The Therapeutic Potential of Oyster Mushrooms in Managing Type 2 Diabetes: A Comprehensive Study

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Abstract - The production of inhouse oyster mushroom seeds and their use as a dietary supplement to manage Type 2 Diabetes Mellitus. Oyster mushrooms are known for their rich nutritional content and potential health benefits, including hypoglycemic effects. The study involved the development of a protocol for producing oyster mushroom seeds and flowers, evaluating their physiochemical properties, and assessing their efficacy in lowering blood glucose levels. A three-month study showed a significant reduction in blood glucose levels among diabetic patients consuming the mushroom supplement, suggesting its potential as a dietary intervention. However, further research over a longer period is recommended to fully understand the long-term benefits and mechanisms.

Keywords **-** Oyster Mushroom, Type 2 Diabetes Mellitus, Dietary Supplement, Blood Glucose, Inhouse Production.

INTRODUCTION

The adage "Food is Medicine," attributed to Hippocrates, underscores the historical recognition of diet's role in health. Diabetes, a global epidemic characterized by elevated blood sugar levels, has become a major health concern. While Type 1 diabetes results from insulin deficiency, Type 2 diabetes is primarily due to insulin resistance. The escalating prevalence of both types, including gestational diabetes, poses a significant public health challenge. This study explores the potential of oyster mushrooms as a dietary intervention for Type 2 diabetes management, building upon the ancient wisdom of harnessing food for therapeutic benefits [(1,2)].

METHODS

Prior to initiating mother culture isolation or transfer, media plates for pure culturing were prepared. Petri dishes or small glass jars served as media containers. Two growth media, agar-based and gelatin-based, were used to culture oyster mushroom mycelium. Potato dextrose agar (PDA) was prepared by boiling potato slices in water, followed by straining and combining with a sugar and agar solution. The mixture was sterilized using an autoclave or pressure cooker. After cooling, the sterilized media was poured into Petri dishes or jars under aseptic conditions. Agar plates were sealed to prevent contamination. The mother culture plate was closely monitored for contamination and discarded if necessary [(3)].

SUBCULTURE

Seeds were soaked for approximately twelve hours, avoiding excessive soaking to prevent fermentation and maintain quality. The soaked seeds were then transferred into containers and sterilized using a pressure cooker. Inoculation with a pure mother culture followed under aseptic conditions to produce clean spawn. The inoculated containers were incubated in darkness until complete substrate colonization. The mature spawn was subsequently stored under refrigeration for future use [(4,5)].

SEED CULTURE

The first batch of spawn was utilized as a "master spawn" to increase spawn quantity for subsequent planting. This involved inoculating a second set of sterilized seeds with the initial spawn. The resulting spawn was termed "planting spawn" and used to inoculate straw for oyster mushroom production. The fully colonized beds were transferred to a cropping room for button development. Various methods, including open, closed, and partially covered bed systems, were employed to initiate button formation. The optimal

method involved complete removal of the polythene cover. The beds were maintained under specific temperature and humidity conditions, with regular monitoring for contamination and pests. Following harvesting, the mushrooms were processed into dietary supplements [5].

Dietary Supplements Preparation

Following oyster mushroom harvest, preparations for dietary supplements commenced. The mushrooms were sliced and dehydrated in a hot air oven at 70 degrees Celsius for four hours, resulting in a greyish-white product. This was subsequently ground and sieved to produce a fine powder. For the biscuit formulation, equal proportions of fresh oyster mushroom powder and wheat flour were combined with organic brown sugar and water to form a dough. The dough was shaped into balls, filled with nuts, and baked in an oven at 180 degrees Celsius for six minutes, followed by an additional 13 minutes at 130 degrees Celsius [6,7,].

Physicochemical Tests

Molisch's, Fehling's, iodine, and Biuret tests were conducted. Molisch's test involved adding Molisch's reagent to the sample followed by concentrated sulfuric acid. A purple ring indicated carbohydrate presence. Fehling's test employed Fehling's solution and a water bath, with red precipitate confirming reducing sugars. The iodine test utilized iodine solution to detect starch, indicated by a blue colour. Biuret's test involved adding Biuret reagent to the sample and observing colour changes for protein content [8]. Proximate composition analysis included determination of crude protein, crude fat, crude Fiber, ash, moisture, and energy value. The Kjeldahl method was used for protein analysis, while crude fat was extracted using a Soxhlet apparatus. Ash content was determined by incineration, and moisture content by drying. Crude fibber was obtained through acid and alkali treatment, and energy value was calculated based on macronutrient composition. Extraction of polysaccharides involved the Soxhlet method using ethanol, followed by isolation using column chromatography. Thin-layer chromatography was employed to assess fraction purity [9].

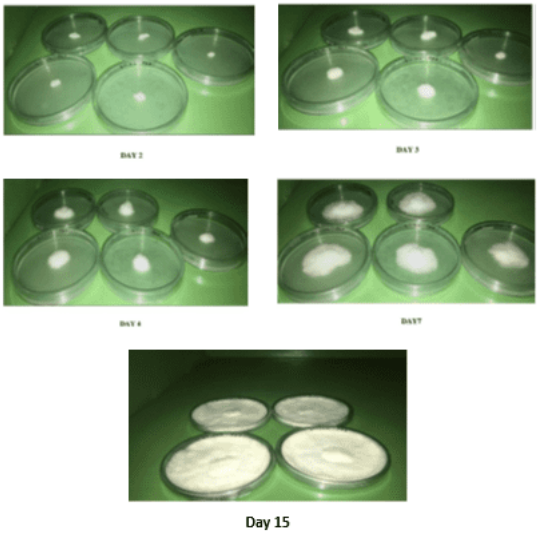
POST MARKETING SURVEILLANCE

The prepared dietary supplement was given to diabetic diagnosed patients who are in the treatment. The continuous follow foe 3 months was done and check the levels of glucose the results reveal there was no synergism on oyster mushroom maintain the blood glucose level neither elevated b=nor decreased during the three months of study [10].

RESULTS AND DISCUSSION

Mother culture preparation

The inoculation of fresh fertile system mushrooms was carried out within an hour of collection. Media was sterilized, and mother culture preparation done under Laminar Air Flow. Incubation occurred in a BOD incubator at 23–25°C until a mother culture was obtained after 15 days. Several attempts were made to optimize sterilization temperature due to contamination concerns with asparagus Niger, eventually resulting in a successful white spawn layer.



Sub Culture

Seeds should be soaked for about twelve hours to avoid seed fermentation and maintain quality for spawn preparation. Afterwards, transfer the soaked seeds into containers like Mason jars or spawn bags and sterilize them using a pressure cooker for 30 minutes at 15 psi. Follow the pressure cooker manufacturer's guidelines for safety. Optionally, seeds can also be boiled with gypsum for at least 60 minutes to reduce microbes. Sterilize the prepared media, transfer the subculture seed obtained from the laminar airflow unit, and incubate in a BOD incubator for 21 days to obtain seed culture. Repeat the subculture process with increased quantity for better results.

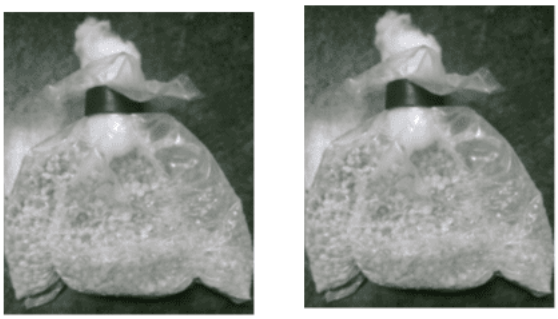


Figure 1: spawn quantity for subsequent planting

Production of Oyster Mushroom

The bag was prepared following specific steps while maintaining 80-85% humidity and 23-25 degrees Celsius temperature. Well-grown mushrooms were cultivated after 21 days. Fully mature spawns mixed with sterilized paddy straws in apatite-sterilized polythene bags yielded white oyster mushrooms after 30 days. Temperature control was achieved with an air-control unit, pest control with organic pesticides, and fumigation with neem oil. Large quantities of oyster mushrooms are produced in-house.

Figure 2: apatite-sterilized polythene bags yielded white oyster mushrooms

Dietary supplements

Powders

The In-house produced Oyster Mushroom were dried at 70 degrees Celsius and powdered. Sieved for uniform particle size and packed.

Figure 3: Powders

Cookies

Oyster mushroom biscuits was prepared using organic ingredients we used for dietary supplements.

Figure 4: Oyster mushroom biscuits

Flower

The flower part of oyster mushroom was packed in tight container was also intended used of oyster mushroom.

Figure 5: Flower part of oyster mushroom

Extraction, isolation and TLC

The extraction was done with the oyster mushroom pieces. Isolation was done in column chromatography. The single spot was spotted furtherly we are going to do HPTLC, NMR studies.

Figure 6: Isolation test

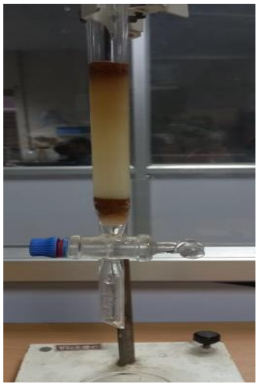


Figure 6: Extraction

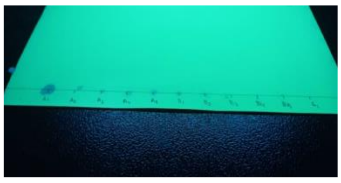


Figure 7: TLC

Crude fat

5g of flour and hexane were added into the Soxhlet apparatus for 16 hours the condensation rate is 2-3 drops/sec. the distillation at 100 degrees Celsius for 30 minutes is dried.

Weight of extract= 0.1334g

Weight of sample = 5g

Fat% = weight of extract / weight of sample x 100

Fat% = 0.1134/5 X 100

Fat % = 2.5%

Moisture content

10g of sample was taken and dried in the hot air oven at 105 degrees Celsius for 5 hours
Weight of taking sample = 10g
Weight of dried sample = 9.2304
Moisture content = weight of taking a sample – the weight of dried sample/weight of original sample X 100
Moisture content = 10 – 9.2304/10 X 100
Moisture content = 7.696%

Ash content

3g sample was taken in the crucible and we put it into the muffle furnace at 550 degrees Celsius for 4 hours
Weight to be taken = 3g
Weight of the residue = 0.2276
Ash content = weight of the residue / weight of the sample X 100
Ash content = 0.2276/3 X 100

Crude fibre

The amount of crude fibre in each sample was determined using standard procedures of AOAC described by Nielsen. About 2 g of each sample was added to a mixture of 200 ml of 1.25% H2SO4 and 0.31 N NaOH, boiled for 30 minutes, and washed with ethanol and petroleum ether twice. The residues obtained were then placed in clean, dry weighed crucibles and dried overnight at 100° C inside the moisture extraction oven. Thereafter, the crucibles were heated in a muffle furnace at 600° C for 6 hours, cooled, and weighed again.
Weight of residue after drying (with crucible) = 60.2535
Weight on ignition (with crucible) = 60.8725 Weight of the sample = 2g
Crude fibre = **weight of the residue after drying – weight on ignition/ weight of the sample X 100**
Crude fibre = 60.8725 – 6025350/ 2 X 100
Crude fibre = 30.95%

PHYSIOCHEMICAL TEST FOR OYSTER MUSHROOM

Molish test

The sample was taken and a molish reagent was added after it turns purple colour so which confirms the presence of carbohydrates

Fehling's test

The Sample was taken and Fehling's solution was added after it turns red precipitate so which confirms the presence of carbohydrates

Iodine test

The sample was taken iodine solution was added after it turns blue colour solution which confirms the presence of starch.

POST MARKETING SURVILANCE

Further studies with a larger patient population are recommended to confirm these findings and to establish standardized guidelines for the use of Oyster Mushroom powder in diabetes management. Regular monitoring and consultation with healthcare professionals are also advised for optimal diabetes management.

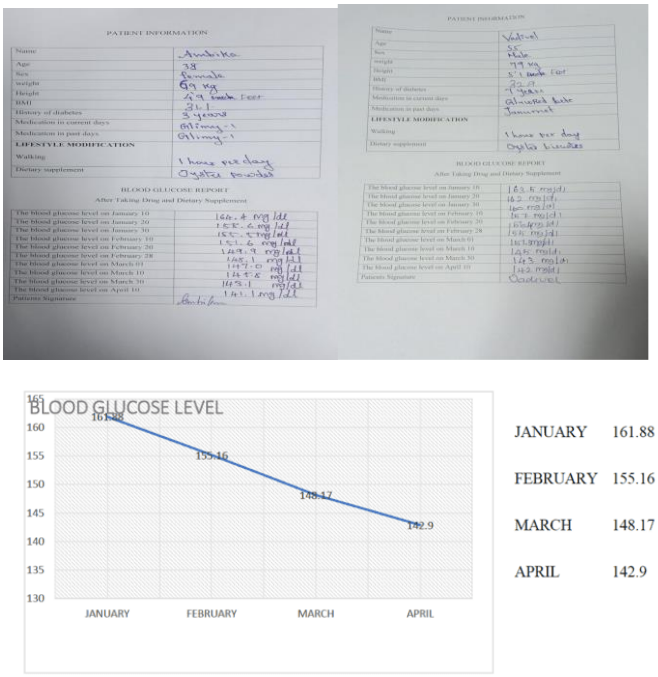


Figure 7: The above-mentioned data shows decrease the glucose level in blood. So, Oyster Mushroom manage the blood glucose level.

CONCLUSION

The In-house oyster mushroom seed and flower were developed and it manage the blood glucose level in human body. 3-month study was not enough so we are going to extend to 2-3 years.

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