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Research Article

EVALUATION OF ANTIBACTERIAL POTENTIALS OF SOME EDIBLE MUSHROOM SPECIES

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Abstract

Edible mushrooms are nutritionally endowed fungi. They serve as repositories of several phytochemical constituents and biomolecules that are responsible for the antimicrobial, antitumor activities etc. Hence a study was undertaken to test the presence of phytochemical constituents and antibacterial activity of five edible mushroom species. Aqueous extract of mushrooms exhibited antibacterial activity against oral bacterial isolates and the degree of inhibition varied with the mushroom species.

Keywords: Antibacterials, Mushrooms, Phytochemicals, Zone of inhibition

Introduction

Mushrooms are known for protein rich food and they have long been appreciated for their flavor and texture. There are thousands of different mushroom species and about 700 species have significant pharmacological properties. Edible mushrooms are valuable source of biologically active compounds (Miles and Chang, 2004). Both fruiting bodies and the mycelium of mushrooms contain compounds with wide range of antimicrobial activity (Mehmet *et al*., 2009). In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs, commonly used in the treatment of infectious diseases. Attempts have been made in many parts of the world to explore the use of mushrooms and their metabolites for the treatment of a variety of human ailments. Hence, this study was attempted with the objective of evaluating the antibacterial activity of aqueous extract of *Agaricus bisporus, Calocybe indica, Hypsizygus ulmaris, Pleurotus ostreatus* and *Pleurotus platypus* against oral bacterial isolates.

Materials and Methods

Collection of mushroom species

Five different mushroom species were used in this study. *Agaricus bisporus* and *Calocybe indica* were procured from Karpaga Vinayagar Mushroom Farm, Madurai. *Pleurotus platypus* and *Hypsizygus ulmaris* were obtained from Tamil Nadu Agricultural University, Coimbatore. *Pleuortus ostreatus* was obtained from Tamil Nadu Agricultural University, Madurai. Fruiting bodies were collected and were dried in hot air oven at 40°C. The dried fruiting bodies were ground to powder and stored in air tight container at room temperature for further work.

Preparation of aqueous extract (Johnsy and Kaviyarasan, 2014)

Ten gram of mushroom powders were extracted by using a soxhlet extractor for 3 hours with 100 ml of distilled water under reflux conditions and the extracts were used for qualitative testing of phytochemicals. The

water extracts were dried in a freeze drier and used for antimicrobial study.

Phytochemical screening

Presence of alkaloids, flavonoids, phenols, saponins, tannins in aqueous extracts of mushroom powders was qualitatively tested.

Alkaloids (Edeoga *et al.,* 2005)

To 1ml of aqueous extract, 5 ml of dilute sulphuric acid was added and distributed into equal portions. Few drops of Mayers reagent was added to one portion and 2-3 drops of Dragendroffs reagent was added to another portion. Formation of white precipitate with Mayers reagent and reddish brown color with Dragendroffs reagent indicates the presence of alkaloids.

Flavonoids (Harborne, 1973)

Five ml of dilute ammonia solution was added to 5ml of aqueous filtrate of each mushroom species. To this mixture, 2 drops of Concentrated H_2SO_4 was added and observed for yellow colouration.

Total Phenols (Harborne ,1998)

One ml of aqueous extract was heated to remove the solvent and the residue was taken in a little of aqueous methanol (Merk, India). To this, 0.5% Ferric chloride solution was added and the change in color indicated the presence of phenols.

Saponins (Edeoga et al .,2005)

Five ml of aqueous extract was boiled with 20 ml of distilled water in water bath and filtered. Ten ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent Froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. Formation of emulsion indicated the presence of saponins in the mushroom aqueous extract.

Tannins (Harborne, 1998)

A few drops of 0.1% Ferric chloride solution was added to the extracts and observed for brownish green or a blue – black coloration.

Antibacterial activity of aqueous extract of mushroom species (Surekha *et al*., 2011)

One gram of each freeze dried powder of mushroom was mixed with 10 ml of sterile distilled water. The contents were stirred for 15 min for effective extraction and centrifuged at 2000g for 15 min and used immediately for antibacterial study.

Bacterial isolates used

The test organisms used in the study were oral bacterial isolates obtained from the culture collection of Department of Botany and Microbiology, Lady Doak College, Madurai. The isolates were grown in specific media and used for the antibacterial studies.

Agar well diffusion method (Surekha *et al.,* 2011)

Inoculum of test organisms was prepared by growing pure isolates in nutrient broth at 37 ^{o}C for overnight. The overnight broth cultures were sub cultured in fresh nutrient broth for growth. Muller Hinton Agar was mixed with 1 ml of test organism and then poured in to sterile petridishes. The plates were allowed to solidify. Wells were made with sterile cork borer and 25 µl of extract was added to each well aseptically. The plates were incubated at 37°C for 48 hrs. The diameter of zones of inhibition was measured.

Results

Phytochemical Studies

Mushroom species (Plate 1) obtained from various places were tested for phytochemical constituents and the results are presented in (Table1). The results show that alkaloids, flavonoids, phenols, saponins and tannins were present in *Agaricus bisporus* and *Pleurotus ostreatus*. *Calocybe indica* showed the presence of all the phytochemicals except alkaloids. Phenols and saponins alone were present in *Hypsizygus ulmaris* whereas only alkaloids and phenols were found in *Pleurotus platypus.*

Antibacterial activity of aqueous extract of mushroom species

In the present study, aqueous extract of five mushroom species was screened for the antibacterial activity (Plates 2-7) and the results are presented in Table.2. Aqueous extract of *A. bisporus* showed more activity against bacterial isolate 4 and 5 whereas *C. indica* exhibited activity against all the bacterial isolates except isolate 2 which showed minimum activity. Aqueous extract of *H.ulmaris* had more inhibition with reference to isolate 1 and 2. Extract of *P.ostreatus* was found to have maximum zone of inhibition against isolate 2. *P.platypus* showed maximum activity against bacterial isolate 5. It is also observed that extract of all the mushroom species showed varied activity against bacterial isolates 4 and 5.

Table 1: Phytochemical constituents of aqueous extract of mushroom species

Phytochemicals	*Agaricus bisporus*	*Calocybe indica*	*Hypsizygus ulmaris*	*Pleurotus ostreatus*	*Pleurotus platypus*
Alkaloids	+ve	-ve	-ve	+ve	+ve
Flavonoids	+ve	+ve	-ve	+ve	-ve
Phenols	+ve	+ve	+ve	+ve	+ve
Saponins	+ve	+ve	+ve	+ve	-ve
Tannins	+ve	+ve	-ve	+ve	-ve

Table 2: Effect of aqueous extract of mushroom species on oral bacterial isolates

Oral Bacterial Isolates	Zone of inhibition in (mm)				
	Agaricus bisporus	*Calocybe indica*	*Hypsizygus ulmaris*	*Pleurotus platypus*	*Pleurotus ostreatus*
Isolate 1	9	14	9	Nil	Nil
Isolate 2	9	5	9	Nil	11
Isolate 3	Nil	10	Nil	6	4
Isolate 4	14	12	7	6	6
Isolate 5	14	12	7	10	9

Plate 1: Edible mushrooms used in this study

Agaricus bisporus *Calocybe indica* *Hypsizygus ulmaris*



Pleurotus ostreatus *Pleurotus platypus*


Plate 2: Pure Culture Of Oral Isolates

Oral isolate 1

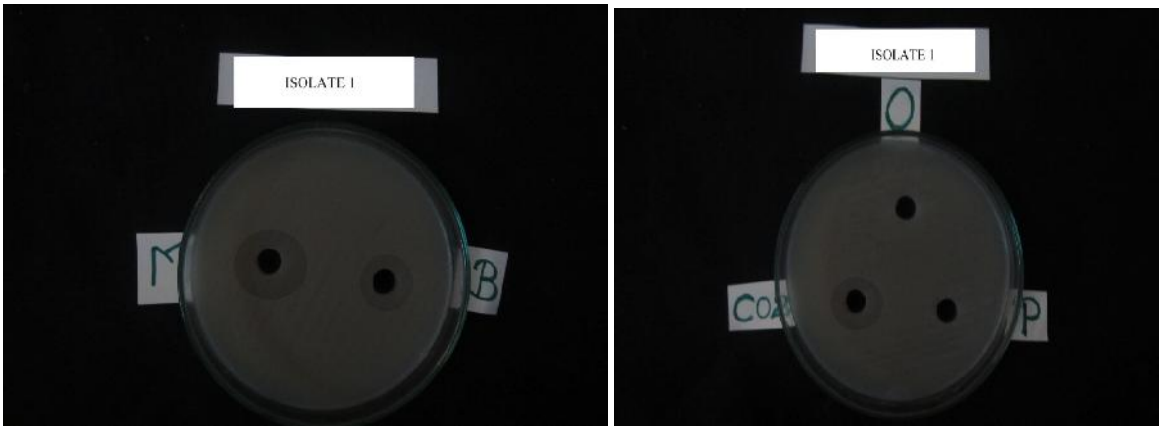
Oral isolate 2

Oral isolate 3

Oral isolate 4

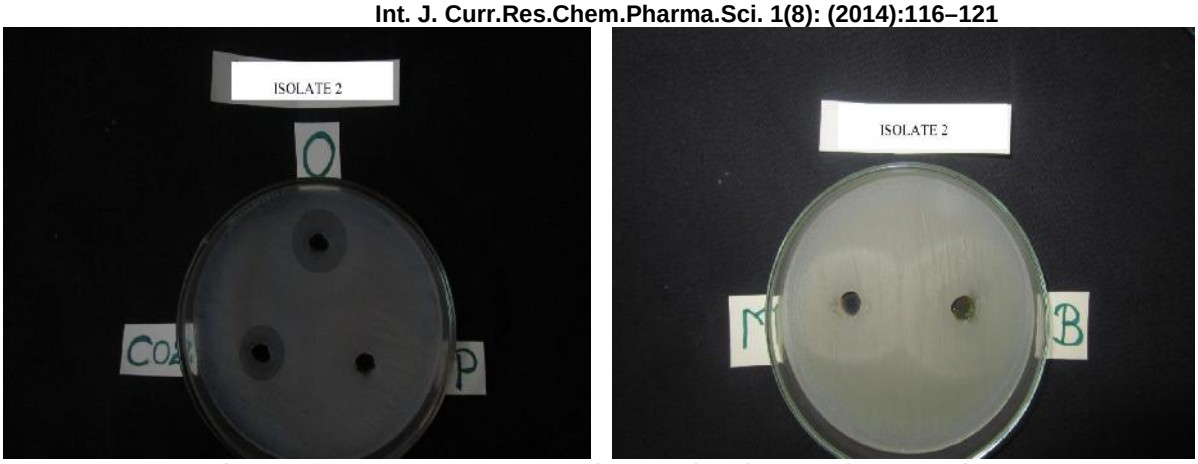
Oral isolate 5

Plate 3: Effect of Aqueous Extract Of Mushroom Species on Oral Isolate 1



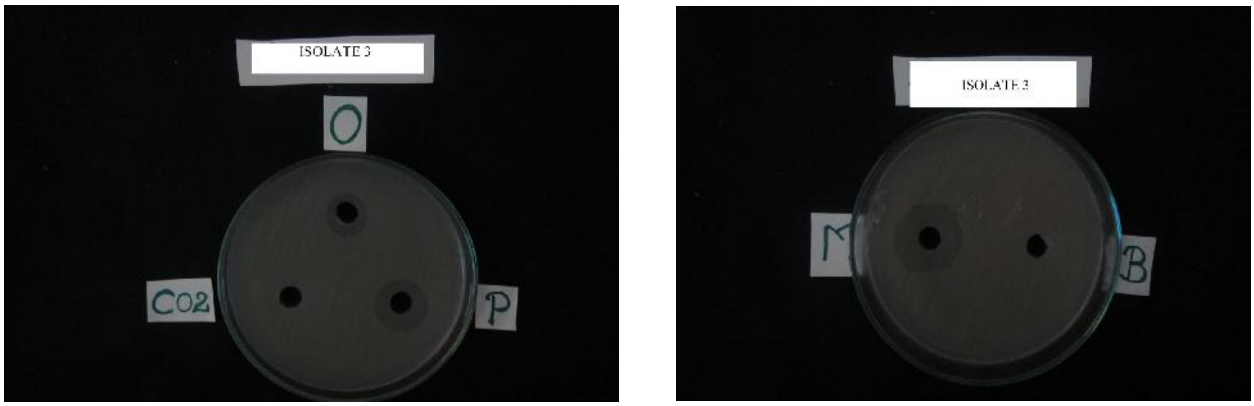
O – *Pleurotus ostreatus*, CO2 – *Hypsizygus ulmaris*, P - *Pleurotus platypus* M- *Calocybe indica* , B – *Agaricus bisporus*

Plate 4: Effect of Aqueous Extract Of Mushroom Species on oral Isolate 2



O – *Pleurotus ostreatus*, CO2 – *Hypsizygus ulmaris*, P - *Pleurotus platypus*
M - *Calocybe indica*, B – *Agaricus bisporus*

Plate 5: Effect of Aqueous Extract Of Mushroom Species on Oral Isolate 3

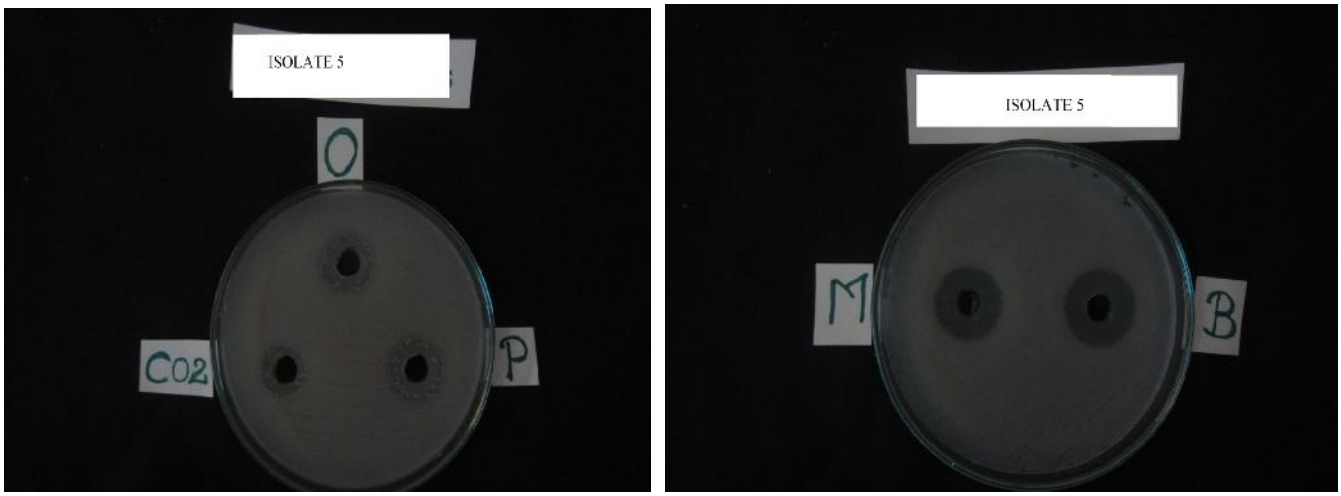


O – *Pleurotus ostreatus*, CO2 – *Hypsizygus ulmaris*, P- *Pleurotus platypus*
M- *Calocybe indica*, B – *Agaricus bisporus*

Plate 6: Effect of Aqueous Extract Of Mushroom Species on Oral Isolate 4

O – *Pleurotus ostreatus*, CO2 – *Hypsizygus ulmaris*, P- *Pleurotus platypus*
M- *Calocybe indica*, B – *Agaricus bisporus*

Plate 7: Effect of Aqueous Extract Of Mushroom Species on Oral Isolate 5



O – *Pleurotus ostreatus*, CO2 – *Hypsizygus ulmaris*, P- *Pleurotus platypus*
M- *Calocybe indica*, B – *Agaricus bisporus*

Discussion

Edible mushrooms are commonly thought to have nutritional value. They have phytochemical constitutents and they produce a wide range of secondary metabolites having medicinal value (Jayakumar *et al*., 2009, Asuquo and Etim, 2011). In this perspective, results of the present study showed the presence or absence of phytochemical constituents in the selected edible mushroom species and their antibacterial activity against bacterial isolates. The extracts of mushroom species showed activity against bacterial isolates as reported earlier (Jonathan *et al*., 2003, Barros *et al.,* 2007). In this study the mushroom species exhibited varying activity against oral bacterial isolates. The spectrum of antibacterial activity may be attributed to the presence of phytochemicals of various chemical types in mushrooms (Johnsy and Kaviyarasan, 2014). The present findings suggest that further extraction and characterization of phytochemicals from edible mushrooms followed by testing their activity against infectious disease causing bacteria may help the researchers to develope novel drugs from mushrooms.

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