

Antimicrobial activity of wild gasteroid fungi *Podaxis pistillaris* and *Phellorinia herculeana* in the Thar Desert of Rajasthan, India

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The Indian Thar desert harbours a treasure trove of fungal diversity especially wild mushrooms. Ethanobotanical information reveals that mushroom flora of Rajasthan has great potential for medicinal value. In the present study, *Phellorinia herculeana* and *Podaxis pistillaris* growing amidst the sand dunes in Jaisalmer and Barmer districts of Rajasthan effectively inhibited the growth of various microbes paving their way for use as therapeutics.

Keywords: *Agaricus blazei*, *Auricularia auricula*, *Cordyceps* spp., *Fomes fomentarius*, *Ganoderma lucidum*, *Phellorinia herculeana*, *Podaxis pistillaris*

INTRODUCTION

Mushrooms have been coined nutraceuticals and are well known for their distinctiveness as nutrient rich healthy foods as well as having curative and therapeutic properties. They have been consumed since ancient times and Greeks believed that they are elixir of life. Mushrooms are a good source of vitamins and minerals providing strength for warriors in battle. The Romans perceived them as "Food of the Gods". For centuries, Chinese culture has treasured mushrooms as a health food and many species of mushrooms like *Agaricus blazei*, *Auricularia auricula*, *Cordyceps* spp., *Fomes fomentarius*, *Ganoderma lucidum* (commonly called Reishi, Lingzhi), *Grifola frondosa* (Maitake), *Lentinula edodes* (Shitake mushroom), *Pleurotus ostreatus* (Oyster Mushroom) are among those macrofungi whose medicinal properties are well characterized. (Chen *et al.* 2008, Choudhury *et al.* 2013). The prominent nutraceutical properties described in mushrooms such as prevention or treatment of Parkinson, Alzheimer, hypertension

along with immunomodulatory, antiviral, anti-tumorous, psychoactive, antiglycaemic and antioxidant properties (Bisen *et al.* 2010; Boh and Beroviè 2007; Heleno *et al.* 2010).

They are also utilized to reduce the likelihood of cancer invasion (Jin *et al.* 2012.) and metastasis due to their antitumorous attributes. Mushrooms act as antibacterial, immune system enhancers and cholesterol lowering agents and additionally, they are important sources of bioactive compounds (Al-Fatimi *et al.* 2006).

More than 2,000 species of mushrooms exist in nature, but around 25 are widely accepted as food and few are commercially cultivated they are of considerable interest because of their organoleptic, medicinal properties and economic value (Ergönül *et al.* 2013; Chang and Miles, 2008). Nutritionally, they are quite rich in proteins, with essential amino acids and fibre, poor fat but with excellent important fatty acids content. Moreover, edible mushrooms provide a nutritionally significant content of vitamins like B₁, B₂, B₁₂, C, D, and E (Mattila *et al.* 2001). Thus, they could be an

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excellent source of many different nutraceuticals and might be used directly in human diet and to promote health for the synergistic effects of all the bioactive compounds present (Barros *et al.* 2007, Vaz *et al.* 2010).

The ecosystem and seasonal variation in India and especially the desert ecosystem of Rajasthan have exotic mushrooms, which possess potential diagnostic properties. The monsoon rains followed by scorching sunlight on the sand dunes of Rajasthan harvests tons of two species of edible mushroom viz. *Phellorinia sp.* and *Podaxis spp.* (Chouhan and Panwar, 2021) which when young are edible and after maturity are used as medicine by the villagers. Ethanobotanical information reveals that the spore mass of these mushrooms is dusted over the wounds to heal them. They are also given to people with bone-cracks and to pregnant women.

In view of the fact of their wound healing property, the spore extracts of *Podaxis pistillaris* and *Phellorinia herculeana* were tested for antibacterial activity against human pathogenic bacteria *Pseudomonas aeruginosa* responsible for pus formation in wound. Thar Desert lies in Rajasthan, India with extremely harsh climatic conditions with an average rainfall below 313 mm in Western Rajasthan, dry hot winds with summer temperature reaching 40 to 45 degrees Celsius, 55.5% average Relative humidity (RH) and extreme summers with desiccating conditions. It is least expected that mushrooms will grow and survive in such conditions of scanty rains during the monsoon periods. Chouhan and Panwar (2021) described the rare gasteroid fungi of the arid and semi-arid areas of the Thar Desert of Rajasthan, surviving under harsh climatic conditions. Mycofloristic surveys carried out after the meager rainfall have revealed the occurrence of different macrofungi in diverse habitats, on a variety of substrates and that are able to survive desiccating environmental conditions. *Phellorinia herculeana* subsp. *herculeana* and *P. herculeana* sub sp. *strobilina* represent the wild, edible and medicinal form of gasteroid fungi which are amongst the most abundant gasteroid fungi appearing in the sand

dunes and sandy places of Rajasthan. *Podaxis pistillaris*, *P. africana*, *P. saharianus* and *P. beringamensis* have also been reported (Chouhan & Panwar 2021). They are species adapted to survival in desert environment and also reflect the rich mycoflora of the State.

With an objective to evaluate the medicinal potential of *Podaxis pistillaris* and *Phellorinia herculeana* against harmful microbes, the antimicrobial activity tests were performed against *Bacillus megaterium*, *Escherichia coli* and *Klebsiella pneumoniae*.

MATERIALS AND METHODS

The specimens of *Podaxis pistillaris* and *Phellorinia herculeana* were collected from sand dunes in western region of Rajasthan. Preserved dried specimens were deposited and maintained at Department of Botany, S. S. Jain Subodh P. G. College, and Jaipur. The human pathogenic bacteria were procured from CSIR-Institute of Microbial Technology (CSIR-IMTECH).

5 gm spore mass of *Podaxis pistillaris* and *Phellorinia herculeana* were dissolved separately in 50 ml ethanol to make ethanolic extract. Both these were kept for 24 hours at room temperature and the alcohol was allowed to evaporate in the atmosphere. In the remaining residue, 50 ml of distilled water was added. The alcoholic extract was then filtered through filter paper. After filtration the aliquots were centrifuged at 10,000 rpm for 20 minutes. The supernatant was filtered through Whatmann No. 1 filter paper. The 10 % alcoholic extract thus prepared was used for investigation of antimicrobial activity using Microbial Bioassay method. Nutrient broth (liquid medium) is prepared and distributed among several test tubes, each with 10ml of medium. These test tubes were then autoclaved and cooled down.

Set1: Test tubes inoculated with 45 hour old culture of *Bacillus megaterium*.

Set2: Test tubes inoculated with 45 hour old culture of *Escherichia coli*.

Set3: Test tubes inoculated with 45 hour old culture of *Klebsiella pneumoniae*.

Each set consisted of 6 test tubes.

A1 –1ml alcoholic extract of *Podaxis pistillaris* + 0.5ml of bacteria *Bacillus megaterium*

Table 1: Comparative analysis of antimicrobial activity of alcoholic extract of *Podaxis pistillaris* and *Phellorinia herculeana* against test bacteria

Treatment	<i>B. megaterium</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
	O. D. (Set 1)	O. D. (Set 2)	O. D. (Set 3)
A1	0.141	0.136	0.181
B1	0.143	0.138	0.288
C1	0.850	0.895	0.743
C2	0.000	0.000	0.000
C3	0.000	0.000	0.000
C4	0.361	0.310	0.427

B1 – 1ml alcoholic Extract of *Phellorinia herculeana* + 0.5ml of bacteria *Bacillus megaterium*.
 C1 – 10ml broth inoculated with 0.5ml of *Bacillus megaterium* (positive control).
 C2–blank.
 C3 –10ml medium with 0.5 ml of bacteria *B. megaterium*+ 0.5ml HCHO solution.
 C4 –10ml medium inoculated with 0.5 ml of bacteria *B. megaterium*+ 1ml chloramphenicol antibiotic

Similarly, 2 more sets of 6 test tubes were inoculated with *Escherichia coli* and *Klebsiella pneumoniae* respectively. These test tubes were

then incubated in BOD incubator for 24 hours at 37°C. After proper incubation, 0.5ml of formaldehyde solution was added to each incubated test tube as a growth stopping agent. The optical density of each set was measured as 530 nm with the help of spectrophotometer and compared with positive control C1 and C4.

RESULTS AND DISCUSSION

After 24 hours of incubation, turbidity was observed in the inoculated test tubes indicating bacterial growth. The optical density (OD) of each set was measured at 530 nm with the help of spectrophotometer and the readings were tabulated. Table 1 shows that OD reading of C1 (inoculated medium but devoid of extract) for each set of bacteria was the highest showing maximum growth of the bacteria in the test tubes. The C1 is the positive control and is used to compare the bacterial growth in tubes having the extract of both the mushrooms. If bacterial growth is less in comparison to C1 in test tubes which have extract present in them shows inhibition of bacterial growth demonstrating their antimicrobial potential.

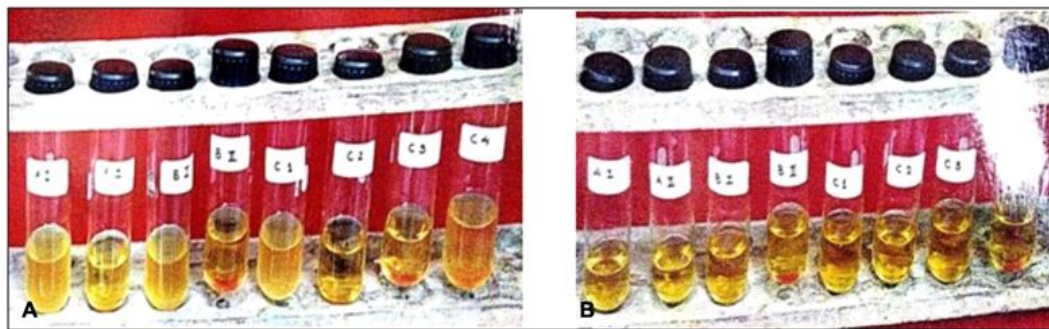


Fig. 1 : Alcoholic extract of *Podaxis pistillaris* against *Bacillus megaterium*; B-Alcoholic extract of *Podaxis pistillaris* against *Klebsiella pneumoniae*



Fig.2: Experiments to comparatively evaluate broad spectrum antibiotic chloramphenicol, *Podaxis pistillaris* and *Phellorinia herculeana* inhibiting the growth of bacteria.

For set 1, 2 and 3 optical density of C1 came out to be 0.850, 0.895 and 0.743 respectively showing maximum bacterial growth.

a) 10% alcoholic extract of *Podaxis pistillaris* showed very less OD value in comparison to C1 (0.141, 0.136 and 0.181 for set 1, 2 and 3 respectively) showing effective inhibition of growth for all types of microbes taken (Fig.1 A).

b) 10% alcoholic extract of *Phellorinia herculeana* also show inhibition in comparison to C1 (0.143, 0.138 and 0.288 for set 1, 2 and 3 respectively) but it showed comparatively less antimicrobial action than *Podaxis pistillaris* (Fig.1 B).

c) The performance of antibiotic was also evaluated (0.361, 0.310 and 0.427 for set 1, 2 and 3 respectively) which shows that broad spectrum antibiotic chloramphenicol is less effective than *Podaxis pistillaris* and *Phellorinia herculeana* in inhibiting the growth of bacteria (Fig.2; Table 1).

The curative values of *Podaxis pistillaris* and *Phellorinia herculeana* are already popular among the local inhabitants of Rajasthan. The positive results for antibacterial properties of these mushrooms have proved the medicinal value of them and paved the way for the vast potential in medicinal field.

The fruiting bodies of *Podaxis pistillaris* have been reported to be used in some parts of Yemen for the treatment of skin diseases, in South Africa as folk medicine against sunburn and in China to treat inflammation (Mao 2000). In countries like India, Afghanistan and Saudi Arabia, *Podaxis pistillaris* and *Phellorinia herculeana* are mainly used as food as the fruiting bodies are known to be rich in proteins containing all essential amino acids, carbohydrates, lipids and minerals (Rathore et al. 2017). In the present study, the current ethno mycological uses of the rare gasteromycetes fungus were investigated and results proved them as probiotic, nutraceutical and medicinal species whose consumption promotes health. Their antibacterial effects have been verified experimentally in the present study.

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