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COMPARATIVE STUDY OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY IN BOILED AND UNBOILED MUSHROOM EXTRACTS OF *AGARICUS BISPORUS*

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ABSTRACT

The extracted product from medicinal mushrooms design to supplement the human diet not as regular food, but as the enhancement of health and fitness, can be classify into the category of dietary supplement. The main objectives of this study was to produce valuable medicinal substances from boiled and unboiled extract of mushroom (*Agaricus bisporus).* The invitro antimicrobial activity of methanolic extracts of *Agaricus bisporus* using the agar well diffusion method against some food borne and clinical pathogens isolated was done. The anti-bacterial and anti-fungal activity was done, for the bacterial organisms such as *Staphylococcus aureus, Pseudomons aeruginosa, Salmonella typhi, Klebsiella pneumoniae, Bacillus subtilis, and Escherichia coli* for fungi *Aspergillus niger, Aspergillus flavus, Pencillium* species and *Mucor* species. Its phenolic compositions and the other antioxidant compound along with the invitro antioxidant activity were also determined. The antioxidant activity done by the oil in water emulsion method. To observe the total oxidizing activity peroxidase and ascorbate oxidase, catalase assays were performed. Enzymatic assays like amylase, cellulase enzyme was done for the mushroom extract to observe the reducing sugar. Comparatively the unboiled mushroom extracts produced a good anti-oxidant and anti-microbial activity.

KEYWORDS: Antimicrobial activity, Antioxidant, Peroxidase, Amylase.

INTRODUCTION

Mushrooms are fungi to be considered a fungus, an organism must be eukaryotic; grow by extending filamentous cells called hyphae, or by budding; obtain nutrients by absorption,

have no chlorophyll, and reproduce via spores.[1] Mushrooms have medicinal value in inhibiting tumor growth and enhancing immune system. The antioxidant activity of five *Agaricus* species mushrooms were screened through chemical, biochemical and electrochemical techniques. The chemical assays allowed an evaluation of their reducing power and radical scavenging activity, while biochemical assays evaluated the lipid per oxidation inhibition capacity, using erythrocytes and brain cells as models. *Agaricus silvaticus* was the most efficient species presenting the lowest EC50 values in the chemical and biochemical assays, and the highest "antioxidant power" in the electrochemical assays.[2] Many investigations made for ethanol extracts of an edible mushroom *Agaricus bisporus,* before and after boiling were investigated for antioxidant, anticancer and antimicrobial activities. To confirm the total antioxidant activity, ABTS, DPPH, free-radical scavenging assay was carried, along with total phenolic and flavanoid concentration. In measuring ABTS and DPPH free-radical scavenging activities, was found to be similar in both the extracts. Moreover, this synthetic antioxidant also shows low solubility and moderate antioxidant activity.[3] Antioxidant components like total phenol and flavanoid were also determined. The culture filtrate of *Phyllosticta* species may have potential source of natural antioxidant.[4] Currently available synthetic antioxidant like butylated hydroxyl toluene (BHT), tertiary hydroquinone and gallic acid esters, has been suspected to prompt or cause negative health effects. Hence, strong restriction has been placed on their application and there is a trend to substitute them with naturally occurring antioxidant.[5] The bioactive compounds together with the high antioxidant activities obtained in some species and the nutrient content in all species may be responsible for their nutritional and therapeutic uses. These results therefore not only make these wild edible mushrooms popular to consume as good food source but may also be valuable in drug development.[6]

MATERIALS AND METHODS

Sample collection

Fresh mushrooms were brought from the supermarket and transferred into the sterile containers aseptically and stored in refrigerator to protect from contamination.

Sample preparation

The collected mushrooms were divided into two parts.

One part of raw mushroom were dried at 60ºC for 48 hours and powdered and another part of the mushroom were boiled for 15 minutes and dried at 60ºC for 48 hours and powdered. One

gram of both dried mushroom powders were dissolved in separate 20 ml of 95% ethanol and kept at 5ºC over night for extraction and the centrifuged, supernatants were stored at 18ºC. This stock solution were used for further investigations.

Antimicrobial activity test

The antimicrobial activities of the extracts were carried out for 24 hours culture of selected organism. The bacteria and fungi used were organisms. *Salmonella typhi, Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, pseudomonas aeroginosa, Aspergillus niger, Aspergillus flavus, Pencillium species.* Muller Hinton Agar media was used for the anti-microbial activity. Agar well diffusion methods was used and 50µl of mushroom extracts were used for testing the activity. After 24 hours of incubation, each plate was examined and zone of inhibition was measured.

Anti-oxidative activity in oil-in water emulsion

1ml of mushroom extract (1gm dried mushroom powder 10ml methanol) and beta hydroxyl acid (BHA) solution (0.02% ethanol) was taken as standard . 3ml of the oil in water emulsion was added to solutions. The mixed emulsion was placed in an 8ml vial with a Teflon cap; oxidation was carried out at 60ºC and the mixture continuously shaken at 250 rpm in an incubator shaken.

The oxidation of oil was monitored by the ferric thiocyanate method in a glass tube containing 7% ethanol (2.3ml) and 30% ammonium thiocyanate (50µl), 50 µl of the reaction mixture. After 3 minutes the absorbance of the colored solution containing ferric thiocyanate complex was measured at 500nm in UV-VIS Spectrophotometer.

Total antioxidant activity

The total antioxidant activity of the sample was evaluated by the method.[7] An aliquot of each sample (0.05ml) was mixed with 0.5 ml of reagent (0.6m sulphuric acid,28 mm sodium phosphate and 4mm ammonium molybdate) in 1.5 ml effendorf tube was taken. The tubes were capped and boiled in a boiling bath at 95ºC for 90 minutes and cooled. The absorbance of each sample was measured at 695 nm against blank in a spectrophotometer.

Assay of peroxidase

Peroxidase activity was assayed according to the method described by.[8] The enzyme reaction was initiated by the addition of hydrogen peroxide. The increase in the optical density at 420nm was for 5 minutes at 1 minute intervals in a UV-visible spectrophotometer.

Assay of ascorbate oxidase

Ascorbate oxidase activity was assayed according to the method of.[9] To 3ml of ascorbate solution (18.8mg ascorbic acid was 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 was measured at an interval of 30 second of a period of 5 minute one enzyme unit is equivalent to 0.01OD.

Assay of catalase

The catalase activity was assayed by the trimetric method based on the procedure described by.[10] 2.5 ml of 0.9% hydrogen peroxide in the same buffer were taken and 0.5 ml enzyme extract was added and incubated at 28ºC for 3 minutes. Unit of catalase activity was expressed as ml of $KMNO_4$ equivalent of hydrogen peroxide decomposed per min per mg protein.

Enzyme assays

The filtrates of fungus were assayed for amylase assays using the modified dinitro salicylic acid (DNSA) reagent method of.[11] The amount of reducing sugar that was released was determined by adding 1ml of DNSA to 1ml of filtrate solution and the absorbance was read at 540nm using a spectrophotometer.

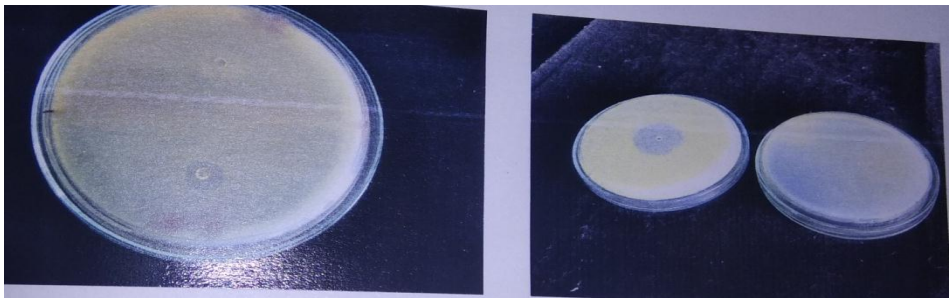
Cellulose activity in the filtrate was determined by the assay medium contained 0.55% Carboxymethyl cellulose (CMC) in 0.55M acetate buffer (pH 6.8) and the reducing sugars, released were measured by the DNSA reagent method of.[12]

RESULT

The antimicrobial activity for the extracts prepared was carried out using the following bacteria and fungi like *Salmonella typhi, Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Aspergillus niger, Aspergillus flavus, Pencillium* species, *Mucor* species by agar well diffusion method was observed. The antioxidant activity was done by oil in water emulsion and results were observed peroxidase, catalase, ascorbate oxidase activity was also observed for the extracts. The total antioxidant activity of the mushroom extract sample was evaluated by the method was observed.[7] The enzyme assay were performed for testing the amylase, cellulase reducing sugars[13] by using starch yeast extract broth and the observed results were noted as figures, graphs and tables.

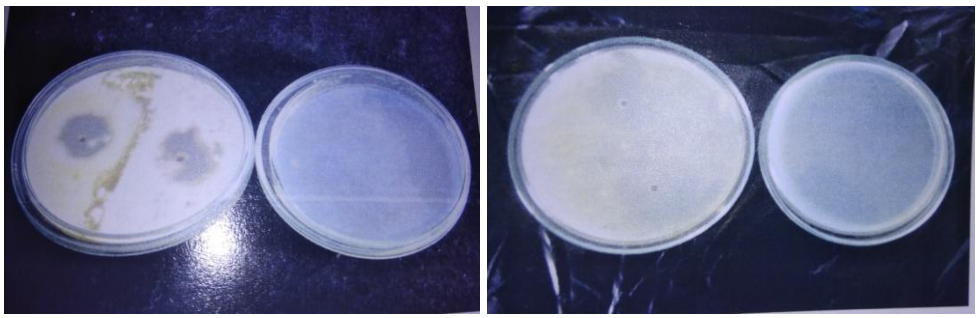
FIGURES

Pseudomonas aeroginosa *Staphylococcus aureus*



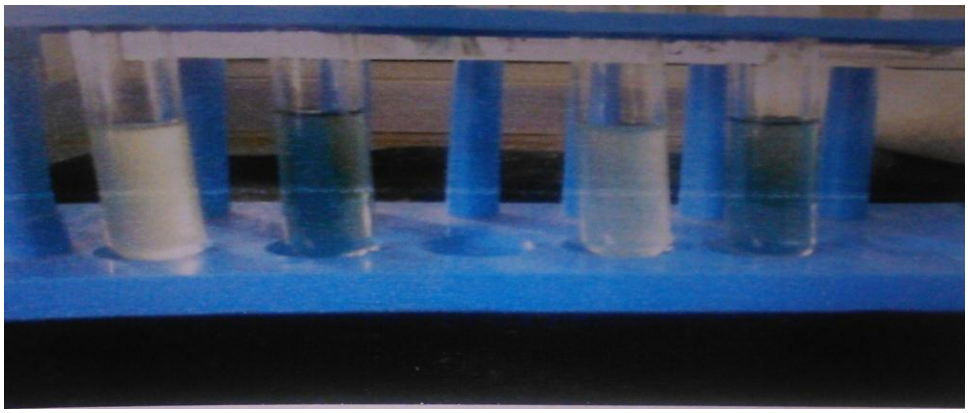
Escherichia coli *Salmonella typhi*

Fig 1: Anti bacterial activity of boiled and unboiled mushroom extracts.



Aspergillus niger *Aspergillus flavus*

Fig 2: Anti fungal activity of mushroom extracts.



Boiled **Unboiled**

Fig -3: Total antioxidant activity of mushroom extracts.

Table-1: Antibacterial Activity of Boiled and Unboiled Mushroom Extracts.

S.No	Organisms	Zone diameter(mm)	
		Boiled	Unboiled
1.	*Staphylococcus aureus*	30mm	32mm
2.	*Pseudomonas aeroginosa*	33mm	No zone
3	*Salmonella typhi*	28mm	30mm
4.	*Klebsiella pneumonia*	No zone	16mm

Table-2: Antifungal activity of Boiled and Unboiled Mushroom Extracts.

S.No	Organisms	Zone diameter(mm)	
		Boiled	Unboiled
1.	***Aspergillus niger***	**32mm**	**16mm**
2.	***Aspergillus flavus***	**30mm**	**15mm**

Table-3: Total antioxidant activity.

Mushroom Extract	Antioxidant activity		Unit of peroxidase activity			Unit of ascorbate oxidase activity		
	Boiled	Unboiled	Time in seconds	Boiled	Unboiled	Time in seconds	Boiled	Unboiled
Agaricus bisporus	**0.061**	**0.853**	**30** **60** **90** **120** **150**	**1.651** **1.664** **1.585** **1.516** **1.464**	**2.021** **2.029** **2.326** **2.340** **2.041**	**10** **30** **50** **70** **90** **120** **140** **160** **180** **200**	**0.516** **2.720** **2.723** **2.733** **2.742** **2.746** **2.755** **2.760** **2.763** **2.770**	**3.000** **3.000** **3.000** **3.000** **3.000** **3.000** **3.000** **3.000** **3.000** **3.000**

Table-4.

Time in seconds	Unit of catalase activity	
	Boiled extract	Unboiled extract
10	3.9	5.6
30	4.6	5.9
60	5.5	6.1
90	6.1	6.3
120	6.5	6.7
240	6.7	7.1
360	6.9	7.4

DISCUSSION

The extracts were prepared from the boiled and unboiled mushrooms using ethanol as a solvent. Antibacterial and antifungal activity of the extract was observed in the Muller hinton agar for the zone of inhibition. Antioxidant and free radicals scavenging activities of edible

mushrooms are isolated and the antioxidant activity of oil in water emulsion method was observed. The total antioxidant activity are boiled and unboiled extracts were observed.[14] Comparatively our extracts showed good results. The enzyme assays are used amylase and cellulose activity is positive. The *Agaricus bisporus* mycelium growth compared with the normal fungal growth in control flask of starch yeast extract broth was observed.[15]

CONCLUSION

The study of mushrooms showed strong inhibitory activity in both bacteria and fungi. Also the inhibitory activity of mushroom extracts in bacteria is effective than fungi. The antioxidant activity and enzymatic activity of mushroom extracts showed good result. From this study we suggest that the mushroom extracts contain active ingredients which can be used for medicinal purpose.

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