased the total phenolic content in *L. edodes* (Berk.) Singer as reported in Korea (29 vs. 54.6 mg/g)[45]. Total phenolics content in uncooked *T. clypeatus* decreased up to one-fifth on cooking (35 vs. 7.3 mg/g). Total phenolics of uncooked *T. clypeatus* were higher than many *Termitomyces* species (*T. badius* Otieno, *T. heimii* Natarajan, *T. mammiformis* R. Heim, *T. medius* R. Heim Grassé, *T. radicatus* Natarajan, *T. striatus* (Beeli) R. Heim and *T. umkowaan* (Cooke & Massee) D.A. Reid) (35.1 vs. 15–22.5 mg/g) and comparable with *T. microcarpus* (Berk. & Broome) R. Heim (35.1 vs. 22.5–37 mg/g)[24,46]. However, very high quantity of total phenolics has been reported in *T. mammiformis* and *T. robustus* (Beeli) R. Heim (211.8 and 178 mg/g, respectively) from Nigeria, but their antioxidant potential was not so high[47].

Tannins

Similar to tannins in plants, mushrooms also possess tannins as important dietary antioxidants owing to their anti-microbial, anti-mutagenic and anti-carcinogenic potential[48,49]. Tannin content of uncooked *T. clypeatus* was higher than *L. squarrosulus*, but it decreased to almost same quantity on cooking in both the mushrooms (Fig. 1b). Tannin content of *L. squarrosulus* was lesser than *L. tuber-regium* (Fr.) Fr. (2.1 vs. 3.3 mg/g) reported from Orissa[50]. Similarly, tannin content of *T. clypeatus* was substantially lower than other *Termitomyces* species from Nigeria (*T. mammiformis* and *T. robustus*) (6 vs. 169–171 mg/g)[47], but higher than *T. umkowaan*[24]. Tannins are known for radical-scavenging, inhibition of lipid peroxidation, protease inhibition and inactivation of pro-oxidant metal ions[51–53]. They are also thought to have the capability to prevent oxidative damage caused by carbohydrates, fats and proteins in the digestive tract.

Flavonoids

The flavonoids have been considered as an important components in polyphenolic group, which are prevalent in many fruits, vegetables and mushrooms. Based on chemical nature, they have been classified into flavones, flavanones, catechins, anthocyanins, isoflavones, dihydroflavanols and chalcones[54–56]. Flavonoids were higher in *L. squarrosulus* than in *T. clypeatus* and significantly decreased upon cooking up to 50 % and 20 %, respectively (Fig. 1c). Its content in uncooked *L. squarrosulus* was higher than other *Lentinus* species (*L. edodes* and *L. tuberregium*) (19 vs. 2–2.5 mg/g)[45,50]. Flavonoids content of *T. clypeatus* was higher than other *Termitomyces* species (*T. badius, T. heimii, T. medius, T. mammiformis, T. microcarpus, T. radicatus, T. striatus* and *T. umkowaan*) (7.7–9.7 vs. 1.4–4.1 mg/g)[46,24]. However, the Nigerian species of *Termitomyces* (*T. mammiformis* and *T. robustus*) possessed higher amount than Indian *Termitomyces* species (23.9–25.7 vs. 1.4–9.7 mg/g)[47]. Flavonoids are known for a wide range of pharmaceutical properties (anti-bacterial, anti-viral, anti-diabetic, anti-inflammatory, hepatoprotective, anti-thrombotic, anti-atherosclerotic, anti-neoplastic, cardioprotective and vasodilatory activities)[54]. Thus, flavonoids content data in uncooked and cooked mushrooms will be very useful for culinary and pharmaceutical applications.

Vitamin C

The vitamin C functions as a powerful antioxidant, pro-oxidant and radical scavenger, but it is prone to chemical degradation by heat or thermal treatment[57,58]. Vitamin C was significantly higher in uncooked *T. clypeatus* than in uncooked and cooked *L. squarrosulus* (Fig. 1d). In both mushrooms, cooking resulted in significant decrease in vitamin C. Its content in uncooked *T. clypeatus* was higher than *T. heimii, T. albuminosus* (Berk.) R. Heim and *T. umkowaan* (1.1 vs. 0.04–0.13 mg/g)[24,59,60] but lower than other species of *Termitomyces* (*T. aurantiacus* (R. Heim) R. Heim, *T. clypeatus, T. eurrhizus* (Berk.) R. Heim, *T. le-testui* (Pat.) R. Heim, *T. microcarpus* and *T. titanicus* Pegler & Piearce) (1.1 vs. 1.91–4.9 mg/g) from Tanzania[61]. In uncooked *L. squarrosulus*, the vitamin C content was

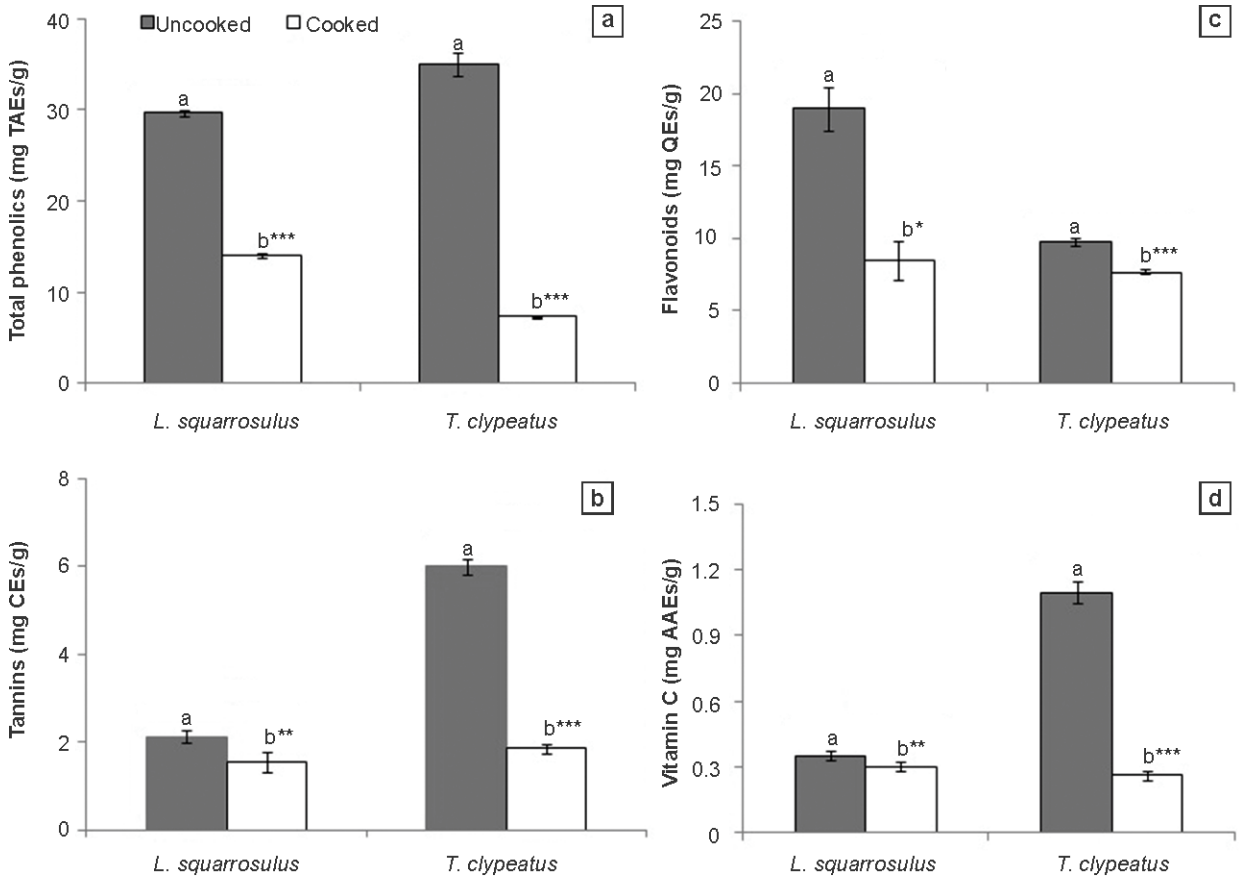

Fig. 1 — Bioactive components: a) Total phenolics (TAEs, tannic acid equivalents), b) tannins (CEs, catechin equivalents), c) flavonoids (QEs, quercetin equivalents), and d) vitamin C in uncooked and cooked *Lentinus squarrosulus* and *Termitomyces clypeatus* (n = 5, mean±SD) (*p <0.05, **p < 0.01, ***p < 0.001, t-test).

significantly higher than other *Lentinus* species in India (*L cladopus, L. connatus, L. sajor-caju* and *L. torulosus*) (0.35 vs. 0.004-0.005 mg/g)[43]. The vitamin C content also followed similar pattern like those of total phenolics and tannins.

Trypsin inhibition and hemagglutinin activities

Uncooked and cooked samples of both mushrooms did not exhibit trypsin inhibition as well as hemagglutinin activities, which is nutritionally not beneficial. These attributes qualify them as better source of nutrition than those mushrooms possessing trypsin inhibition and hemagglutination activities.

Bioactivity

Currently, there is a great demand for natural antioxidants as they are generally regarded as safe compared to the synthetic antioxidants. The synthetic antioxidants (butylated hydroxytoluene BHT; butylated hydroxyanisole, BHA) are widely used in food industries and they are carcinogenic, damage the liver and linked to stomach cancer if used in higher quantities[62,63].

Wong *et al*[64] reported that, at least two methods could be employed for their fair assessment as the antioxidant capacity of test samples influenced by various factors. The present study embodies five assays to arrive at tangible conclusions on the bioactive potential of wild mushrooms studied.

Total antioxidant activity

The total antioxidant activity was higher in uncooked *L. squarrosulus* than in *T. clypeatus* and decreased significantly on cooking (Fig. 2a). However, antioxidant potential of *T. clypeatus* was close to that of *T. umkowaan* as reported from the Southwest India[24].

Ferrous ion-chelating capacity

The ferrous ion-chelating capacity was slightly higher in *T. clypeatus* than in *L. squarrosulus* with significant decrease on cooking at 0.6 mg/mL concentration (Fig. 2b). This capacity of *T. clypeatus* was comparable to *T. umkowaan*[24]. The metal-chelators convert the pro-oxidant metals into stable compounds thereby reduce the damaging effect[9,65].

DPPH radical-scavenging activity

The DPPH radical-scavenging activity was slightly higher in *T. clypeatus* than in *L. squarrosulus* (49.8 vs. 46.1 %) with significant decrease on cooking in both mushrooms at 1 mg/mL concentration (Fig. 2c). Scavenging activity of *T. clypeatus* was three-fold higher than *T. umkowaan*[24]. In spite of low total phenolics, tannins and vitamin C, uncooked as well as

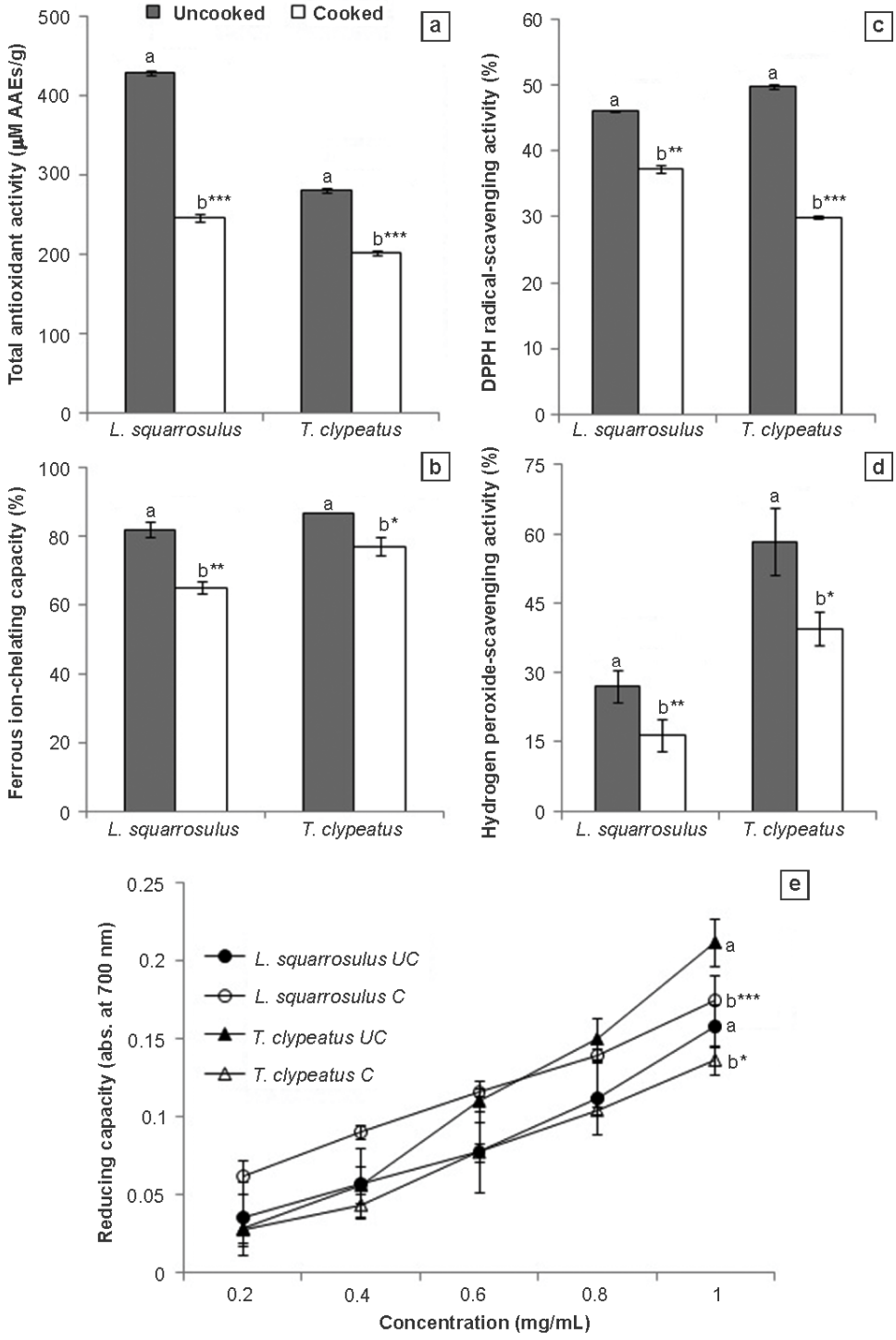

Fig. 2 — Bioactivity: a) Total antioxidant activity (AAEs, ascorbic acid equivalents); b) Fe^{2+} chelating capacity (600 µg/mL), c) DPPH radical-scavenging activity (1 mg/mL), d) hydrogen peroxide-scavenging (peroxidase) activity, and e) reducing power in uncooked and cooked *Lentinus squarrosulus* and *Termitomyces clypeatus* (n = 5, mean±SD) (**p* <0.05, ***p* <0.01, ****p* <0.001, *t*-test).

cooked *L. squarrosulus* showed higher total antioxidant activity, almost close to *T. clypeatus* in ferrous ion-chelation and DPPH radical-scavenging activities. This could be due to very high flavonoid content present in *L. squarrosulus.*

Hydrogen peroxide-scavenging activity

The hydrogen peroxide-scavenging potential was very high in uncooked as well as in cooked *T. clypeatus* compared to *L. squarrosulus* (Fig. 2d). It was significantly reduced in both mushrooms on cooking. Iron as a pro-oxidant, generates free radicals via lipid peroxidation and it speeds up peroxidation by decomposing lipid hydroperoxides into secondary free radicals leading to a cascade of events. Reactive oxygen species (ROS) and other free radicals cause progressive damage to the immune system leading to several problems (degeneration and ageing) against which antioxidants fight by different mechanisms as first line of defence to protect the cell membrane from the harmful effects of lipid peroxidation[3,7,9,66].

Reducing capacity

The reducing power at 1 mg/mL concentration in uncooked *T. clypeatus* was higher than *L. squarrosulus*. It has decreased significantly on cooking in *T. clypeatus* but increased in *L. squarrosulus* (Fig. 2e). Reducing power of *T. clypeatus* was considerably higher than *T. umkowaan*[24]. But, cooking significantly lowered the reducing power, which was almost equal in *T. clypeatus* as well as *T. umkowaan*.

Bioactivity vs. bioactive components

The PCA performed on antioxidant activities vs. bioactive principles and proximal properties resulted in two components (Eigen value, <1), which accounted for 100 % variance. In uncooked *L. squarrosulus*, rotated score plot revealed 51.2 % variance for component 1, while 48.8 % for component 2 (Fig. 3a). Total antioxidant (taa) and hydrogen peroxide-scavenging (prx) activities were clustered with flavonoids (flv) and tannins (tan) in group 1, while ferrous ion-chelating capacity (fic) clustered with crude protein (crP), crude lipid (crL) and total carbohydrates (crb) in group 2. In cooked *L. squarrosulus*, rotated score plot revealed 51.45 % variance for component 1, while 49.55 % for component 2 (Fig. 3b). In group 1, the DPPH radical-scavenging (dpp) and peroxide-scavenging (prx) activities were clustered with crude lipid (CrL), total phenolics (phn) and tannins (tan), while ferrous ion-chelating capacity (fic) and reducing power (rpo) were clustered with vitamin C (vtC), crude protein (crP) and crude fibre (crF) in group 2.

In uncooked *T. clypeatus*, rotated score plot revealed 52.7 % variance for component 1 and 47.3 % variance for component 2 (Fig. 4a). The DPPH radical-scavenging activity (dpp), reducing power (rpo), total antioxidant activity (taa) and ferrous ion-chelating capacity (fic) clustered with total phenolics (phn), tannins (tan), vitamin C (vtC) and total carbohydrates (crb). In cooked *T. clypeatus,* rotated score plot revealed 68.7 % variance for component 1 and 31.3 % variance for component 2 (Fig. 4b). In group 1, DPPH radical-scavenging activity (dpp), total antioxidant

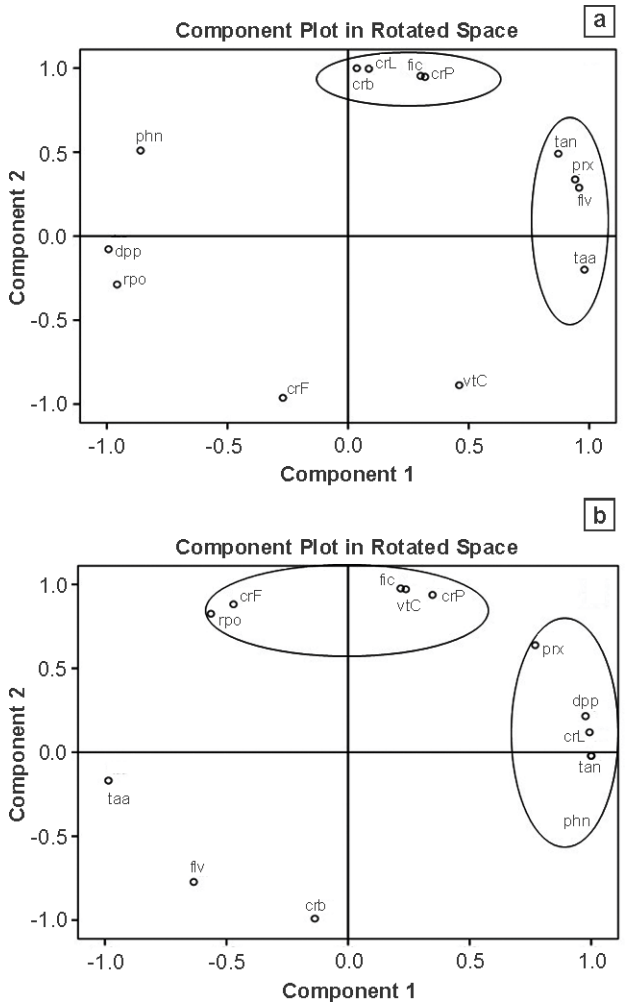

Fig. 3 — Principal component analysis of *Lentinus squarrosulus*: a) Uncooked and b) Cooked [(Bioactive principles: total phenolics (phn), tannins (tan), flavonoids (flv) and vitamin C (vtC); proximal properties: crude fibre (crF), crude lipid (crL), crude protein (crP) and total carbohydrates (crb); vs. antioxidant activities: total antioxidant activity (taa), free radical-scavenging activity (dpp), ferrous ion-chelating capacity (fic), reducing power assay (rpo) and hydrogen peroxide-scavenging activity (prx)].

activity (taa) and reducing power (rpo) were clustered with flavonoids (flv), crude lipids (crL) and total carbohydrates (crb), while in group 2, peroxide-scavenging activity (prx) and ferrous ion-chelating capacity (fic) were clustered with crude protein (crP).

The above results based on comparison of antioxidant activity vs. proximal and bioactive components clearly indicate that *L. squarrosulus* as well as *T. clypeatus* possess very good antioxidant potential, which is governed by proximal as well as bioactive components.

In spite of mushrooms being part of human diet for several centuries owing to their taste and flavor, they

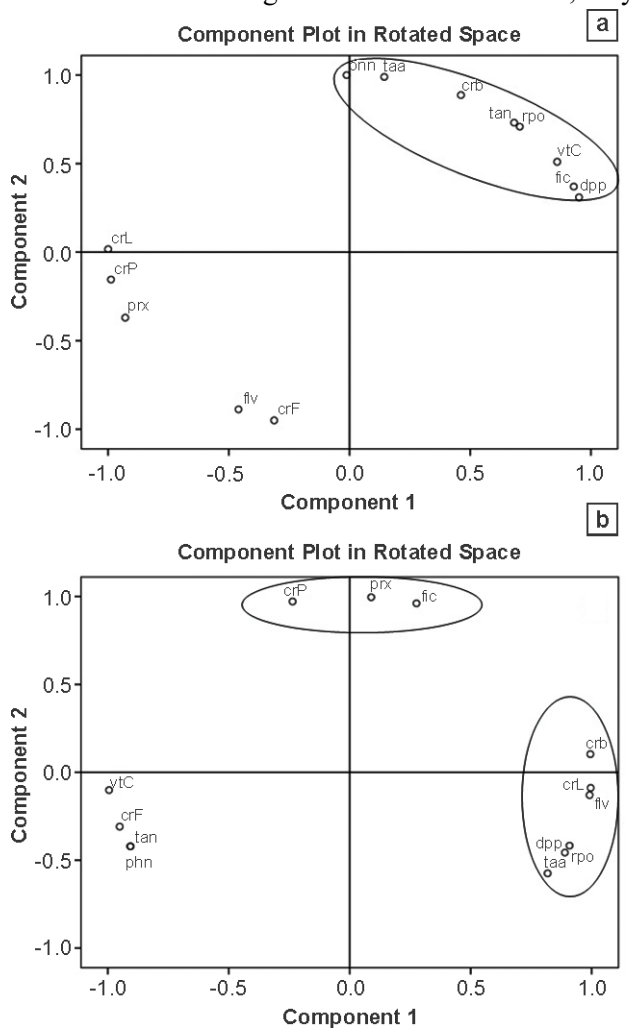

Fig. 4 — Principal component analysis of *Termitomyces clypeatus*: a) Uncooked and b) Cooked [(Bioactive principles: total phenolics (phn), tannins (tan), flavonoids (flv) and vitamin C (vtC); proximal properties: crude fibre (crF), crude lipid (crL), crude protein (crP) and total carbohydrates (crb); vs. antioxidant activities: total antioxidant activity (taa), free radical-scavenging activity (dpp), ferrous ion-chelating capacity (fic), reducing power assay (rpo) and hydrogen peroxide-scavenging activity (prx)].

are still underexplored source for food and nutraceuticals[67]. There are over 2000 mushrooms known to possess nutritional, medicinal and health-promoting compounds[68,69]. According to Manzi and Pizzoferrato[70], uncooked mushrooms yield more beneficial products than cooked mushrooms corroborating with the present study.

Macrofungal polysaccharides have many applications in the field of cancer biology, immunology and microbiology[71,72]. The β-glucans are dietary fibres in mushrooms possess cholesterol and glucose lowering effects[70]. *Lentinus* spp. are rich source of β-glucans (called lentinans), which possesses anti-tumor and immune boosting properties[73]. A glucan molecule (PS-I) isolated from a hybrid of *L. squarrosulus* + *Pleurotus florida* Singer known to stimulate and activate the cells of the immune system (macrophages, splenocytes and thymocytes) *in vitro* indicating their potential application in the cancer and anti-microbial therapies[74]. Instant noodles preparations incorporate β-glucan-enriched materials (BGEMs) derived from *L. edodes* resulted in low oil uptake on frying (reduced up to 22 %) is one of the healthier options[75]. This opens up several possibilities to employ mushroom-derived BGEMs incorporation in food products. Some *in vivo* studies (rat model) on *L. squarrosulus* revealed interesting results[43,76]. The LD_{50} was not seen up to a concentration of *L. squarrosulus* at 5 g/kg body weight and at low concentration (0.25 g/kg body weight) could heal ulcers. The effective concentration was much lower than its lethal dose indicating safety of using *L. squarrosulus*[42]. The bioavailability of *L. squarrosulus* mycelium extract *in vivo* in rats showed the presence of absorbable antioxidants, which has the ability to move into the circulating plasma thereby resulting a sharp increase in antioxidant capacity proving its versatility in formulations of nutraceuticals[76]. Abdullah *et al.*[77] also studied the anti-ulcerogenic activity of *L. squarrosulus* and proposed its use as a potent nutraceutical agent. Thus, wild *L. squarrosulus* of Southwestern India serves as a potent future nutraceutical source.

Among the 23 species of indigenous mushrooms from India, based on the antioxidant index some *Termitomyces* species [(e.g., *T. heimii, T. mammiformis, T. schimperi* (Pat.) R. Heim and *T. tyleranus* Otieno] are considered to possess very high value. Species of *Termitomyces* such as *T. heimii* and *T. mammiformis* contain high amount of gallic acid, gentisic acid, protocatacheuic acid, and tannic acid[78].

In a study, it was observed that the polyphenol-rich fraction of *T. heimii* possessed many active compounds (like ascorbic acid, β-carotene, flavonoids, pyrogallol, and lycopene + cinnamic acid) and showed EC_{50} as low as 0.021 mg/mL and 0.19 mg/mL for hydroxyl radical-scavenging and super oxide dismutase-scavenging, respectively[60].

Conclusion

The results of the present study indicates that both the mushrooms possess considerable quantities of bioactive compounds and antioxidant potential, which qualifies them to be used as potent nutraceutical sources. Also based on the results, *T. clypeatus* can be advocated as another high-value food for future applications.

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