

# Traditional Knowledge and Study of Medicinal Properties of the Weeping Milk Cap Mushroom *Lactarius volemus* (Agaricomycetes) in México

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**ABSTRACT:** Gathering and consumption of wild edible mushrooms are traditional practices in Mexico, influenced by the local culture of indigenous communities. In the Mestiza community of El Salto, municipality of Puente de Ixtla, Morelos, Mexico, traditional knowledge promotes the use and management of natural resources for meeting nutritional, economic, social, cultural, and environmental needs. Main mushroom species are gathered in the community for consumptions or marketing, involving women and men in the processes of collecting, consuming, cleaning, and selling. The traditional consumption of mushrooms supported healthy eating patterns, and *Lactarius volemus* was found to be a species of the most cultural importance (i.e., the highest frequency of mentioning in the interviews). Antioxidant and antibacterial properties of *L. volemus* extracts were analyzed and described. *L. volemus* showed significant DPPH free radical scavenging activity of 355.63 µg/mL, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging activity of 742.13 µg/mL, as well as bacteriostatic and bactericidal effects. Analyses of the minimal inhibitory concentration showed a high degree of susceptibility; the most potent minimal bactericidal concentration was 28.94 mg/mL recorded against *Escherichia coli* and *Streptococcus agalactiae*. Strategies for promoting mushroom consumption in indigenous and peasant communities, based on local cultural traditions, are discussed.

**KEY WORDS:** *Lactarius volemus*, antibacterial properties, antioxidant properties, traditional knowledge, medicinal and wild mushrooms

**ABBREVIATIONS:** ABTS, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; GAE, gallic acid equivalent; MBC, minimal bactericidal concentration

## I. INTRODUCTION

Fungi and mushrooms are important for human populations in agriculture, industry and forestry. Several indigenous cultures from central Mexico have concurred in the state of Morelos, including Tlapanecas from the state of Guerrero, Otomíes from the state of Puebla, and Mazahuas from the state of Mexico. This biocultural heritage within a region combined biological and cultural diversity, allowing the traditional use and management of many wild mushroom species. More than 1047 species of wild mushrooms have been recorded in Morelos,<sup>1,2</sup> of which 38 species from coniferous and subtropical forests have been identified, which are sold and consumed in main popular markets of Morelos. Positive correlations have been found between the consumption frequency of edible mushrooms (wild, cultivated) and the nutritional and metabolic status in indigenous and peasant communities.<sup>3</sup>

Mushrooms are often used for food, economic subsistence, agriculture, medicine, and social activities<sup>4</sup> described 371 species that are consumed in Mexico. The most important Macromycetes with culinary uses

are concentrated in this region, where they coexist with pine and oak vegetation. The nutritional properties of mushrooms in Mexico are well documented, but the use of mushrooms in traditional medicine is low or non-existent. Although there is both cultural knowledge and scientific evidence of their antioxidant, immunomodulatory, antibacterial, anticarcinogenic and anti-inflammatory properties, among others.

There are 35 Nahua communities in the state of Morelos, concentrated in Hueyapan, Tetela del Volcán, Tetelcingo, Cuautla, Santa Catarina, Tepoztlán, Cuentepec, Temixco, Xoxocotla, and Puente de Ixtla. This has resulted in a wealth of traditional knowledge. This work was conducted in the Nahuatlán community of El Salto, in the municipality of Puente de Ixtla, Morelos. For this reason, mushrooms are an essential biological resource for the population during the rainy season, as well as a source of income for families through the sale of edible mushrooms.

The objective of this research is to identify the roles of the different actors involved in the process of collection, knowledge transfer, and consumption of edible mushrooms, as well as to generate scientific information that will strengthen our knowledge of mushrooms and their health benefits for the inhabitants of the community of El Salto, specifically the medicinal properties of *Lactarius volemus* (Fr.) Fr. (= *Lactifluus volemus*, Russulaceae, Agaricomycetes).

## II. MATERIALS AND METHODS

### A. Study Area

In this work, we studied the Nahuatlán community of El Salto, municipality of Puente de Ixtla, Morelos, in which wild edible mushrooms are important sources of food or income during the rainy season, also, the positive impact on health. The gathering process, traditional knowledge, and mushroom consumption are described, including the analysis of antioxidant and antibacterial properties of the most culturally important species *L. volemus*, known traditionally as the weeping milk cap mushroom.

The community of El Salto is located at the mountainous region Sierra Madre del Sur, municipality of Puente de Ixtla, state of Morelos (coordinates N 18°17'31.8" W 99°27'15.2"), 1,819 m altitude. The climate is semi-warm sub-humid, with average monthly temperatures of 7–14°C. Rainfall occurs during the summer, between June and October. A total population of 125 inhabitants,<sup>5</sup> 65 females and 60 males. The vegetation is typical of lowland deciduous forest, several southern areas are oak forest, predominately *Quercus magnoliifolia* Née and *Q. castanea* Née. The rural community is difficult to access,<sup>6</sup> most people are under poverty (60–80%) or extreme poverty (20.2%) conditions.

### B. Data Collection

The sample size was 15% of the total population.<sup>7</sup> A total of 18 open-ended, unstructured interviews were conducted using a dialogic technique,<sup>8,9</sup> in order to record species of wild edible mushrooms important to community members, focusing on consumption, income, traditions, and cultural importance as the frequency a species was mentioned in the interviews.<sup>10,11</sup> Individual interviews were conducted by participant observation during the rainy season, beginning in July and ending in October.<sup>12</sup> Qualitative information was categorized and expressed as percentages, and the frequency of mention recorded.<sup>13</sup> Species identification was performed on the basis of morphology according to standard guides.<sup>14–16</sup>

### C. Preparation of Mushroom Extract

Mature basidiocarps of *L. volemus* were gathered in the community forest, dried at 40°C in a forced air-drying oven. Dried mushrooms (10 g) were macerated for 24 h a hydroalcoholic solution (40% ethanol, 60%

water). Mushroom extracts were concentrated to 10 mL in a rotary evaporator at 40°C, filtered (0.45 µm; Merck, Millipore, Molsheim, France), and kept at 4°C until use. Conductivity, resistance, and pH were determined in a multiparameter equipment (Mettler Toledo, Columbus, OH, USA).

#### D. Antioxidant Assay

The concentration of phenolic compounds in the mushroom extract was expressed as gallic acid equivalents (GAE), according to the Folin-Ciocalteu method previously described by Adebayo et al.<sup>17</sup> The reaction containing mushroom extract, Folin and Ciocalteu's phenol reagent, and sodium carbonate was incubated in the dark for 2 h, and the absorbance measured at 740 nm in a microplate reader (Epoch, Biotek, Winooski, VT, USA).

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was determined spectrophotometrically in the mushroom extract. The reaction initiated adding 40 µL of 0.4 mM DPPH in methanol, incubated in the dark, and the absorbance measured at 517 nm in a microplate reader (Epoch, Biotek, Winooski, VT, USA). The following antioxidant standards were used as reference activities: BHA (0.0–25.0 µg/mL), BHT (0.0–25.0 µg/mL),  $\alpha$ -tocopherol (0.0–25.0 µg/mL), and ascorbic acid (0.0–40.0 µg/mL). DPPH free radical scavenging activity was determined according to Adebayo et al.<sup>17</sup>

The reaction to determine 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging activity contained the mushroom extract and the adjusted ABTS solution; the absorbance was measured at 734 nm in a microplate reader (Epoch, Biotek, Winooski, VT, USA). The following reference antioxidant standards were used: BHA (0.0–40.0 µg/mL), BHT (0.0–40.0 µg/mL),  $\alpha$ -tocopherol (0.0–100.0 µg/mL), and ascorbic acid (0.0–40.0 µg/mL). The scavenging activity was calculated, as previously described.<sup>17</sup>

#### E. Antibacterial Properties

The methodology established by Adebayo et al.<sup>17</sup> was followed. All bacterial strains studied are deposited at the Centre of Biotechnology of Edible, Functional, and Medicinal Mushrooms (CB-HCFM), Campus Puebla. Antibacterial tests of the *L. volemus* extract were carried out using the strains of the following Gram-negative bacteria; *Escherichia coli* (CPB-3, CPB-8, CPB-12,); *Pseudomonas aeruginosa* (CPB-6, CPB-13); *Salmonella typhi* (CPB-1 and *Stenotrophomonas* spp. (CPB-7,)) and Gram-positive bacteria *Bacillus subtilis* (CPB-5, CPB-9,); *Listeria monocytogenes* (ATCC-19111, CPB-11,); *Staphylococcus aureus* (CPB-2, CPB-10); and *Streptococcus agalactiae* (CPB-4). The broth microdilution method in a microplate was used to assess the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).<sup>17</sup>

#### F. Statistical Analysis

Experiments were carried out in triplicate. The results are expressed as means  $\pm$  standard deviation (SD, n = 3). Statistical analysis of experimental data was carried out using the Tukey's test from SAS 9.4 software (2012 SAS Institute Inc., Cary, NC, USA),  $P < 0.05$  was considered significant.

### III. RESULTS

#### A. Traditional Knowledge

Twelve women and six men participated in interviews, as well as five key informants. Most mushroom gatherers were men (66%). All family members participated in mushroom gathering, particularly on weekends

(Fig. 1A), promoting knowledge exchange among generations. Mushroom gathering by families involved animal transport (e.g., horses, mules) along forest paths (Fig. 1B). Mushroom patches known previously in the forest were preferred during gathering. Several species were associated to specific trees, e.g. *Lactarius indigo* (“hongos azules,” blue mushrooms) were found close to *Quercus* spp.

Mushroom gathering is a temporary activity during the rainy season, complementary to other main household laborers. Most women were housewives (67%), supporting agricultural (11%) and trade (22%) activities. Men are occupied in diverse activities, such as agriculture (37%), factories (38%), or trade (25%). The participation of men and women in mushroom gathering, selection, and trade was recorded, along with other products derived from family agriculture and livestock, showing traditional use and management of regional biological resources to meet their daily needs. Most products are sold or swapped in popular market days at Puente de Ixtla, Morelos. The exchange of knowledge is fundamental for community development.

Main period for gathering edible mushrooms was from August to October, reaching peak values (~ 50%) in September. Gatherers identified edible mushrooms mainly based on color, shape, place of growth, and type of vegetation. Thirteen species of edible mushrooms were identified, recording their local common names (Table 1).

The most frequently mentioned species were *L. volemus* (“hongo de leche”; “milk mushroom”) and *L. indigo* (“azules”; “blue mushroom”), which were recorded in all interviews. Common names in indigenous languages or Spanish are assigned by association to plants where mushrooms fruiting bodies or features and similarities with common things. This is the case of *Amanita rubescens*, whose common name is “lengua de venado” (“deer tongue”), *Cantharellus cibarius* as “Flor de calabaza” (“pumpkin blossom”), as well as other examples shown in Table 1.

## B. Biological Activity

*L. volemus* is a mushroom species of cultural importance in the community studied. Accordingly, its antioxidant and antibacterial activities were assessed. The hydro-alcoholic extract of *L. volemus* was characterized



**FIG. 1:** Mushroom gathering at El Salto, municipality of Puente de Ixtla, state of Morelos, Mexico. (A) Family members in the forest after harvesting. (B) Young people gather mushrooms based on traditional knowledge.

**TABLE 1:** Wild edible mushrooms gathered at the community of El Salto, Morelos, Mexico

Taxonomic name	Common name	Frequency of report (%)	Cultural value
<i>Amanita caesarea</i> (Scop.) Pers.	Yema de huevo	16.6	3
<i>Amanita rubescens</i> Pers.	Lengua de venado, mantecado	11.1	2
<i>Boletus</i> spp.	Pancitas	11.1	2
<i>Cantharellus cibarius</i> Fr.	Flor de calabaza, trompa de marrano	22.2	4
<i>Lactarius deliciosus</i> (L.) Gray	Chicalo	11.1	2
<i>Lactarius indigo</i> (Schwein.) Fr.	Hongo azul	100	18
<i>L. volemus</i> (Fr.) Fr.	Hongo de leche	100	18
<i>Lyophyllum decastes</i> (Fr.) Singer	Clavitos	11.1	2
<i>Pleurotus djamor</i> (Rumph. ex Fr.) Boedijn	Oreja de cazahuate	33.3	6
<i>Ramaria botrytis</i> (Pers.) Bourdot	Moteado, frijolito	16.6	3
<i>Ramaria flava</i> (Schaeff.) Quél.	Escobetas	16.6	3
<i>Russula brevipes</i> Peck + <i>Hypomyces lactifluorum</i> (Schwein.) Tul. & C. Tul.	Enchilado	16.6	3
<i>Volvariella bombycina</i> (Schaeff.) Singer	Pollitos de cazahuate	11.1	2

as follows: concentration,  $463.0 \pm 5.48$  mg/mL; pH  $5.71 \pm 0.01$ ; conductivity,  $58,672 \pm 2.37$   $\mu$ S/cm; resistance,  $9.0 \pm 0.0$  ohms; total dissolved solids,  $23.47 \pm 2.56$ ; and sugars,  $42.42 \pm 0.24^\circ$  Brix.

### C. Total Phenolic Content

There was a high concentration of phenolic compounds in the *L. volemus* extract of 5301.27 GAE  $\mu$ g/mL (Table 2)<sup>17</sup> reported a concentration of 129.06 GAE  $\mu$ g/mL for a hydroalcoholic extract from *Pleurotus ostreatus*.

### D. Antioxidant Properties

The antioxidant activity of *L. volemus* showed significant DPPH and ABTS radical scavenging activity (Table 2). An activity of 355.63  $\mu$ g/mL was recorded in the DPPH assay, which was significantly higher than those from antioxidant standards BHT (1.99  $\mu$ g/mL), BHA (25.80  $\mu$ g/mL), ascorbic acid (19.43  $\mu$ g/mL), and alpha-tocopherol (1.80  $\mu$ g/mL).

**TABLE 2:** The total phenolic content and antioxidant activity of hydro-alcoholic extracts of *L. volemus*

Sample	DPPH ( $\mu$ g/mL)	ABTS ( $\mu$ g/mL)	Total phenolic content (GAE $\mu$ g/mL)
<i>L. volemus</i>	$355.63 \pm 16.67^a$	$742.13 \pm 25.20^a$	$5301.27 \pm 116.24$
Antioxidant standards			
BHT	$1.99 \pm 0.09^c$	$33.71 \pm 2.80^b$	—
BHA	$25.80 \pm 0.09^b$	$38.13 \pm 0.93^b$	—
Ascorbic acid	$19.43 \pm 1.69^b$	$3.58 \pm 0.07^c$	—
Alpha-tocopherol	$1.80 \pm 0.06^c$	$3.88 \pm 0.42^c$	—

Means in a column followed by different letters indicate significant difference,  $P < 0.05$ , according to the Tukey's test.

In the ABTS assay, the activity was 742.13  $\mu\text{g/mL}$ , being also significantly higher than those from antioxidant standards BHT (33.71  $\mu\text{g/mL}$ ), BHA (38.13  $\mu\text{g/mL}$ ), ascorbic acid (3.58  $\mu\text{g/mL}$ ), and alpha-tocopherol (3.88  $\mu\text{g/mL}$ ).

Thus, *L. volemus* showed significantly higher antioxidant activity than that edible mushroom *P. ostreatus* (0.63  $\mu\text{g/mL}$ ) and *P. pulmonarius* (0.49  $\mu\text{g/mL}$ )<sup>17,19</sup> reported a higher DPPH radical scavenging capacity in small-sized compared with large-sized *L. volemus*.

### E. Antimicrobial Activity

The susceptibility of diverse bacteria to the *L. volemus* extract was evaluated (Table 3). Analyses of the MIC recorded two bacterial species showing high degree of susceptibility (*E. coli* CPB-3, CPB-8; *S. agalactiae*), seven species showing average degree of susceptibility (*B. subtilis* CPB-5, CPB-9; *E. coli* CPB-12; *L. monocytogenes*; *P. aeruginosa* CPB-6, CPB-13; *S. aureus* CPB-2, CPB-10; *Stenotrophomonas* sp.), and a species having low degree of susceptibility (*S. typhi*). The most potent MBC was 28.94 mg/mL recorded for *E. coli* (CPB-3) and *S. agalactiae*; followed by 57.88 mg/mL for *B. subtilis* (CPB-5, CPB-9), *E. coli* (CPB-8, CPB-12), and *L. monocytogenes*; 115.75 mg/mL for *P. aeruginosa* (CPB-6, CPB-13), *S. aureus* (CPB-2, CPB-10), and *Stenotrophomonas* sp.; and 231.5 mg/mL for *S. typhi*.

## IV. DISCUSSION

In the community studied, interestingly, most mushroom gatherers were men (66%), although all family members may participate. Other studies have reported that women were mainly involved in mushroom gathering and selling in communities from the states of Mexico, Oaxaca, Tabasco and Veracruz.<sup>18,19</sup> Mushroom gatherers were 20 to 80 years old, using and naming wild mushrooms according to their morphology (color, shape), the place where they grow, and the type of local vegetation, as found in previous research work from the states of Chihuahua and Chiapas,<sup>20–22</sup> Mexico,<sup>23,24</sup> Tlaxcala,<sup>25</sup> and Veracruz.<sup>26</sup> A close relationship was found between the most frequently mentioned species and their detection in field

**TABLE 3:** MIC and MBC of the hydro-alcoholic extract from *L. volemus*

Strain	Test bacteria	MIC (mg/mL)	MBC (mg/mL)
CPB-1	<i>Salmonella typhi</i>	231.5	231.5
CPB-2	<i>Staphylococcus aureus</i>	115.75	115.75
CPB-3	<i>Escherichia coli</i>	57.88	28.94
CPB-4	<i>Streptococcus agalactiae</i>	57.88	28.94
CPB-5	<i>Bacillus subtilis</i>	115.75	57.88
CPB-6	<i>Pseudomonas aeruginosa</i>	115.75	115.75
CPB-7	<i>Stenotrophomonas</i> sp.	115.75	115.75
CPB-8	<i>Escherichia coli</i>	57.88	57.88
CPB-9	<i>Bacillus subtilis</i>	115.75	57.88
CPB-10	<i>Staphylococcus aureus</i>	115.75	115.75
CPB-11	<i>Listeria monocytogenes</i>	115.75	57.88
CPB-12	<i>Escherichia coli</i>	115.75	57.88
CPB-13	<i>Pseudomonas aeruginosa</i>	115.75	115.75

trips, collected fruiting bodies, and edible mushrooms sold at the market of Tilzapotla Municipality. Wild edible mushrooms were usually harvested a day before to be sold at the market of Puente de Ixtla, Morelos, Mexico, or even early morning of the same day. Wild edible mushrooms played an important role in the diet of the community studied from August to October, an average of 78% of mushrooms gathered were used for household consumption, while the rest 22% were sold in regional markets along with other wild edible plants collected, such as mountain coriander or “tepecilantro” (*Peperomia bracteata*). Mushrooms were sold by piece (fruiting body), bundle (several fruiting bodies), or in a pot (ca. 3.0 kg). Mushroom selling by bundle is common (46%), costing from \$20.00 to \$30.00 Mexican pesos (USD 1.00–1.50). A pot weighing approximately 3 kg of fresh mushrooms cost USD 280.00 to \$350.00 Mexican pesos (USD 14.04–17.55). Income from mushroom selling are important for the household economy.

Wild edible mushrooms, gathered and consumed by households from the community of El Salto, Morelos, Mexico, are cooked traditionally in different ways. They can be stewed (37.0%), grilled (14.8%), eaten in “quesadillas” (18.5%), as a complement of vegetables, soups, creams, and meat stews (3.7%), or even eaten raw (11.11%). Interestingly, interviews indicated that the consumption of mushrooms is considered beneficial to human health. During the rainy season, edible mushrooms replace meat due to its protein content.

The consumption of *L. volemus* as a food has only been reported from America (Mexico), Asia (China, Lao, Nepal), and Europe (Bulgaria, Russia, Turkey, Ukraine).<sup>27,28</sup> Functional and medicinal properties have also been reported in this species.<sup>29,30</sup> The current study showed that *L. volemus*, known traditionally as milk mushroom, is the most culturally important species in the Mestiza community of El Salto, municipality of Puente de Ixtla, Morelos, Mexico. Its antioxidant and antibacterial properties are relevant for healthy diets, as has been shown in Mexican rural communities.<sup>30,31</sup> The consumption of antioxidant compounds, important components of all fungal species,<sup>32–34</sup> is considered beneficial to health and plays an important role in cellular maintenance, as an adjuvant in the prevention and treatment of cellular aging and other diseases, especially in cancer patients.<sup>35–37</sup> Strategies for promoting mushroom consumption in indigenous and peasant communities, based on local cultural traditions, are being developed.

The consumption of antioxidant compounds, important components of all fungal species is considered beneficial to health and plays an important role in cellular maintenance, as an adjuvant in the treatment and prevention of disease, especially in cancer patients, and as a preventive for common diseases.<sup>34–38</sup> Found phenolic compounds in aqueous extracts from wild Mexican mushrooms belonging to *Morchella* sp. (19.86 mg GAE/100 g), *Gyromitra* sp. (18.65 mg GAE/100 g), and *Floccularia* aff. *luteovirens* (17.84 mg GAE/100 g).

Similar results were reported by Öztürk et al.<sup>38</sup> Conversely, Zang et al.<sup>39</sup> reported a bactericidal effect of a *Grifola frondosa* extract on *E. coli* and *S. aureus* and attributed this effect to the concentration of polysaccharides in the extract. Perez et al.<sup>3</sup> and Adebayo et al.<sup>17</sup> observed bacterio-static effects of an extract of the edible mushrooms *Hericium erinaceus* and *Pleurotus* spp. Similar bacteriostatic and bactericidal effects were reported for *Agaricus*<sup>38</sup> and *Pleurotus*<sup>40</sup> species reported a bactericidal effect of a *Grifola frondosa* extract on *E. coli* and *S. aureus*, due to the presence of polysaccharides.

This work demonstrates for the first time the bacteriostatic and bactericidal activity of *L. volemus*, highlighting the great beneficial potential of the consumption of these edible mushrooms by the population. The community of El Salto has a high level of poverty, which limits access to health services. However, the activities carried out by its inhabitants using indigenous biological resources can help to address common problems, such as gastrointestinal and respiratory infections, and improve the general health of consumers. Our results suggest that the consumption of edible mushrooms contributes to the improvement of the general health of the inhabitants of the community.

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The data are available from the corresponding author on request.

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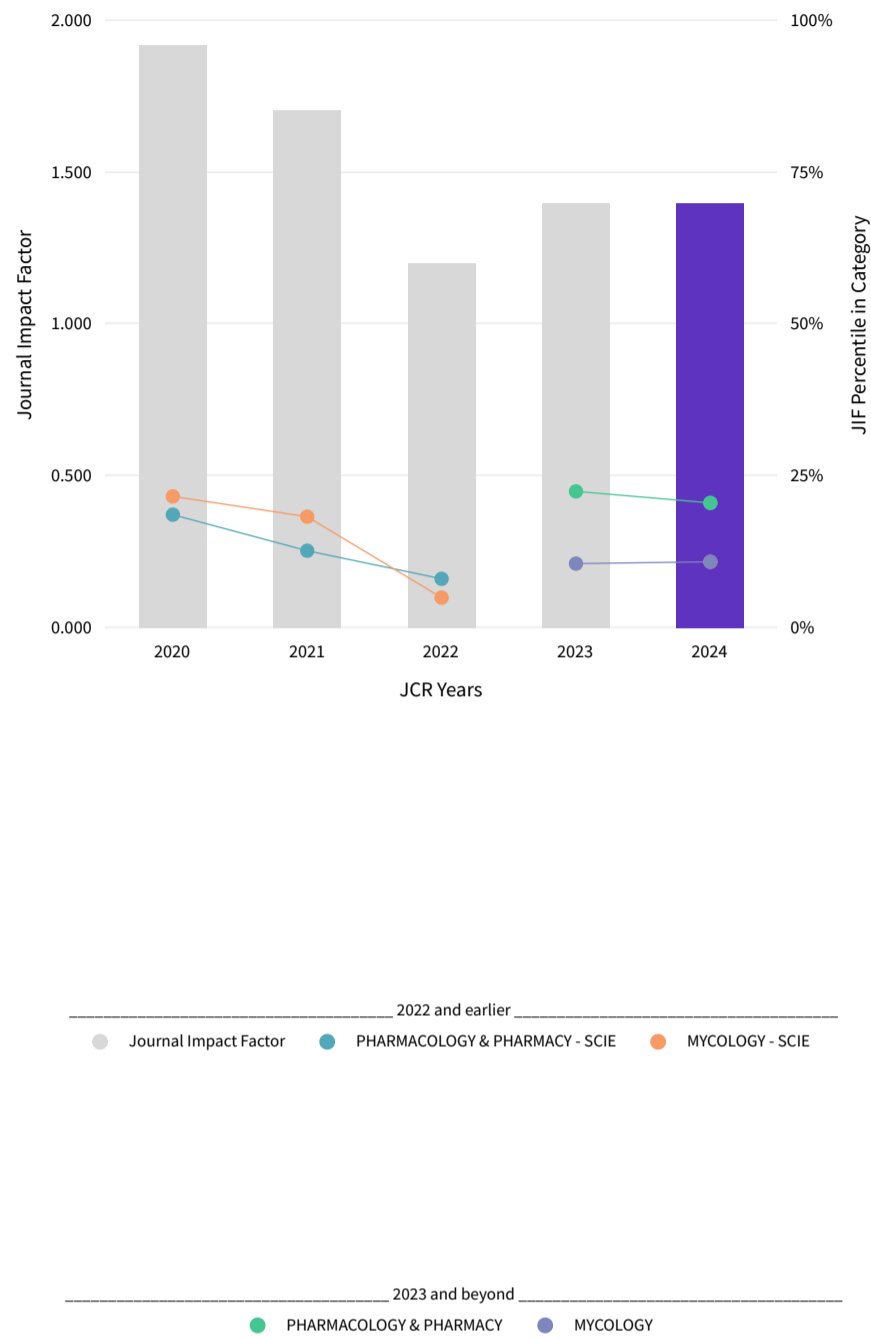
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