First ethnomycological record of *Fistulinella wolfeana* as an edible species and some of its nutritional values

Primer registro etnomicológico de *Fistulinella wolfeana* como una especie comestible y algunos valores nutricionales

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RESUMEN

Registramos por primera vez el uso de *Fistulinella wolfeana* como especie comestible en las comunidades otomíes de Tesquedó, Xajay y Tenasdá, en Amealco de Bonfil, Querétaro, México, la cual forma parte de los recursos naturales utilizados durante la estación de lluvias. Además, esta especie está ampliamente distribuida en el Eje Volcánico Transversal, y tiene el potencial de ser utilizada como recurso no maderable. Se describe el valor nutricional de las muestras en fresco, así como en seco. Además, tiene una alta actividad antioxidante, alcanzando casi 90% de inhibición en diluciones 1:10 de extracto metanólico, comparable con valores para otros miembros de la familia Boletaceae.

PALABRAS CLAVE: hongos comestibles, etnomicología, valor nutritivo, actividad antioxidante.

ABSTRACT

We registered for first time the use of *Fistulinella wolfeana* as an edible species in the Otomi communities of Tesquedó, Xajay and Tenasdá, in Amealco de Bonfil, Querétaro, México, which is part of the natural resources used during the rainy season. In addition, this species is widely distributed in the Volcanic Transmexican Belt, and it has the potential to be used as non-timber resource. The nutritional value for fresh, as well as dried samples, is described. Additionally, it has high antioxidant activity, reaching almost 90% of inhibition in 1:10 dilutions of methanolic extract, comparable with values for other members of the Boletaceae family.

KEYWORDS: edible mushroom, ethnomycology, nutritional value, antioxidant activity.

INTRODUCTION

Mexico is a high diversity center of the Boletaceae family, with 212 known taxa (García and Garza-Ocañas, 2001) of 877 species described in the world (Kirk *et al*., 2008). *Fistulinella wolfeana* Singer & J. García was described as a Mexican new species (Singer *et al.*, 1991), however, its edibility was not determined. González and Valenzuela (1993) developed an inventory of Boletaceae and Gomphidiaceae from the State de México, and reported their seasonality and distribution, but they did not

Recibido / Received: 19/02/2016
Aceptado / Accepted: 30/11/2016

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mention them as an edible species. This is a very common species in Mexican forests (García *et al*., 1998; García, 1999; García and Garza-Ocañas, 2001). Villanueva *et al.* (2008) mentioned *F. wolfeana* as a mycorrhizal species with Quercus spp., and García (1999) described its distribution along the Mexican central states, such as Jalisco, Guanajuato, Querétaro, Morelos, the State of México and Hidalgo, but it could be found in other states.

Gomphidiaceae from the State de México, and reported their seasonality and distribution, but they did not mention them as an edible species. This is a very common species in Mexican forests (García *et al*., 1998; García, 1999; García and Garza-Ocañas, 2001). Villanueva *et al.* (2008) mentioned *F. wolfeana* as a mycorrhizal species with Quercus spp., and García (1999) described its distribution along the Mexican central states, such as Jalisco, Guanajuato, Querétaro, Morelos, the State of México and Hidalgo, but it could be found in other states.

Estrada-Torres and Aroche (1987), Burrola-Aguilar *et al.* (2012) and Lara-Vázquez *et al.* (2013) studied the ethnomycological knowledge of some Otomí communities in State of México and reported 28 edible mushrooms species. At the national level, Queretaro State is the third largest Otomí population (INEGI, 2009), however, there has been no reported edible mushrooms species related to this ethnic group. There is only evidence that two species are consumed in Querétaro, particularly near the "Cerro el Zamorano" in the municipality of Colón (Landeros *et al*., 2006), *Clavulina amethystina* named "menudo de vaca" and *Lycoperdon perlatum* as "huevo" (egg).

The aim of the present work is to report the edibility of *F. wolfeana* for the first time, the ethnomycological importance as a non-timber forest product, and its use as a food resource, with a general description of its nutritional value and antioxidant capacity.

MATERIALS AND METHODS

Collected material

The specimens were bought in Tesquedó community, and were used to determine the species taxonomic keys of Singer *et al.* (1991). About 700 g of fresh mushroom were collected from Xajay community (a locality near to Tesquedó), covered with aluminum foil, placed inside a plastic bag and transported to the laboratory in a cooler with refrigerant.

Color determinations

Color determination was made on pileus, context and pores, of three fresh mushrooms of *F. wolfeana*. Measurements were

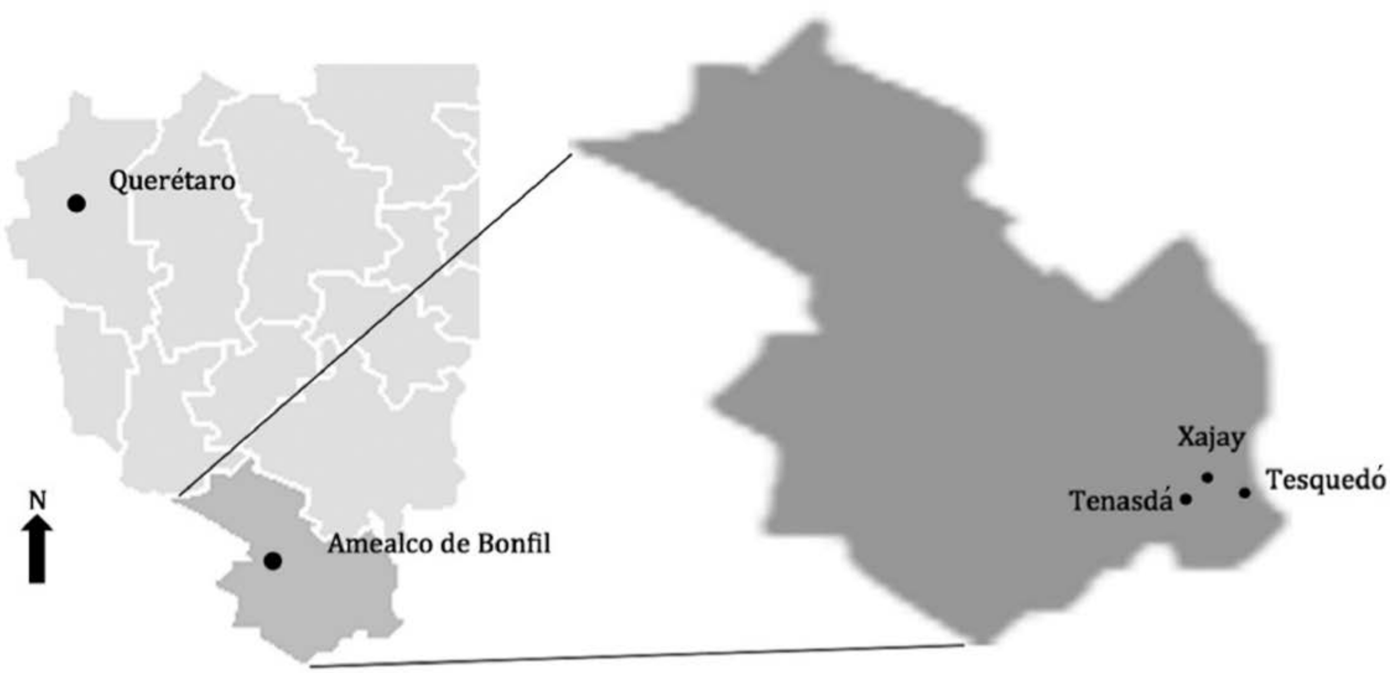

Figure 1. Location of the study area.

taken five times per structure (pileus, context and pores) with a Minolta spectrophotometer (CM-2002). The parameters L* (Luminosity), a* (red/Green coordinates), b* (yellow/blue coordinates), c* (chrome and clarity) and h° (tone angle) were averaged and compared using standard deviation. For pore surface and context, precautions were taken to avoid any damage that could induce color changes, caused by touching these parts. The colors are important since wild mushrooms are an important source of bioactive compounds, such as pigments (Ferreira *et al*., 2009).

Proximal analysis

One sample of 300 g of fresh mushrooms and one sample of 300 g of dried mushrooms were processed to determine their nutritional values. The proximal analysis was conducted in the Unit of Chemical Services at the Faculty of Chemistry of the Universidad Autónoma de Querétaro, using the methods established by the Mexican norms: NMX-F-607-NORMEX-2002) for ash determination, NMX-F-613-NORMEX-2003 for raw fiber determination, NMX-F-608-NORMEX-2002 for protein determination, NMX-F-089-S-1978 for ethereal extracts determinations (Soxhlet method), and NMX-AA-051-SCFI-2001 for the use of atomic absorption spectrophotometer (Perkin Elmer, 2002).

Antioxidant activity

Mushroom extracts were prepared according to Elmastas *et al.* (2007) with some modifications. For methanolic extraction, 1 g of powdered lyophilized mushrooms was resuspended in 40 mL of methanol and then mixed with a homogenizer (IKA T25 Basic) into Falcon tubes, and were covered with aluminum foil and placed on an orbital shaker (Orbital shaker Midi OM10E) for 24 h. The extract was filtered with a Whatman no. 2 paper and the remaining extract was collected. Methyl alcohol was removed in a rotatory evaporator at 40°C and 70 rpm at 175 mbar to obtain the dried extract (Büchi, Heating Bath B490, Rotovapor R-205 and Vacuum V-800). The final amount of the extract was resuspended in 2 mL of HPLC water and stored at 4°C for one day, to avoid oxidative damage.

The radical scavenging activity was determined with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, as reported by Brand-Williams *et al.* (1995), and was modified to read in a 96-well microplate. Aliquots of 280 µL of DPPH (100 µM) in methanol were deposited with 20 µL of mushroom extract at 1:10 and 1:100, and completed to 300 µL each. Samples were incubated in the dark for 45 min, and then measured at 490 nm (Microplate reader). Samples were processed in triplicate. Trolox reagent was used as standard, and was prepared at 0, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8 and 1 µM to determine Trolox equivalents.

RESULTS AND DISCUSSION

Ethnomycological importance

Fistulinella wolfeana was found in local markets for the first time in the Tesquedó community, and then was mentioned by several people who live in the three communities (Tesquedó, Xajay and Tenasdá). It is known as "Ushki jieth´e" in otomí dialect, meaning "salted mushroom", beacuse people attribute it a salty taste, even though some call it "sweet mushroom", due

Figure 2. People from Tesquedó community who collect *Fistulinella wolfeana* for sale.

to a mild flavor when the mushroom is mature. It is collect either for self-consumption, economic purposes (Figure 2), or even to exchange for main supplies, such as vegetable oil, bread, rice or corn. This is important to emphasize, because there are no previous reports of its edibility. Therefore, *F. wolfeana* could be used both as a food source or an economical income source. An important feature is that people dry and keep the mushrooms (Figure 3) for almost a year, to consume them during dry season, when there are not mushrooms and food is scarce. The preservation is possible because this species is rarely invaded by larvae, even when it is left on the soil exposed to sunlight. In fact, this is the first report of conservation of a bolete, because *Boletus* spp., and other genera of the Boletaceae family, are frequently infected by larvae, therefore, people cannot conserve them. Estrada-Torres and Aroche (1987) indicated that people from Acambay, Estado de México, dry their mushrooms hanging them with a thread, and exposing them to the sunlight; but there was no mention of any bolete among them. In our study, we have observed that people place the mushrooms over a rock or a tree during the day, and at night they keep them inside to avoid rehydration, and repeat the same process for few days until the mushrooms are completely dry. During this study, *F. wolfeana* was consumed for two consecutive seasons by the authors and at least seven more people, who indicated that the taste was exquisite.

Figure 3. Dry specimens of sun-dried *Fistulinella wolfeana*.

Species description

Fistulinella wolfeana Singer & J. García, in Singer, García & Gómez, Beih. Nova Hedwigia 102: 82 (1991)
Figure 4

Pileus 30-150 mm, plane-convex, plane-concave or concave, brown pinkish to violet, glabrous; pores 1-1.5 mm diameter, rounded, pinkish, orange-reddish when it is injured, sometimes emarginated; individual tubes 7-20 mm long, white becoming pinkish in older specimens; stipe cylindrical to bulbose at the base; context 15-50 mm, mostly white, unchanging or slightly pinkish in the pileus, yellowish to the base of the stipe, and taste of pileus is mild.

Spores 10-14 × 4-5.6 μm, yellowish to ochraceous yellow, subfusoid, some with suprahilar depression; basidia 23-28 × 9-11 μm, 4-spored; pleurocystidia 36-55 × 6-12 μm, mostly ventric to fusiform, hyaline to yellowish in KOH and reddish in Melzer´s reagent; cheilocystidia 38-60 × 5-12 μm, ventric to fusiform mostly, ochraceous; hymenophoral trama bilateral, with the center gelatinized, slightly ochraceous; epicutis of the pileus with hyphae 2-7 μm diam., composed of a gelatinized ixotrichodermium, with parallel elements cystidia like, with rounded apex mostly yellowish with ochraceous lipid content; cells of the stipe 21-30 × 4-6(-7) μm, versiform, with cystidia like elements and dermatopseudocystidia, 4-spored caulobasidia and terminal elements with rounded apex.

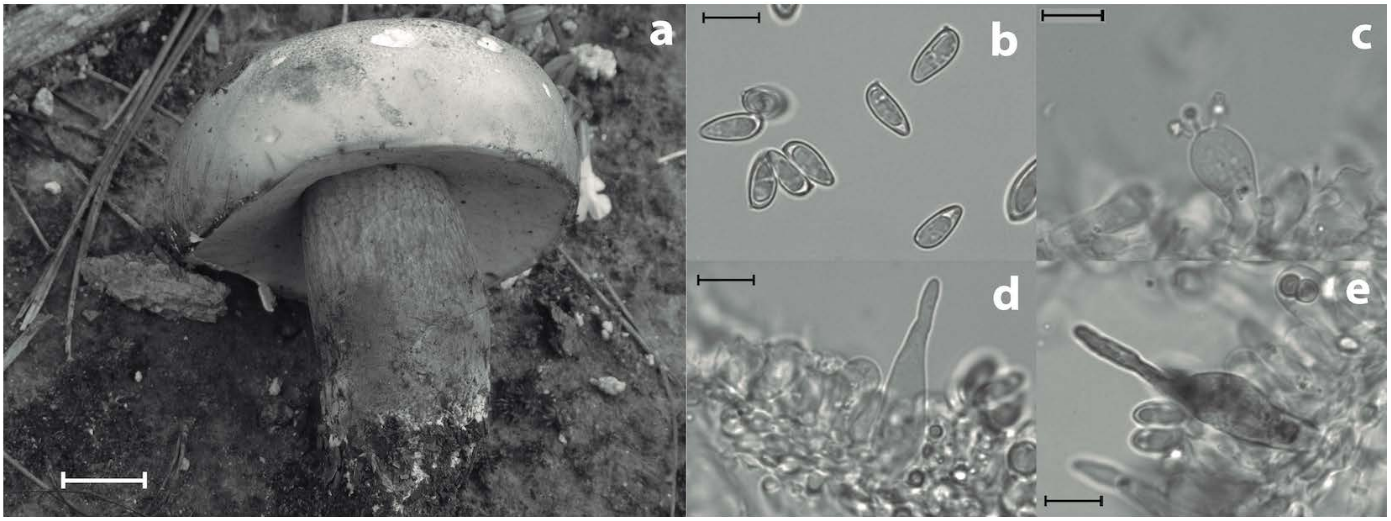

Figure 4. Basidiome (a). Spores (b), Basidia (c), Cheilocystidia (d), Pleurocystidia (e). Scale bar (a) = 5 cm, (b, c, d, e) = 10µm.

Comments. *Fistulinella wolfeana* could be confused with some species of Tylopilus, because both genera have rose tubes and pinkish pores, which change to orange or ochraceous reddish when touched. Tylopilus indecisus has a pale brown slightly reticulated stipe and thin spores (10.5-15.5 × 3.5-4.5). Tylopilus plumbeoviolaceus has a very bitter taste and reticulated stipe surface. Finally, T. subcellulosus has narrower and shorter spores (9.5-12 × 3.5-4) and bitter taste, compared with F. wolfeana. On the other hand, *F. wolfeana* has viscid surface pileus, while all Tylopilus species have subtomentosus, glabrous or smooth dry surface.

Material studied. *Robles 10*, 11 Aug 2013, (QMEX); *Robles 162*, 15 Sept 2013 (QMEX), Amealco, Querétaro, México.

Habitat. In hardwood forest, mainly by *Quercus* spp. with some mixed reforested areas of *Pinus* spp.

Color determination

There is a lack of reported quantitative color determination for wild mushrooms, and most determinations have been done on cultivated species with industrial purposes (Mohapatra *et al*., 2010; Qi *et al*., 2014). We made a complementation of the original color description, with the values obtained in the test. Pileus and pores show similar values, while context presented differences due to its whitish color. According to color scores, luminosity (L*) had the following order: context > pores > pileus; coordinates red/green (a*): pores > pileus > context; and coordinates yellow/blue (b*): pores > pileus > context. On the other hand, c* and h° parameters had the following order, chrome (c*): pores > pileus > context; and tone angle (h°): context > pores > pileus. The parameter in the pileus had the major deviation in color, including pinkish, pinkish-violet or pinkish-brownish, probably due to sun exposure. Pores change their color, from white to pinkish-lilac, when touched. It could explain the minimal variation and similarity between the values of both parts. The high value in context explains the white color and the low variation in color change, as indicated by Singer *et al*. (1991) in the original description. The above values, suggest a variation depending on where the mushroom is growing, and if it is directly influenced by the sunlight, because specimens found in the inner part of the forest had lower L* values, indicating a higher color tone, while those exposed to sunlight had paler tones, showing higher L* values. Pileus and pores had similar values; context does not change since it is inside, and only pileus shows a considerable variation (Table 1). Thus, this difference may suggest a variation in the amount of pigments that decrease in exposed specimens. It would be convenient to determine pigments, to find out which are the most important that make up the colors of this species, and how they are modified by environmental conditions.

Table 1. Color values of pileus, pores surface and context of *Fistulinella wolfeana*

Parameters	Pileus	Pores	Context
L*	46.37 ± 5.4	49.26 ± 0.9	74.62 ± 0.8
a*	8.62 ± 0.9	8.90 ± 0.5	0.88 ± 0.4
b*	25.09 ± 1.1	25.53 ± 1.7	16.67 ± 0.8
c*	26.74 ± 1.3	27.27 ± 1.9	16.71 ± 0.8
h°	70.24 ± 0.8	70.64 ± 0.9	87.18 ± 1.1

L* (Luminosity), a* (red/green coordinates), b* (yellow/blue coordinates), c* (chrome and clarity) and h° (tone angle). Values averaged ± standard deviation.

Proximal analysis

Results of proximal analysis are shown in Table 2, which are compared with data from other foods, such as mushrooms and some meats frequently consumed. Protein levels, in dry weight, were similar to those in Agaricus bisporus and Pleurotus ostreatus, but with less than half of fat and carbohydrates. Therefore, *F. wolfeana* is a food with high protein content.

In addition, its sodium content, was very low compared to another mushrooms species. This is important because of potential health problems, such as hypertension that can be caused by excess of sodium. Finally, *F. wolfeana* had more fiber content, in both fresh and dry weight, than any of the commercial mushrooms compared, which means that it can help lower cholesterol levels and promote digestion.

Regarding to comparison with some meats of frequent consumption, protein values (in dry weight) were similar to those in chicken, beef, tuna fish and blacktip shark. *Fistulinella wolfeana* had fewer carbohydrates and fats than meat; such as the case with commercial mushrooms. Two important aspects to consider in F. wolfeana, the presence of fiber and the low amount of sodium, indicate that this species is a good nutritional alternative, even compared to meat.

Table 2. Comparison of fresh and dry proximal analysis of *Fistulinella wolfeana* with other foods

Species/Food		Carbohydrates	Ash	Fiber	Fats	Moisture	Protein	Sodium (mg/100g)
Fistulinella wolfeana	Fresh	18.01	1.49	2.93	0.46	73.69	3.42	-
	Dry	30.21	4.48	23	0.71	11.77	29.83	4.5
Boletus gruop dried	Fresh	N	N	N	N	N	N	N
	Dry	19.46[5]	1.71[5]	8.74[5]	1.08[5]	N	0.12[5]	N
Agaricus bisporus	Fresh	0.4[4]	0.82[5]	2.3[4]	0.3[4]	78.3-90.5[2]	1.8[4]	21[3]
	Dry	51.3-62.5[2]	7.7-12[2]	8-10.4[2]	1.7-8[2]	N	23.9-34.8[2]	N
Pleurotus ostreatus	Fresh	Trace[4]	0.89[5]	N	0.2[4]	73.7-90.8[2]	1.6[4]	6[3]
	Dry	57.6-81.1[2]	6.1-9.8[2]	7.5-8.7[2]	1.6-2.2[2]	N	10.5-30.4[2]	N
Chiken	Fresh	25.1[1]	1.28[1]	N	7.75[1]	74.9[1]	19.7[1]	51.55[1]
	Dry	N	N	N	N	N	N	N
Cow	Fresh	27[1]	0.97[1]	N	2.57[1]	73[1]	22.3[1]	54.9[1]
	Dry	N	N	N	N	N	N	N
Tuna fish	Fresh	N	0.90[6]	N	0.76[6]	75.24[6]	22.37[6]	N
	Dry	N	N	N	N	N	N	N
Blacktip shark	Fresh	N	1.15[6]	N	1.56[6]	77.78[6]	20.04[6]	N
	Dry	N	N	N	N	N	N	N

Data taken from [1]De Moreno *et al.* (2000); [2]Juárez-Luna (2012); [3]Sánchez *et al.* (2007); [4]Sadler (2003); [5]Manzi *et al.* 2004; [6]Castro-González *et al.* (2007); N= No data available.

Comparison with other wild mushrooms

The comparison of proximal analysis results of *F. wolfeana* with wild mushrooms, are shown in Table 3, and data are shown in grams per 100 g of dry material. The average amount of protein in *F. wolfeana* is 29.83 g; Amanita caesarea had a higher value (34.77 g), while Armillaria tabesceus had a lower content (22.90 g). However, the contents of fat, carbohydrates and ash were the lowest for these two species. Therefore, we consider that *F. wolfeana* is a good diet alternative for people looking for foods low in fat and carbohydrates. This species is abundant in oak forests in Mexico, and large specimens grow there, which can weigh up to half a kilogram when fresh; that is why it is a highly prized species, because with a few basidiomata, they can cook for a family (six to eight members).

During the rainy season, *F. wolfeana* can replace other foods, such as meat, which in many cases is not accessible for most of the people of the communities. Also, this species could be dried and preserved for almost a year in a freezer.

Table 3. Basic comparison of *Fistulinella wolfeana* with other wild edible mushrooms (g/100g), data of dry material

Mushrooms species	Proteins	Fat	Carbohydrates	Ash
Amanita caesarea	<u>34.77</u>	3.50	55.63	6.05
Armillaria mellea	24.47	2.10	65.47	7.95
Armillaria tabesceus	22.90	2.54	<u>66.87</u>	7.63
Boletus aureus	27.17	4.47	62.10	6.25
Cantharellus cibarius	21.57	2.88	66.07	<u>9.44</u>
Fistulina hepatica	22.60	3.17	66.00	8.20
Fistulinella wolfeana	29.83	0.71	30.21	4.48
Hygrophorus russula	32.47	<u>6.00</u>	53.33	8.18
Lepista nuda	34.37	3.22	56.33	6.03
Ramaria largentii	28.80	5.67	58.87	6.67
Russula delica	26.10	2.88	66.07	<u>9.44</u>

Data taken from Ouzouni *et al*. (2009). The underlined number represents the highest value for each category.

Antioxidant activity

Many common wild boletes are known to have a considerable antioxidant activity, comparable with our results (Figure 5), as indicated by several previous studies (Elmastas *et al*., 2007; Ribeiro *et al*., 2006; Sarikurkcu *et al*., 2008; Tsai *et al*., 2007). Looking for organic compounds profiles in boletes, we could find different values for fatty acids and phenolic compounds, that show high levels of linoleic, oleic and stearic acids (Kalaç, 2009). In general, mushrooms have low fat content, but in some cases, unsaturated acids such as oleic acid (C18:1) and linoleic acid (C18:2) are predominant over saturated fats (León-Guzmán *et al*., 1997; Ribeiro *et al*., 2009; Guillamón *et al*., 2010). Some members of the Boletaceae family have shown the same predominance of unsaturated acids (Pedneault *et al*., 2006). In this work, we report an increase in fat content when mushrooms were dried, but more studies are needed to determine the profile of fatty acids in raw, dry and even cooked mushrooms, in order to know if heat modifies the amount of fat. This could have implications as people dry them to consume them later.

It is important to notice that some species are considered health enhancers by people who consume them (Garibay-Orijel *et al*., 2007; Alonso-Aguilar *et al*., 2014), which makes sense, because it has been reported that the consumption of wild mushrooms play a role in the prevention of cardiovascular diseases (Guillamón *et al*., 2010). Bautista (2013) published one of the most recent and complete reports about Mexican medicinal mushrooms traditionally used by Nahuas, Tseltales and Oto-

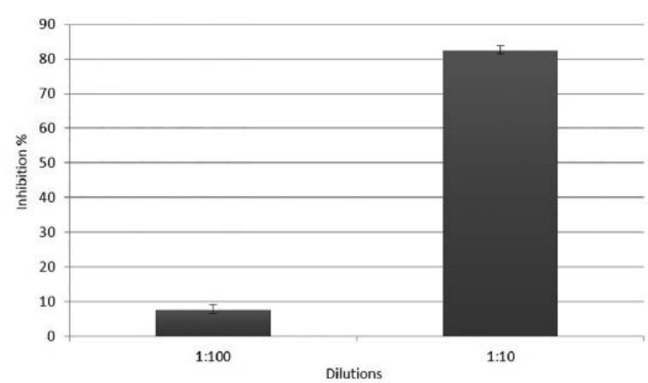

Figure 5. Percentage of radical scavenging activity with *2,2-diphenyl-1-picrylhydrazyl* (DPPH) from methanol extracts diluted 1:10 and 1:100. Data were averaged from N=6, bars are standard deviation.

míes. The author cited a traditional medicine man, stating that: "not all the mushrooms are medicinal, but all edible mushrooms are potentially medicinal". In the case of F. wolfeana, we could hypothesize that it has medicinal properties, but its use as one of the Mexican traditional wild mushrooms has not been reported. Elmastas *et al.* (2007) and Puttaraju *et al.* (2006) indicated that boletes contain phenolic compounds, suggesting that it is possible to find them in Fistulinella genus. In addition, many phenolic compounds have antioxidant potential (Ribeiro *et al.*, 2006). These components could also have the capacity to repel insects, as we have noticed in all the mushrooms collected for this work.

Conclusions

We have reported here that *Fistulinella wolfeana* as a very common species in oak forests in Mexico, which produce abundant and robust basidiomata. We also described its important nutritional value and as a multifunctional non-timber resource that can be consumed when fresh, but also preserved and consumed after drying, a practice carried out in the three communities that comprised our study. Additionally, it is used as exchange for basic supplies, an activity that has been disappearing because of predominance of the modern monetary system in small villages. We reported a high antioxidant activity, but only using a 1:10 dilution, which decreases at higher dilutions and is considerably higher when not dissolved. Therefore, we can conclude that *F. wolfeana* is a good source of antioxidants. This work should encourage future studies on the bioactivity of Mexican members of the Boletaceae family..

ACKNOWLEDGMENTS

We thank to CONACyT for the scholarship grant for postgraduate studies, Universidad Autónoma de Querétaro and PRODEP (SEP) for supporting this research. To all who helped in the ethnomycological work and especially to Sabina Wischin, who helped in determining the antioxidant activity and results interpretation.

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