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(Original Research)

Phytochemical Profiling of Selected Wild Edible Mushrooms from the Thar Desert, Pakistan

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Abstract

Khumbi or wild mushrooms have been long valued in the rural communities of Thar Desert, Pakistan, as an edible food item and as a source of income during monsoon season. The purpose of this paper was to carry out preliminary mycochemical screening on several types of wild mushrooms. Specifically, three of the most common species found in the area, Agaricus sp., *Macrocybe gigantea* and a type of desert truffle (hypogeous) fungus, were studied using methanol and water-based extractions of their fruiting bodies to qualitatively assess the presence of various secondary metabolites such as: alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, and proteins. The results demonstrated that each of the three species had phenols and proteins; Agaricus sp. had significant amounts of flavonoids and saponins; *Macrocybe gigantea* had alkaloids and a moderate amount of terpenoids; and the truffle-type fungus had very little alkaloids, however, it had large amounts of tannins. These initial findings indicate that wild mushrooms from Thar Desert may be a rich source of bioactive compounds that can provide the basis for developing nutraceuticals. Therefore, it is recommended that additional studies be conducted to quantify the amount of these compounds and evaluate their toxicity so that they can be commercially developed and utilized as a source of income for the people who harvest them.

Keywords: Thar Desert, Phytochemical, Mushrooms

Introduction

In the Thar Desert of southern Pakistan, there are only limited rainfalls from time to time during the short period of the monsoon. This is when people living locally collect wild mushrooms for both consumption and for selling purposes (Khaskheli, 2020). The Khumbi mushroom grows rapidly

immediately after rainfall and has traditionally been an essential component of the local diet in the desert, a place where conventional agricultural practices are quite difficult to be accomplished (Chandani, 2019) and thus provide significant sources of food and income to households in this region.

Worldwide, mushrooms are increasingly recognized not only as nutritious foods but also as reservoirs of biologically active secondary metabolites such as polysaccharides, alkaloids, phenolics, triterpenes, and terpenoids (Zhang et al., 2023). These compounds are associated with a range of beneficial properties, including antioxidant, antimicrobial, immunomodulatory, and anticancer activities (Bolesławska et al., 2024). Wild edible species, in particular, tend to be rich in phenolic compounds and proteins, contributing further to their health-promoting potential (Hossen et al., 2021).

Desert fungi are not studied very much, b ut there has been some important research on Terfezia arenaria, a type of truffle found in t he Sahara Desert. Research shows that its m ethanol extracts have a lot of flavonoids and polyphenols, and they also show strong anti microbial properties (Harir et al., 2019).

Even though Thar mushrooms are import ant for culture and nutrition, there is no detai led study of their chemical properties. There is very little information available about the active compounds in these mushrooms. It is i mportant to understand the secondary metab olites of these locally important mushrooms to assess their health benefits and to make su re they are safe for people to eat.

Accordingly, this study was designed to collect representative wild edible mushrooms from the Thar Desert after monsoon, to prepare methanolic and aqueous extracts of fruiting bodies, to conduct qualitative mycochemical screening for important secondary metabolites (alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, proteins) using standard assays, and to interpret preliminary results to guide future quantitative and bioactivity studies.

Materials and Methods

Mushroom Collection and Identification

Th study area was Tharparkar District, Sindh, Pakistan, during the post-monsoon season (July–August). Fruiting bodies of edible mushrooms ("khumbi") were collected by local foragers and identified in the field using macroscopic morphology. Three representative types were selected: *Agaricus* sp. (gilled mushroom), *Macrocybe gigantea* (large, umbrella-type), and a presumed desert truffle (hypogeous fungus). The Samples were air-dried, preserved, and deposited at the University Herbarium; morphological identification was supported by reference literature and, if possible, by molecular ITS-rDNA sequencing.

Preparation of Extracts

The fruiting bodies were cleaned, shade-dried, and ground into a fine powder. Two solvents were used i.e., 80% methanol (v/v) and distilled water. For each species, 10 g of dry powder was extracted with 100 mL of solvent by maceration on a shaker for 48 hours at room temperature. Extracts were filtered (Whatman No. 1) and concentrated under reduced pressure (rotary evaporator for

methanol) or freeze-dried (aqueous). Final crude extract stocks were stored at 4°C.

Qualitative Phytochemical Screening

Standard colorimetric and precipitation assays were performed on each extract to test for Alkaloids (Mayer's reagent test), Flavonoids (Alkaline reagent test), Phenols (Ferric chloride test), Tannins (Gelatin test), Saponins (Froth test), Terpenoids (Salkowski's test) and Proteins (Biuret test). Each test was run in triplicate, and results recorded as absent (−), weak (+), moderate (++), or strong (+++).

Results

Qualitative results were tabulated in a phytochemical profile table (Table 1). The data was presented to compare the relative intensity (on a scale of 0–3) of major classes (alkaloids, flavonoids, phenols) across species/solvents.

Table 1: The qualitative screening results for the three species in both methanolic and aqueous extracts.

Secondary Metabolite	***Agaricus* sp. (MeOH)**	***Agaricus* sp. (H_2O)**	***Macrocybe gigantea* (MeOH)**	***Macrocybe gigantea* (H_2O)**	**Truffle-type (MeOH)**	**Truffle-type (H_2O)**
Alkaloids	++	+	+++	++	+	+
Flavonoids	+++	++	+	+	+	±
Phenols	+++	+++	++	++	++	++
Tannins	+	+	++	+	+++	++
Saponins	++	+	+	±	+	±
Terpenoids	+	±	++	+	+	+
Proteins	+++	+++	+++	+++	+++	++

–, absent; ±, trace/very weak; +, weak; ++, moderate; +++, strong

In recent study of the phytochemical profiles, each of the three mushroom species stood out in its own way. Even the type of extract made a difference. Methanolic extracts brought out more metabolites than aqueous ones, hands down. *Macrocybe gigantea* exhibited the highest alkaloid and terpenoid levels, particularly in methanol. Phenols and proteins were consistently strong across all species and solvents, indicating their abundance. *Truffle type* showed markedly strong tannin content in methanolic extract, while *Agaricus sp.* displayed strong flavonoids and phenols, especially in methanol. Saponins appeared moderate in *Agaricus sp.* but weak or trace in the other species. Overall, methanolic extracts yielded richer secondary metabolite profiles than aqueous extracts (Table 1).

The methanolic extract of *Agaricus* sp. gave strong (+++) positive responses for flavonoids, phenols, and proteins, and moderate (++) for alkaloids and saponins. *Macrocybe gigantea* showed the strongest alkaloid signal (+++ in MeOH) and moderate terpenoids. The truffle-like species exhibited

the strongest tannin reaction (+++ in MeOH), moderate phenols, and moderate proteins (Fig. 1).

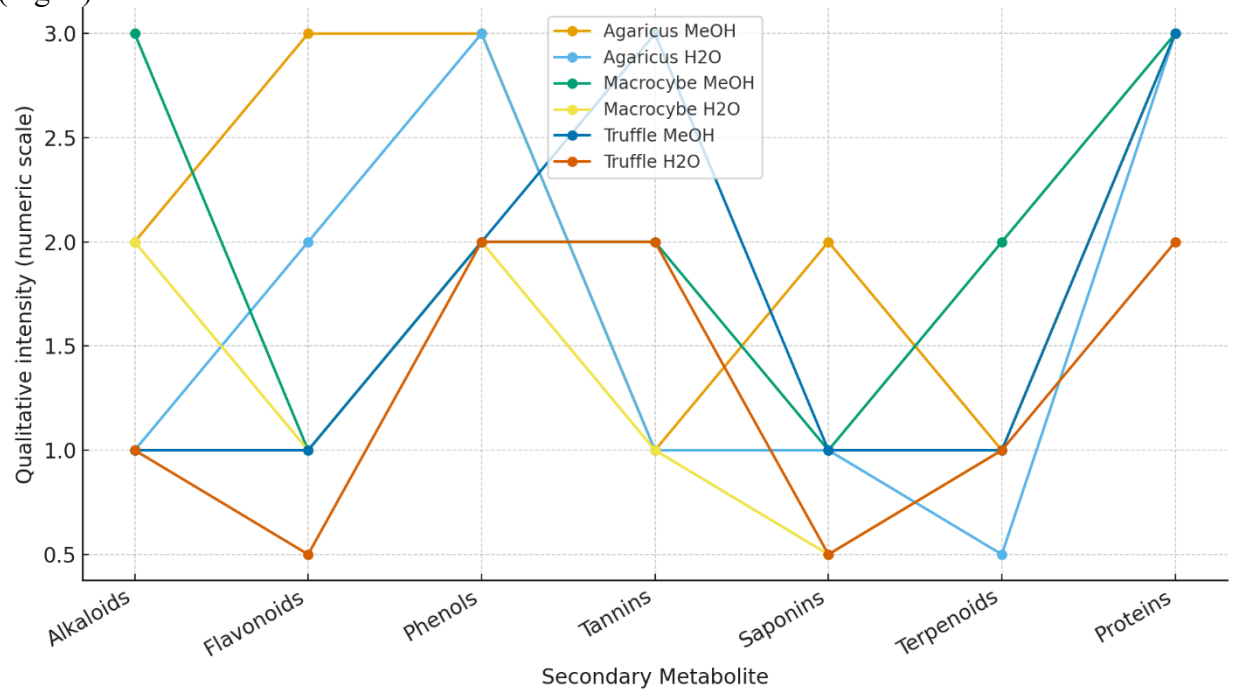

Figure 1: Phytochemical screening of various secondary metabolites of mushrooms

Discussion

The detection of phenols and proteins in all three species suggests that Thar edible mushrooms may indeed provide not just macronutritional benefits but also antioxidant potential. Phenolic compounds are well-known antioxidants, and wild mushrooms globally have been documented to contain substantial phenolic concentrations (Hossen et al., 2021).

The very strong flavonoid response in *Agaricus* sp. (methanolic) is noteworthy. Flavonoids are among the most biologically active mushroom metabolites, contributing to free-radical scavenging and anti-inflammatory activities (Hayat et al., 2024). Meanwhile, the robust presence of alkaloids in *Macrocybe gigantea* aligns with reports that some mushrooms (e.g., *Hygrophorus eburneus*) contain bioactive alkaloids, including β-carbolines. The moderate terpenoid detection in *Macrocybe* may reflect triterpenes or terpenoid-type compounds, which are common in medicinal fungi (e.g., *Ganoderma*) and are responsible for many health effects (Zhang et al., 2023).

Interestingly, the truffle-type species had the strongest tannin signal. Tannins can contribute to astringency and have both health-promoting (antioxidant) and possible antinutritional effects. Their presence indicates that truffle-like fungi in Thar might contribute to dietary polyphenols but may also require further toxicological evaluation (Hossen et al., 2021). The preliminary phytochemical profiles of these Thar mushrooms suggest they could be valuable dietary and therapeutic resources. Local communities already highly value them for nutrition and income (Khaskheli, 2020). The

strong phenol and flavonoid content in *Agaricus* sp. might support its further development as a functional food. However, given the detection of alkaloids and tannins (which at high concentrations may have adverse effects), further quantitative analysis (e.g., total phenolic content, HPLC profiling, GC–MS) and safety evaluation (cytotoxicity, acute toxicity) are needed before recommending large-scale consumption or valorization for nutraceutical products (Zhang et al., 2023). Phytochemical screening in wild mushrooms from other regions has revealed a similar diversity of secondary metabolites. For instance, a study on termite-associated wild edible mushrooms found alkaloids, flavonoids, and phenols in their methanol extracts (Tharu, 2022). Another study on wild mushrooms demonstrated a variety of bioactive compounds, including phenolics and minerals, with measurable antioxidant activity (Bolesławska et al., 2024).

Conclusions

Wild edible mushrooms from the Thar Desert, such as *Agaricus* sp., *Macrocybe gigantea*, and a truffle-like species, show a promising diversity of secondary metabolites based on preliminary mycochemical screening. These mushrooms pack phenols, proteins, flavonoids, alkaloids, tannins, and terpenoids, all of which point to real potential as nutraceutical resources. However, rigorous quantitative and bioactivity analyses including safety assessment are needed to validate their health benefits and promote sustainable, safe use. This study provides a baseline for further phytochemical, pharmacological, and conservation-oriented research on Thar's unique myco-flora.

Acknowledgements

Not Applicable.

Conflict of Interest

Not Applicable.

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