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EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL POTENTIALITY OF SOME EDIBLE MUSHROOMS

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ABSTRACT:

Edible mushrooms are the fungi fruiting bodies which have high protein content along with several vitamins and minerals. Mushrooms have medicinal value in inhibiting tumour growth and enhancing immune system. The antimicrobial activity of 90% ethyl acetate and antioxidant activity of fruiting bodies of mushrooms of four edible mushrooms were investigated. The antimicrobial activity of four mushroom samples was tested against different bacteria using agar well diffusion method with ampicillin as positive standard reference to determine the sensivity of the bacterial strains. Two mushrooms samples showed significant inhibitory activity against *Staphylococcus aureus* which is a normal inhabitant in humans. The antioxidant activity, peroxidase, number of ascorbate oxidase units and catalase activity were significant more with *Agaricus bisporus* than the other edible mushrooms used in the study. All the activities measured are to find out for the presence of reactive oxygen species which play an important role in cell death and signal transduction.

Keywords: Edible mushrooms, Antimicribial activity, Antioxidant activity

[I] INTRODUCTION

Mushrooms are spore bearing fruiting bodies of fungi, typically produced above ground on soil or on food surface. They may be edible, poisonous or indigestible.

Edible mushrooms are commonly thought to have little nutritional value of which many species are high in fiber and provide vitamins such as thiamine, riboflavin, niacin, biotin, cobalamines, ascorbic acid and a significant source of vitamin D along with some minerals like selenium, potassium and phosphorus. Many species have medicinal value to inhibit tumor growth and enhance the immune system.

Natural compounds from edible foods having antioxidant activity have been used as potential therapeutic agents of aging [1]. Superoxide dismutase and catalase represent potential protein drugs for antioxidant therapy [2].

A number of highly reactive oxygen species, which include the superoxide anion, the hydroxyl radical and hydrogen peroxide are produced as unavoidable side products in the normal metabolism. Organisms have a comprehensive array of antioxidant defense mechanisms to reduce free radical formation or reduce their damaging effects. These include enzymes such as superoxide dismutase and catalase to decay superoxide and peroxidase respectively and essential radicals scavengers like ascorbic acid and reduced glutathione [3]. An improved antioxidant status helps to minimize the oxidative damage and thus to delay or prevent pathological changes. Potential antioxidant therapy should be therefore included either as natural free radical scavenging antioxidant enzymes or as agents which are

capable of augmenting the activity of the antioxidant enzymes [4].

The present study investigates the antimicrobial, antioxidant activity as well as protein and sugar content of some locally available edible mushrooms.

[II] MATERIALS AND METHODS

2.1. Mushroom samples:

The mushroom samples *Pleurotus sajor-caju, Volvariella volvaceae* were collected from Institute of Horticulture located in Visakhapatnam, *Agaricus bisporus* and *Pleurotus ostreatus* were obtained from local markets.

2.2. Preparation of mushroom extract:

The tissues of mushrooms were thoroughly washed. The enzyme extract (w⁄v) were separately prepared by grinding the 0.5g of wet tissue in chilled motor using 5ml of phosphate buffer, pH 7.5. The homogenate was centrifuged at10, 000 rpm for 15min and the supernatant was used as enzyme source for all the antioxidant experiments and 10% ethyl acetate extracts for antimicrobial experiments.

2.3. Antimicrobial studies:

The test organisms include in the study are *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 3021), *Escherichia coli* (NCIM 2066), *Klebsiella pneumoniae* (NCIM 2957) and *Proteus vulgaris* (NCIM 2027). These strains are obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune.

The bacteria were grown in Muller Hinton media at 37^{o}C and maintained on nutrient agar slants at 4 ^{o}C and stored at -20 ^{o}C. Inoculum of test organisms was prepared by growing pure isolates in nutrient broth at 37 ^{o}C for over night. The over night broth cultures were sub cultured in fresh nutrient broth and grown for 3hrs to obtain log phase culture. The agar plates were prepared by pour plate method using 20ml nutrient medium. The molten sterile nutrient agar medium is cooled to 45^{o}C and mixed thoroughly with 1ml of growth culture of concentrated test organism and then poured into the sterile petridishes and allowed to solidify. Wells are made with sterile cork borer and 25µl of extract (1mg\ml) was added to each well aseptically. Ampicillin (10µg/disc) is included as standard antimicrobial agents and tested along with the extract. The agar plates were incubated at 25 ^{o}C for 48hrs. The diameter of zones of inhibition was measured in mm using Himedia one reader.

2.4. Total antioxidant activity:

The total antioxidant activity of the sample

was evaluated by the method of Prieto *et al* method [5].

An aliquot of each sample (0.05ml) was mixed with 0.5ml of reagent (0.6M H_2SO_4, 28mM sodium phosphate and 4mM ammonium molybdate) in 1.5ml eppendorf tube. The tubes were capped and boiled in a boiling water bath at 95^{0}C for 90min and cooled. The absorbance of each sample was measured at 695nm against blank in a spectrophotometer. A typical blank contained 0.5ml of reagent solution and 0.05ml of buffer and treated in the same manner as test. The antioxidant capacity was expressed as micromoles of ascorbic acid equivalents of antioxidant capacity.

2.5. Assay of peroxidase:

Peroxidase activity was assayed according to the method described by Seevers *et al.,* 1971 [6]. The reaction mixture taken in a 3ml cuvette containing 0.5ml of 20mM sodium acetate buffer, pH-5.0, 0.5ml of 1.3mM benzidine, 0.1ml of diluted mushroom extract. The enzyme reaction

was initiated by the addition of hydrogen peroxide.

The reference cuvette contained all the components of the reaction mixture except hydrogen peroxide which was substituted by an equal volume of the buffer, the increase in the optical density at 420nm was recorded for 5min at 1min intervals in a UV visible spectrophotometer. The units of peroxidase activity were expressed as an increase in optical density at 420nm per min per mg protein.

2.6. Assay of ascorbate oxibase:

Ascorbate oxidase activity was assayed according to the method of Vines and Oberbacher [7]. To 3ml of ascorbate solution (18.8mg ascorbic acid dissolved in 300ml of 0.1M phosphate buffer, pH-5.6), 0.1ml of enzyme extract was added and the change in the absorbance at 265nm is measured at an interval of 30sec of a period of 5min. One enzyme unit is equivalent to 0.01 O.D change per min per mg of protein.

2.7. Assay of catalase:

The catalase activity was assayed by the titrimetric method based on the procedure described by Radhakrishnan and Sarma [8]. 2.5ml of 0.9% hydrogen peroxide (v/v) in the same buffer were taken and 0.5ml of enzyme extract was added and incubated at 28^0C for 3min. The reaction was then arrested by adding 5ml of 2N H_2SO_4 and the residual H_2O_2 was titrated with 0.1N $KMnO_4$ solution. Blank was carried out without extract. Unit of catalase activity was expressed as ml of 0.1N $KMnO_4$ equivalents of hydrogen peroxide decomposed per min per mg protein.

[III] RESULTS

3.1. Antimicrobial activity:

The antimicrobial activities of four edible mushrooms are presented in Table 1. *A.bisporus* and *P.ostreatus* exhibited different levels of antimicrobial activity against bacteria and the other two samples showed no activity. The positive control, Ampicillin showed 11-15mm zone of inhibition which is taken as a standard. Most significant inhibitory activity was observed with *S.aureus* with both the samples and *A.bisporus* showed more activity towards *P.vulgaris*.

3.2. Antioxidant activity, peroxidase, ascorbate oxidase and catalase:

The antioxidant, peroxidase and ascorbate oxidase enzyme activities of four mushroom samples were tested and are tabulated in Table 2. The antioxidant activity of samples was expressed as equivalents of α-tocopherol (µg/gm wt) which was found to be 1.7 in *A.bisporus* and *P.ostreatus*, 1.1 in *V.volvace* and 0.3 in *P.sajor-caju*. Peroxidase activity was found to be more with *A.bisporus* expressed as an increase in optical density at 420nm per min per mg protein and no peroxidase activity was observed in *P.ostreatus*. Maximum ascorbate oxidase activity in terms of enzyme equivalents to 0.01 OD change per min at 420nm is shown by *A.bisporus* with 16 enzyme units followed by 7 enzyme units with *V.volvace*. Units of catalase activity was measured by decomposing time of hydrogen peroxide by mushroom sample which is highest with *A.bisporus* of 4min and the other samples did not show much catalase activity [Table 3].

S.No.	Microorganism (Bacteria)	Zone of growth inhibition in mm				
		Ampicillin (10µg/disc)	*Agaricus Bisporus*	*Pleurotus Ostreatus*	*Volvariella Volvace*	*Pleurotus sajor-caju*
1.	*Escherichia coli*	14	16	15	ND	ND
2.	*Bacillus subtilis*	15	15	16	ND	ND
3.	*Staphylococcus aureus*	15	24	24	ND	ND
4.	*Klebsiella pneumonia*	11	16	15	ND	ND
5.	*Proteus vulgaris*	15	22	18	ND	ND

Table: 1. Antimicrobial activity of some edible mushrooms on different bacterial species.

Mushroom extract (10%)	Antioxidant capacity	Units of Peroxidase activity	No. of ascorbate oxidase enzyme units
Agaricus bisporus (A.b.)	1.7	0.15	16
Pleurotus ostreatus (P.o.)	1.7	0.0	0
Volvariella volvace (V.v.)	1.1	0.04	7
Pleorotus sajor-caju (P.s.)	0.3	0.01	1

Table: 2. Total antioxidant, peroxidase and ascorbate oxidase enzyme activity of edible mushrooms.

Time of decomposing of H_2O_2	Units of catalase activity			
	Agaricus bisporus (A.b.)	***Pleurotus ostreatus (P.o.)***	***Volvariella volvace (V.v.)***	***Pleorotus sajor-caju (P.s.)***
10 sec	0.6	0.8	0.8	0.9
30 sec	0.5	0.8	0.8	0.9
60 sec	0.4	0.8	0.8	0.8
90 sec	0.2	0.8	0.8	0.8
120 sec	0.2	0.8	0.8	0.8
240 sec	0.1	0.8	0.8	0.8
360 sec	0.1	0.8	0.8	0.8

Table: 3. Units of catalase activity expressed as ml of 0.1N Potassium permanganate equivalents of hydrogen peroxide decomposed per min per mg protein.

The protein concentration of mushroom samples was determined by Lowry method [9] using crystalline BSA as standard and all samples showed high protein content with *A.bisporus* the highest with 96µg/10µl. The sugar content in all the mushroom samples was found to be less.

[IV] DISCUSSION

The ethyl acetate extracts of *A.bisporus* and *P.ostreatus* exhibited a broad spectrum of antimicrobial activity which may be due to the presence of antioxidant compounds. Reactive oxygen species (ROS) such as super oxide, hydrogen peroxide and hydroxyl radicals are molecules that contain oxygen and have

higher reactivity than ground state molecular oxygen. In our body, ROS play an important role in cell death and signal transduction by ionizing radiation. When water, the most copious intracellular material is exposed to ionizing radiation, decomposition reactions occur, which form a variety of free radicals and molecular products. These products can peroxides membrane lipids and attack proteins or DNA. In order to avoid these unnecessary reactions there is a requirement of enzymatic and non-enzymatic defense systems that deal with ROS produced as a consequence of aerobic respiration.

It was observed that from all the results obtained, *Agaricus bisporus* has higher ontioxidant, catalase, peroxidase activity and high ascorbate oxidase content, high protein content when compared with other samples.

[V] CONCLUSION

Mushrooms are good to health as they are rich in proteins and have good antioxidant capacity which is essential in fighting against diseases.

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