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Antimicrobial Activity of cultivated Edible Mushrooms, Agaricus Bisporus and pleutrous sajor caju

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ABSTRACT

The edible mushroom which are *Agaricus bisporus* and *Pleurotus sajur-caju* that are the beneficial for the health. In this study the chemical composition of antimicrobial properties genetic variation with the thee Pleurotus strains were explored these mushrooms were Effective against the bacteria such as *Escherichia coli*, *staphylococcus aureus*, *vibrio cholerae*, and antimicrobial properties are Gram-positive bacteria. While *P. sagar caju* showed a broader spectrum including antifungal effect. The *agaricus bisporus* and *Pleurotus sajor-caju* of natural antimicrobial agents are useful in food preparation and complementary theories in managing microbial infection of the patients. The results indicate that the bioactive substances found in these mushrooms, such as terpenoids, polysaccharides, and phenolic compounds, may be useful in the creation of natural antibacterial agents. It is advised that these chemicals be isolated and characterized further as they may help develop substitute solutions for the food preservation and pharmaceutical industries.

Keywords: *Edible mushrooms, Antimicrobial Activity, Agaricus bisporus, Pleurotus sajur-caju..*

1. INTRODUCTION

Antimicrobial is a substance that to kills or slow down to the growth of microorganisms such as bacteria. fungi or protozoans. Antimicrobial substances either kill microbes (Microbicidal) or prevent the growth of microbes (Micro biostatic) (Tiwari, *et al* 2021). The history of microorganisms begins with the observations of Pasteur and Jobert, who discovered that one type of bacteria could prevent the growth of another. But the exact mechanism and reason of inhibition was unknown for many years. Later on, it was found that, microorganisms produce certain compounds which inhibit or kill the other competitive microorganisms (Hume, 2017). These compounds are commonly known as antibiotics. Antibiotics can be defined as the substances that are produced by one organism that kill or prevent the growth of another organism at very low concentration (Etebu, *et al* 2016). Antimicrobial includes not just antibiotics, but synthetically formed compounds as well (Gan *et al* 2021). The discovery of different antimicrobials made the way for better health for millions around the world. Most of the infections can be cured easily with short course of antimicrobials (Aminov 2017).

However, the future effectiveness of the antimicrobial's therapy is somewhat to be doubt. Microorganisms are becoming resistant to more and more antimicrobial agents. Currently, bacterial resistance is combated by the discovery of new drugs (Scarborough, *et al* 2024). However, microorganisms are becoming resistant more quickly than new drugs are being made available; thus, future research antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials or how to treat infections with alternative means (Terreni, *et al* 2021)..

Non-Pharmaceutical antimicrobials:

A wide range of chemical and natural compounds are used as antimicrobials. Organic acids are used widely as antimicrobials in widely as antimicrobials in food products e.g. citric acid, lactic acid, acetic acid and their salts, either as ingredients, or as disinfectants. Traditionally, various plants have used to prevent or cure infectious diseases (Coban, 2020).

Many of these plants have been investigated scientifically for antimicrobial activity and large numbers of plant products have been shown to inhibit the growth of pathogenic microorganisms. A number of these agents appear to have structures and modes of action that are distinct from these of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal so, it is worthwhile to study plants and plant products for activity against resistant bacteria (Vaou, *et al* 2021)

Mushroom

Although, most health care professionals skilled in the art of botanical medicine are aware of the antimicrobial properties of certain mushroom and fungi, few may realise that mushroom are rich sources of natural antibiotics (Lim, et al 2022).

Mushroom is the fleshy, spore-bearing fruiting body of a fungus, normally produced above ground on soil or on its food sources. Since the cultivated button mushroom, Agaricus bisporus, is the standard for the term "mushroom," the word is most commonly used to refer to fungus (Basidionmycota, Agaricomycetes) that have a stem (stipe) a cap (pileus) and gills (lamella/lamellae) on underside of the cap (Yekini et al 2021).

The world “Mushroom” can also be used for a wide variety of gilled fungi, without stems, and the term is used even more generally to describe both the fleshy fruiting bodies of some Ascomycota and the woody or leathery fruiting bodies of some Basidiomycota (Díaz-Godínez, et al 2021)

Identification

Identifying mushrooms require a basic understanding of their microscopic structure. Most are Basidiomycetes and gilled. Their spores called basidiospores are produced on the gills and fall in fine rain powder from under caps. At the microscopic level the basidiospores are shot off basidia and then fall between the gills in the dead air space (Kamalakannan *et al* 2020).

As a result, for most mushroom, if the cap is cut off and placed gill-side-down overnight, a powdery impression reflecting the shape of the gills or pores. or spines etc is formed. The colour of powdery print, called a spore print, is used to help classify mushroom and can help to identify them. Spore print colours include white, brown black, purple-brown, pink, yellow and cream. The presence of juices upon breaking, brushing reactions, odour. taste, shades, of colour, habitat and season are all considered by both amateurs and professional mycologists (Charles, 2020).

In general, identification of genus can often be accomplished in the field using a local mushroom guide. Identification of species, however, requires more effort; one must remember that, a mushroom develops from a button stage into a mature structure, and only the latter can provide certain character needed for the identification of the species (Pouliot, *et al* 2021).

Classification

Typical mushrooms are the fruit bodies of members of the order Agaricales, whose type genus is Agaricus and the species is the field mushroom, Agaricus campestris (Joshi, *et al 2021).* However, in modern molecularly defined classifications, not all members of the order gilled fungi, collectively called mushroom, occur in other order in the class *Agaricomycetes* e.g. chanterelles are the *Cantharellales*, false *chanterelles* like Gomphus are in Gomphates, milk mushroom (Lactarius) and russulas (Russula) as well as Lentinula’s are in the Russalates, while the tough leathery genera Lentinus and Panus are among the polyporales. but *neolentinus* is in the *Gleophvllates*, and the little pin-mushroom genus. Rickenella, along with Similar genera, are in the *Hymenochaetales.* Within the main body of mushroom, in the Agaricales, are common fungi like the common fairy ring mushroom (*Marasmius oreades*), shiitake, enoki, oyster mushroom, fly agaricus and other Amanitas, magic mushroom like species of Psilocybe, paddy straw mushroom, shaggy manes etc (Liu *et al* 2024).

A typical mushroom is the Lobster mushroom, which is a deformed, cooked-lobster-coloured parasitized fruitbody of a Russula or Lacterius coloured and deformed by the *mycoparasitic Ascomycetes*, *Hypomyces lactifluorum*, other mushrooms are non-edible and they termed as 'Mushroom' loosely, so that it is difficult to give full account of their classification (Laperriere *et al* 2018). Some have pores underneath, others have spines, such as the hedgehog mushroom and other tooth fungi, and so on. Mushroom has been used for polypore’s, puffballs, jelly fungi, coral fungi, bracket fungi, stinkhorns & cup-fungi. Thus, the term mushroom is more one of the common applications to microscopic fungal fruiting bodies than one having precise taxonomic meaning. There are approximately 14,000 described species of mushrooms (Bunyard *et al* 2020).

Types of mushrooms

1) Edible Mushrooms: - Edible mushrooms are known to be safe to eat because they have been eaten frequently with no ill effects. They are used extensively in cooking, in many cuisines. Many species are high in Ibera and provide vitamins such as thiamine, riboflavin, niacin, biotin, cobalamins, ascorbic acids. Some mushrooms can become significant sources of vitamin D after exposure to U.V. light (El-Ramady et al 2022). They are now recognized as a nutritious food as well as an important source of biologically active compounds of medicinal value. Mushrooms are also a source of some minerals, including potassium, phosphorus and selenium. Mushrooms have long been appreciated for their flavour and textumulates a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids. Also mushroom phenolic compounds has been found to be an excellent antioxidant and synergist that is not mutagenic (Das et al 2021)

2) Toxic mushroom: - Many species that can produce secondary metabolites that can be toxic, mind altering, antibiotic, antiviral or bioluminescent. There are only a small number of deadly species, several others can cause particularly several and unpleasant symptoms. e.g. Amanita phalloides (Fokunang et al 2022).

3) Psychoactive mushrooms: - The hallucinogenic qualities of mushrooms have long been used in many indigenous medical systems across the globe. They have served as sacraments in ceremonies meant to promote visionary states and promote physical and mental healing. The Velda ceremony is one such custom (Guzmán 2019). Psychedelic effects are a feature of psilocybin mushrooms. Often referred to as "magic mushrooms," "zoomers," or "shrooms," they are freely sold in smart stores across the globe or on the illicit market in nations where their sale is prohibited. According to (Pereira (2014). psilocybin mushrooms have been known to induce profound and transformative insights that are sometimes referred to as mystical experiences.

Psilocybin a naturally occurring chemical in certain psychedelic mushrooms like Psilocybin census is being studied for its ability to help people suffering from psychological disorders such as obsessive-compulsive disorder. It has been observed that little doses can prevent migraine and cluster headaches. Amantia muscaria is also psychoactive. The active constituents are Ibotenic acid and muscimol. The Muscaria chemotaxonomic group of amanitas contains no amatoxins or phallotoxins and are hepatoxic (Geiger et al 2018).

4) Medicinal mushrooms: - Many species of medicinal mushrooms have been used to folk medicines for thousands of years. Medicinal mushrooms are now the subject of study for many ethnobotanists and medicinal research. The ability of some mushrooms to inhibit tumour growth and enhance aspects of the immune system has been a subject of research for approximately 50 years (Pal 2019). Mushrooms have hypoglycaemic activity, anti-cancer activity, anti-pathogenic activity and immune system enhancing activity. Oyster mushroom naturally contains the cholesterol drug lovastatin. Mushroom produce large amount of vitamin D when exposed to U.V. light (Sharma *et al* 2022).

The edible mushrooms *Agaricus bisporus* and *Pleurotus sajor caju* have high nutritional value, enhance immune system, potential of host mediated response and act as antimicrobial agent (Sousa *et al* 2023).

Benefits of Mushrooms: -

Nutritional Value of mushrooms:

Table 1: 100 grams of uncooked, white button mushrooms contain the following nutrients:

1	Folate (vitamin B9)	16.0 mcg
2	Niacin (vitamin B3)	3.6 mg
3	Pantothenic Acid (vitamin	1.5 mg
4	Riboflavin (vitamin B2)	0.4 mg
5	Thiamin (vitamin B1)	0.1 mg
6	Vitamin B6	0.1 mg
7	Copper	0.3 mg
8	Iron	0.5 mg
9	Magnesium	9.0 mg
10	Phosphorus	86.0 mg
11	Potassium	318 mg
12	Selenium	9.3mg
13	Zinc	0.5 mg

Health & Nutritional Benefits of Eating Mushrooms:

Mushroom have less calories and contain approximately 80 to 90 % water. In addition, they are high in fibre and low in fat, carbohydrate, and salt. This is the reason why mushroom is considered good for those aiming for weight loss. (Assemie, & Abaya, (2022).

Mushrooms are a great source of potassium; in fact, some people claim that they contain more potassium than bananas. since potassium helps lower blood pressure and diminished the risk of stroke, mushrooms are recommended to people suffering from hypertension (Arzon et al (2021). Copper, a mineral with cardio-protective qualities, is abundant in mushrooms. A single serving of mushrooms is said to provide about 20 to 40 % of the daily needs of copper (Kaur et al 2022). Mushrooms are believed to help fight against cancer. They are an excellent source of selenium, an antioxidant that works with vitamin E to protect cells from the damaging effects of free radicals (Anwar et al 2018).

White button mushroom has been found to restrain the activity of aromatase, an enzyme involved in estrogen production, and 5-alpha reductase, an enzyme that converts testosterone to DHT (Bayala et al 2020).

White button mushrooms may lower the incidence of prostate and breast cancer, according to research. In fact, extract of white button mushrooms has been found to help in diminishing cell proliferation as well as tumor Size (Wang et al 2021).

Lentinan, a beta-glucan found in shiitake mushrooms, has been linked to immune system stimulation and is therefore thought to be beneficial in the fight against AIDS. It also helps fight infection and exhibits anti-tumor activity (Vetvicka et al 2021). Being rich in fibre, protein and vitamin B, mushrooms help maintain a healthy metabolism (Rathore et al 2017).

It has been found that mushroom extract helps stop migraine headaches and is beneficial for people suffering tronn nental illnesses, like obsessive compulsive disorder (Badri 2018). Oyster mushroom said to be useful in strengthening of veins and relaxation of the tendons.

2. MATERIAL AND METHOD

1) Collection of mushroom samples:

Two mushroom species were collected. one species i.e. *Pleurotus sajor caju* was collected from The Agriculture college, Kolhapur and the second species i.e. *Agaricus bisporus* was collected from the local market.

2. Collection of test culture:

Different bacterial cultures i.e. *E. coli. Proteus vulgaris, Bacillus cereus -Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus* were collected from institutional laboratory. These cultures were inoculated on sterile nutrient agar slants. For *E. coli* Mac-Conkey's agar was used. All these slants were incubated at 37^{0}C for 24 h. The slants were preserved in refrigerator till use (Saif (2018).

3. Preparation of Mushroom extract:

i) Ethanol extract: - The pre washed disinfected fruiting bodies of mushroom were cut into pieces. Crushed in mortar and pestle with intermittent addition of ethanol. Then the muslin cloth was filtered by the mixture. Collected filtrate was kept at 50'C for about 24 hrs. Then concentrated ethanol extract was collected on rubber cork bottle and stored in refrigerator for further use.

By following the same procedure, the two more extracts were prepared that are:

ii) Acetone Extract

ii) Water Extract

4. Antimicrobial activity of mushroom Extracts against laboratory cultures:

The same general procedure was followed to check the antimicrobial activity of mushroom extract of both species in ethanol water and acetone on different bacterial cultures. (Shah. *et al* 2018)

The procedure is as follows:

- The bacterial cultures were spread inoculated on sterile nutrient agar plates.
- Wells were bored using a sterile cork borer of 4mm diameter. On each plate four wells were bored.
- 40mg/ml of ethanol extract was poured in single well. Other two wells were poured with the same concentration of acetone and water extract.
- The plates were kept in refrigerator for diffusion of extract for half an hour.
- After diffusion the plates were kept for incubation at 37°C for 24 hrs.
- After incubation, the plates were observed for zone of inhibition and results were recorded.

5. Comparison of antimicrobial activity showed by the extracts of mushroom spp. with commercially available antibiotics:

- The bacterial cultures i.e. *E. coil, Proteus vulgaris, Bacillus cereus. Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus* were spread inoculated on sterile Nutrient agar plates,

- The discs of antibiotics viz: Azithromycin, tetracycline, cephalosporin and gentamycin were kept at different four points marked on cach plate at specific distance.
- Plates were kept in refrigerator for diffusion of antibiotics for half an hour.
- After diffusion the plates were incubated at 37^0C for 24h.
- The plates were examined for a zone of inhibition following incubation.

3. RESULTS AND DISCUSSION

Result of antimicrobial activity of *Agaricus bisporus* on different cultures are laboratory shown in Table: 4:1.

Table 2: Antimicrobial activity of extracts of *Agaricus bisporus.*

Sr no.	Name of Organisms	Ethanol extract (Zone in mm)	Acetone extract (Zone in mm)	Water (Zone in mm)
1	*E. coli*	28	22	35
2	*P. vulgaris*	21	18	25
3	*B. cereus*	25	28	35
4	*B. subtilis*	17	20	25
5	*S. aureus*	15	18	26
6	*M. luteus*	8	10	15

As shown in table 4.2, all test organisms were found to be sensitive to the extracts of *Pleurotus sajor caju.*

Ethanol extract showed highest inhibitory activity against *E. coli* and it is followed by *B. Cereus. B. subtilis* and *S. aureus* showed moderate sensitivity while the least sensitivity was shown by *M. luteus.*

In case of acetone extract test organisms *E. coli* and *B. cereus* were found to be highly sensitive and gave same zone of inhibition. *B. subtilis, p vulgaris* and *S. aureus* showed zones ranging between 17-21 mm. *M. Iuteus* was least sensitive.

Water extract showed the highest activity amongst all other extracts showing 35 mm of zone against two test organisms i.e. *E. coli* and *B. cereus.* Other organisms showed better sensitivity while *M. luteus* give least sensitivity

3. Comparison of antimicrobial activity of extracts of *A. bisporus* with commercially available antibiotics:

Table 3: Comparison of extracts of *Agaricus bisporus* with commercially available antibiotics:

Sr No.	Name of organism	Ethanol extract	Acetone extract	Water extract	Azithro mycin	Tetra cyline	Cephalic sporin	Gentamycin
1	*E. coli*	28	22	30	23	22	30	29
2	*P.vulgaris*	21	18	23	25	20	35	32
3	*B. cereus*	25	28	35	17	12	28	27
4	*S. Subtill*	17	20	23	11	12	15	17
5	*S. aureus*	15	18	24	9	13	18	25
6	*M. luteus*	8	10	13	24	11	16	21

The antimicrobial activity of extracts of *A. bisporus* was compared with four commercially available antibiotics viz., Azithromycin, tetracycline, cephalosporin and gentamicin. The result of comparison is shown in table 4:3

The antimicrobial activity of extracts and antibiotics when tested against *E. coli*, it showed highest sensitivity to antibiotic cephalosporin which is similar to water extract i.e. 30 mm diameter. Gentamycin found to be second antibiotic which when tested gave 25 mm diameter of zone which not much different than ethanol extract. Acetone extract and tetracycline showed similar activity against *E. coli.*

Second test organisms i.e. *P. vulgaris* which showed maximum zone i.e. 35 min for antibiotic cephalosporin followed by gentamycin (32mm) Remaining antibiotics as well as extracts showed antimicrobial activity ranging from l8-25 mm diameter of zone.

In case of *B. cereus,* water extract showed highest activity. It was found to be equally sensitive to both cephalosporin and acetone extract followed by activity of gentamycin (27 mm).

B. subtilis was found to be most sensitive to water extract followed by acetone extract while its sensitivity to all antibiotics and ethanol extract was negligible.

Fifth test organism was *S. aureus* which showed highest sensitivity to gentamycin followed by water extract. Activity shown by cephalosporin Was found to be similar with acetone extract 18 mm Remaining antibiotics and ethanol extract showed negligible activity.

M. luteus was found to be highly sensitive to azithromycin 24 mm followed by Gentamycin (2l mm), while all antibiotics and extracts left showed negligible antimicrobial activity against *M. Iuteus.*

4. Comparison of antimicrobial activity of extracts of *Pleurotus sajor caju* with commercially available antibiotics: -

Table 4: comparison of extracts of *Pleurotus sajor caju* with commercially available antibiotics.

Sr No.	Name of organism	Ethanol extract	Acetone extract	Water extract	Azithro mycin	Tetra cyline	Cephalic sporin	Gentamycin
1	*E. coli*	32	28	35	23	22	30	29
2	*p.vulgaris*	22	19	24	25	20	35	32
3	*B.cereus*	28	28	35	17	12	28	27
4	*S.Subtill*	19	21	25	11	15	25	29
5	*S.aureus*	16	17	26	9	13	18	25
6	*M.luteus*	9	10	15	24	11	16	21

The antimicrobial activity of extract of mushroom was compared with the same antibiotics.

In case of *E. coli*, it showed more sensitivity of water extract and that to the antibiotic cephalosporin and followed by gentamycin.

P. vulgarius was strongly inhibited by antibiotic cephalosporin and gentamycin than extracts of mushroom.

B.cereus showed high sensitivity to water extract. It showed moderate activity to the antibiotic's cephalosporin and gentamycin and activity to other antibiotics was found to be negligible.

B.subtilis was showed similar activity to water extract and cephalosporin it was largely inhibited by gentamycin.

S.aureus showed better inhibitory zones against mushroom extract and commercially available antibiotics.

In case of *M. luteus* it was found that, it was sensitive to commercially available antibiotics than the mushroom extracts.

Photographs no. 1

Antimicrobial activity of extracts of *pleurotus sajor caju*

Bacillus cereus

Proteus vulgaris

Micrococcus luteus

Bacillus subtillis

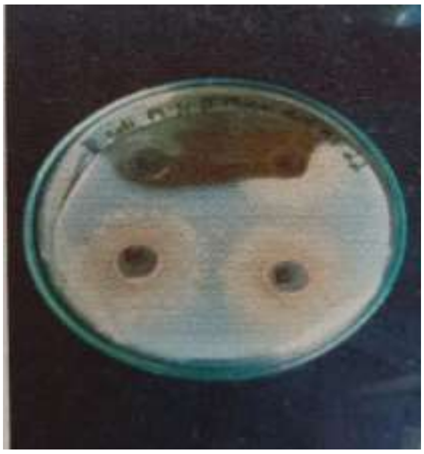
Staphylococcus aureus

E. coli

Photographs no. 2

Antimicrobial activity of extracts of *Agaricus bisporus*

Bacillus cereus



E. coli

Proteus vulgaris

Staphylococcus aureus

Bacillus subtilis

Micrococcus luteus

Photographs no. 2

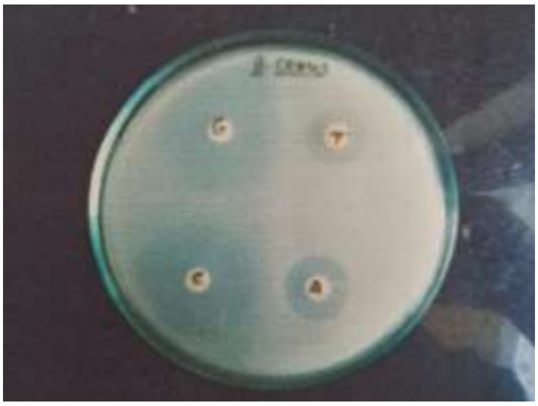
Antimicrobial activity of extracts of *Agaricus bisporus*

Bacillus cereus

Staphylococcus aureus

Micrococcus luteus

E. coli



Bacillus subtilis

Proteus vulgaris

4. CONCLUSIONS

- Both the mushroom species i.e. *Agaricus bisporus* and *Pleurotus sajor caju* are having antimicrobial potential.
- All the extracts i.e. ethanol, acetone and water possess antimicrobial property.
- Water extract was found to be highly effective amongst all in its antimicrobial activity.
- Most sensitive bacterial cultures to mushroom extracts are *B. cereus* and *E. coli.*
- Comparison between antimicrobial activity of mushroom extracts and antibiotics viz; Azithromycin, tetracycline, cephalosporin and gentamycin proved to be equally active as antimicrobials.

Further study of determining spectrum of antimicrobial substance in mushroom against different pathogens and active ingredient may prove it a good candidate for antimicrobial therapy on wide range

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