International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.5, pp 991-995, 2015

Ultrasonic-Assisted Extraction and Antibacterial Activites of Protein Recovred from White Button Mushroom (*Agaricus bisporus*)

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Abstract: This study is aimed at the use of Ultrasonic assisted method to enhance the yield of bioactive components in which protein which is our area of concentration is included .The mechanical effect of ultrasound is believed to accelerate the extraction of components from Mushroom due to disruption of cell wall and enhance mass transfer of cell content. Mushrooms were collected, powdered and divided into four portions, one portion was subjected to cold extraction by making into mixture with Phosphate buffer saline and incubated. The other three portions were also made into mixture with buffer and ultrasonicated for 10min 20mins and 30mins respectively, the four samples were centrifuged to precipitated out protein, the precipitated proteins were separated out by salting out method and crude protein extract was collected and partially purified by dialysis method. The concentration of protein present were estimated and was found to be 142 µg/ml , 228µg/ml,360µg/ml,356µg/ml for conventional,10mins,20mins,and 30mins respectively. Protein was further purified by Biogel P30 column chromatography and characterized by SDS-PAGE. In this the mushroom subjected to 20mins Ultrasonication was found to have higher efficiency. The mixture obtained was used for anti-microbial study which was found effective against gram positive and gram negative bacteria like *Staphylococcus aureus-5021, Bacillus substillis-2717,Psedomonas aeruginosa-2492, Klebsiella pneumonia-2957, Escherichia coli*-2810.

Key Words : White button mushroom, protein extraction, Ultrasonic assisted extraction.

1. Introduction

In recent years, application of chemically synthesized antibiotics instead of conventional ones has got serious attention due to anti-microbial resistance which is now a major concern on physicians, patients, pharmaceutical producers and public some examples of micro-organism are *Escherichia coli* and *Klebsiella species* which produce beta-lactamase and present resistance to third generation cephalosporin.

Mushrooms are considered as one of the curiosities of nature and are widely consumed for their own flavour throughout the world and the use of mushroom as food is probably as old as civilization. Mushrooms are superior nutritional supplement with magnificent medicinal values and have been evaluated for their nutritional status and medicinal values on the basis of their chemical composition.(1) They have several biological activities like antiproliferative, immunomodulatory, antiviral, antifungal and anti bacterial effects etc.(2-4).

The medicinal bioactive compounds present in mushroom include: polysaccharides, proteins, peptides, glycoprotein, nucleosides, triterpenoids, lectins, lipids and their derivatives. These active constituents can be extracted by conventional method of extraction but have limitations like-time consumption, requires large amounts of solvent, incomplete extraction and hardly automated processes.However various novel techniques which include ultrasonic assisted extraction, microwave assisted extraction etc. have been developed to overcome limitations of conventional methods by shortening extraction times that decrease solvent consumption, increase yield and enhances the quality of extracts.(5-12)

The aim of current research is to discover the use of ultrasonic assisted extraction method to enhance extraction of protein present in mushroom (*Agaricus bisporus*) and to venture future prospects of edible mushrooms for their antimicrobial potentiality.

2. Experimental

2.1. Dried *A.bisporus* preparation

A.bisporus was purchased in a local commercial marker in Chennai, Tamil Nadu, India. All the mushrooms rinsed for 15 min under running tap water and air dried for 48 hours at room temperature, grind with mixer grinder and seal in air-tight plastic bags stored under dry and dark conditions until used.

2.2. Extraction of proteins by cold extraction process

The total soluble protein from fruiting bodies of white button mushroom, *A.bisporus* was extracted using the method as described with modification.(13) Five gram of white button mushroom was mixed in 10mMPhosphate buffered saline(PBS),pH 7.4 containing 150mM of sodium chloride. The suspension was kept at 4^0C shaker incubator for overnight and then centrifuged at 1000xg, 4^0C for 30min. The supernatant was collected as crude protein extract, precipitated by salting out process with ammonium sulphate at 60% saturation and dialysed against same PBS twice to remove off the salt.

2.3 Extraction of protein by Ultrasonic-assisted extraction method

The ultrasonic-assisted extraction of proteins from dried *A.bisporus* was performed using an ultrasonicator (Branson). Five grams of dried A.bisporus powders were extracted with PBS buffer at pH 7.4 in a beaker held in the ultrasonicator at different length of time (10,20,30 minutes)with uniform ultrasonic power in room temperature. After filtration to remove debris fragments, the filtrate was used to precipitate protein by salting out and continuous of dialysis.

2.4 Column Chromatography

Protein extract after dialysis was solubilized in 2ml of PBS buffer, pH 7.4, and loaded on a Biogel P30 column which preciously equilibrated with PBS buffer. The column was washed with the same buffer and elutes were collected in 3ml volumes at the flow rate of 30 mL/hr and monitored by UV spectrometer at 280nm. Protein concentration in the crude as well as purified preparation was estimated by using Bovine Serum Albumin as standard.

2.5 Polyacrylamide gel electrophoresis

Electrophoretic analysis of purified protein was done on 10% denaturating PAGE (SDS-PAGE). Electrophoresis was performed at constant voltage of 100V for 4h in Bangalore Genei Vertical mini Gel apparatus. After electrophoresis the gel was stained with Coomassive Brilliant Blue G 20 and decoloured with decolouring solution. Molecular weights of the protein samples were determined by comparison with the molecular mass marker proteins.

2.6 Assay of Antibacterial Activity

Antibacterial activity of *Agaricus bisporus* protein extract was examined against several species of gram positive and gram negative bacteria by using cup plate method. Late exponential phase of the test bacteria were prepared by inoculating 1% (v/v) of the cultures into the fresh Muller-Hinton broth and incubating on an orbital shaker at 37^0C and 100rpm overnight. Before using the cultures, they were standardized with a final cell

density of approximately 108 cfu ml^{-1}. Muller Hinton agar were prepared and inoculated from the standardized cultures of the test organisms then spread as uniformly as possible throughout the entire media. Agar well was made with sterile borer, test proteins introduced into the well and incubated at 37^0C for 24hrs. Antibacterial activity was evaluated by measuring the diameter of inhibition zone(mm) on the surface of plates and the results were reported as Mean+-SD after three repeats.

3. Results and Discussion

Aim of this study is to compare between conventional method of extraction and Ultrasonic assisted extraction and to find out which give a better yield of Protein from white button mushroom(*Agaricus bisporus)*. Ultrasonic assisted extraction was used to increase the yield of protein extraction from White button Mushroom (*Agaricus bisporus)*. The great extraction efficiency by Ultrasonic treatment its mainly attributed to its mechanical effect which leads to disruption of cell wall, particle size reduction and facilitation of mass transfer between immiscible phase through a super agitation and the most important mechanical effect of Ultrasonic treatment is microstreaming and micro jetting . Ultrasonic assisted extraction method offer high reproducibility at shorter time, simplified manipulation,lower energy input and less solvent consumption.

White button Mushroom (*Agaricus bisporus)* was collected and rinsed under running tap for 15 minutes, it was dried over night at room temperature and kept in a sealed and air tight plastic bags and stored under dry and dark condition for its further use. The stored mushrooms were divided into four parts one of which was subjected to cold extraction and the other three to ultrasonic assisted extraction. For the cold extraction the mushroom was mixed with 100ml phosphate buffered saline of pH7.4, it was kept overnight at 4^0c in shaker incubator after which it was centrifuged at 4500rpm for 20 minutes and supernatant obtained which contains crude protein extract.

To other three portion, each was mixed with 100 ml phosphate buffered saline and ultrasonicated for 10mins 20mins and 30mins respectively, it was filtered to remove the debris and the filtrate contains crude Protein. From the crude sample obtained from the four samples protein was separated out by salting out method, this was done by adding the amount of ammonium sulphate required to produce 60%, the addition was done slowly with simultaneous stirring on a magnetic stirrer which lasted for about 30 minutes after which it was taken for centrifugation at 4500rpm for 20minutes, the pellets were re-suspended in phosphate buffered saline and stored in a refrigerator for further estimations. The stored pellets were subjected to partial purification by dialysis method.The partially purified protein was estimated using Lowry's method, from the estimated protein, the portion subjected to 20 minutes ultrasoniction was found to contain more amount of protein.Furthermore, the partially purified Protein was further purified by Biogel P30 column chromatography which was eluted with phosphate buffered saline, the absorbance of the elutions were seen and 20 minutes ultrasonication was found to have the highest peak.

Table:1 Concentration of proteins after purification by column chromatography

Type	Concentration
Cold extraction	142 µg/ml
10 minutes of ultrasonication	228µg/ml
20 minutes of ultrasonication	360µg/ml
30 minutes of ultrasonication	356µg/ml

From the crude sample characterization of Protein by SDS-PAGE was carried out and the mixture of protein was observed. The effectiveness of the extracted protein was checked by conducting antimicrobial studies on the following gram positive bacteria and gram negative bacteria- *Staphylococcus aureus-5021, Bacillus substillis-2717,Psedomonas aeruginosa-2492, Klebsiella pneumonia-2957.*using Mueller Hinton agar as medium and the extracted protein was found to be effective against gram positive and gram negative Bacteria.

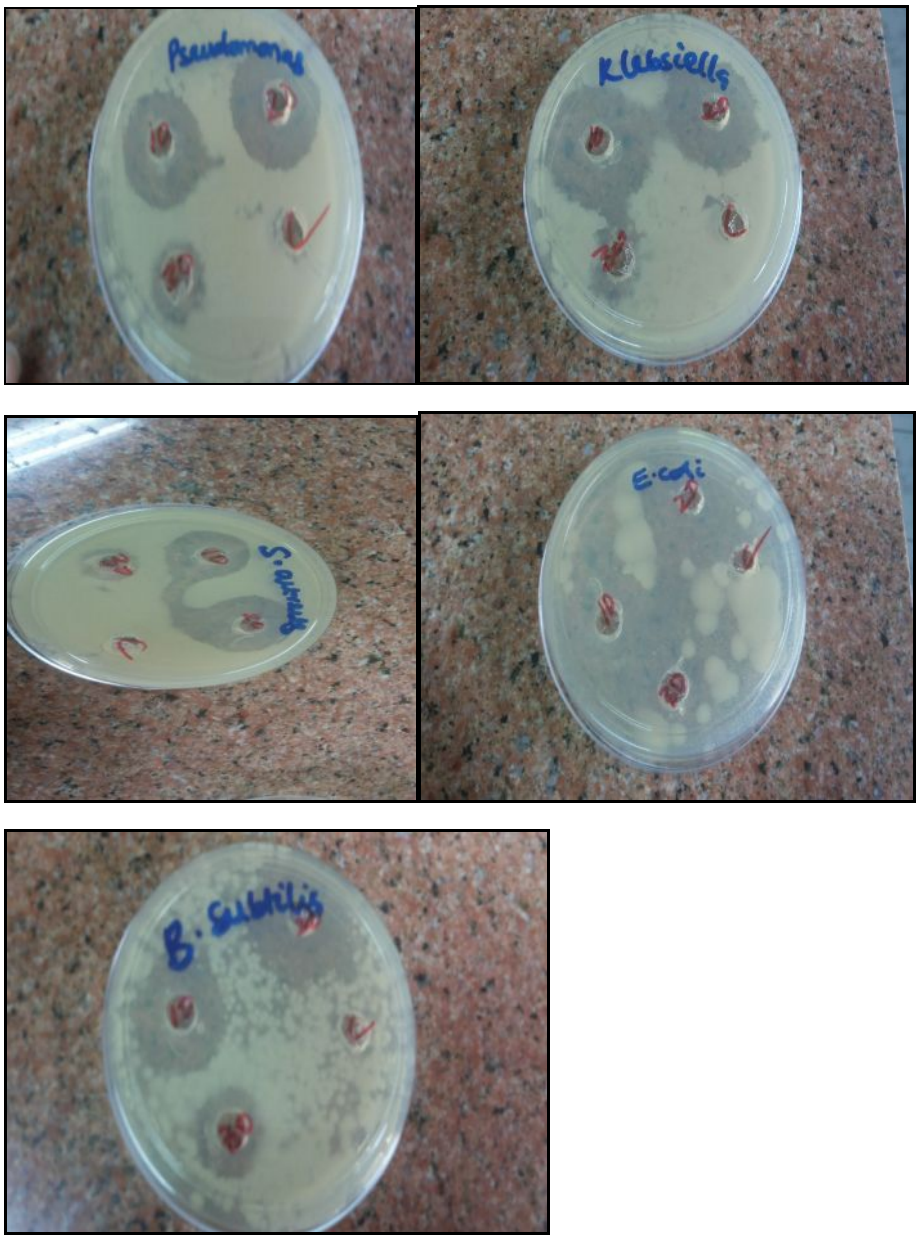

Fig1. Antibacterial effect of protein from A.bisporus against Staphylococcus aureus-5021, Bacillus substillis-2717,Psedomonas aeruginosa-2492, Klebsiella pneumonia-2957

Table 2: Antibacterial activity of the isolated proteins

Sample	Staphylococcus aureus 5021	Bacillus subtilis 2717	Escherichia coli 2810	Pseudomonas aeruginosa 2492	Klebsiella pneumoniae 2957
Cold Extraction	3 mm	2mm	6mm	5mm	6mm
10 Minutes	10mm	10m	18mm	16mm	10mm
20 Minutes	18mm	16mm	19mm	20mm	10mm
30 Minutes	11mm	14mm	16mm	10mm	13mm

The future work of this study presents the structural elucidation of protein components by using,NMR, Mass spectroscopy and Electron spin resonance spectroscopy etc, also the samples can further be purified by High performance liquid chromatography. The yield of protein from white button mushroom(*Agaricus bisporus)* can be increased by altering pH, temperature and ultrasonic power etc, furthermore this parameters can be optimized using response surface methodology.

Acknowledgement

The authors are grateful to SRM College of pharmacy, SRM University to provide facilities to full fill the work.

4. References

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