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Pharmaceutical Evaluation and Formulation Development of Mushroom (Agaricus Bisporus) For Anti-Diabetic Activity

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ABSTRACT
The aim of the study is to develop a formulation of Atenolol for the nasal drug delivery system with consideration to parameters require for nasal administration, to get maximum utilization and efficacy of the drug also delivery of drug in case of emergency situation. The use of Atenolol in conventional dosage form possesses several disadvantages like the absorption in GI tract is low, hence low bioavailability, require large amount of dose and has to pass first pass metabolism. This encourages us to formulate the dosage form with Atenolol as a novel drug delivery whih will show maximum potential utilization of the Atenolol. The in vitro studies of microspheres were performed. The scattering electron microscopy (SEM), study was also performed. The SEM demonstrated spherical particle with rough surface. The mean partical force size increased with the increase in polymer concentration. The adhesive
of microspheres was equivalent to that of Eudragit RL 100. The results indicate that Atenolol solvent evaporation microspheres formulated with HPMC is a promising nasal delivery system.
Keywords: *Microspheres, Atenolol, Eudragit RL 100, HPMC, Mucoadhesive.*

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I. INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind.Herbs had been used by all culture throughout history. It was an integral part of the development of modern civilization.

Herbal medicinal products are defined as any medicinal products, exclusively containing one or more active substances. WHO report 80% of the world population relies on the drug from natural origin. A number of traditional herbal medical practices have been adopted for the diagnosis, prevention and treatment of various diseases. The objective of development of herbal formulation is to provide the synergistic, potentiated, agonistic/ antagonistic pharmacological agents with in themself and work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects.

India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. These systems have rightfully existed sideby-side with Allopathy and are not in „the domain of obscurity", as stated by Venkat Subramanian. Millions of Indians use herbal drugs regularly, as spices, home-remedies, health foods as well as over-the-counter (OTC) as self-medication or also as drugs prescribed in the non-allopathic systems. The more than 500,000 non-allopathic practitioners are trained in the medical colleges (>400) of their respective systems of health and are registered with the official councils which monitor professionalism.

Hence, these systems are not folklore or traditional herbal practices. There are basic axioms of these systems leading to a logical and systematic structure of pathogenesis and diagnosis, which serves also as a determinant for therapy.

The developer of a potent natural product penicillin, Nobel-laureate Ernst Boris Chain wrote an inspiring article entitled "The quest for new biodynamic substances". In 1967, he wrote, "In China and India there has been an extensive drive aimed at the systemic study of medicinal plants

traditionally used in these countries in folklore medicine; this has failed, so far, to bring to light new classes of compounds with interesting pharmacologic activities. As far as drug research is concerned, therefore, we cannot expect many major surprises to come from the study of plant constituents." The diversity of medical uses of plant is at times daunting for a new entrant to the field. But for a multidisciplinary research and a development network the options of research approach provide deep motivation for identification of new

pharmacophores. Besides expanding the herbal therapeutic and preventive armamentarium, new pharmacophores may help to evolve new targets of drug action as well as a possibility for combinatorial chemistry on the novel pharmacophores. For example, curcumin has been a target molecule for a significant endeavor for a large number of combinatorial compounds. The Council of Scientific and Industrial Research in India has initiated sizeable and meaningful efforts for the development of herbal- based formulations for diabetes, arthritis and hepatitis by a national network programme. The industry, the academia and the government research laboratories work in close collaboration. Interesting and novel activities have been detected with the selected plants and some of the active ingredients of therapeutically demonstrable effects e.g. glycaemic control and inhibition of HbA1c (glycosylated haemoglobin) level coupled with a reduction in in vitro formation of Amadori products. The diverse approaches to herbal drugs have led to interesting hits and novel activities, which need further in depth drug development efforts, both as herbal as well as new single molecule drugs.2 Ayurveda is concerned with healthy living along with curative measures that involve consideration an individual physically, mentally and spiritually. Ayurveda compounded referring a system of knowledge, helps in detoxification and cleansing, boosting the effectiveness of the immune system and helps the body to cope with stress. One of the main strategies in ayurvedic medicine is to increase body‟s natural resistance to the disease.

II. MATERIALS AND METHODS

Mushroom is a saprophytic fungus that grows on dead and decaying organic matter. Due to the absence of chlorophyll, it is unable to synthesize its own food and hence is dependent upon the organic matter/substrate for food. The first record of cultivation of mushroom dates back to the reign of Louis XIV (1637-1715). French scientists were the first to detail record the mushroom cultivation techniques which is valid even now. In the same context, an article was published in Paris in 1707, following that mushrooms were cultivated in the foothills of France in 1800. In these regions horse dung was used (which itself got pasteurized due to high temperatures), as the substrate for spawn inoculation and mushroom production. Subsequetly, this technique spread to neighbouring areas and local inhabitants started mushroom cultivation in cases, mines and other moist areas. In 1810, mushroom cultivation began in specially designed crop rooms which got further cultivation in many parts of the world.

Morphology

Mushrooms can be defined as “a macro-fungus with distinctive fruiting bodies, epigeous or hypogeous, large enough to be seen with naked eyes and picked up by the hands”. The mushroom fruiting body may be umbrella like or of various other shapes, size and colour. Commonly it consists of a cap or pileus and a stalk or stipe but others have additional structures like veil or annulus, a cup or volva. Cap or pileus is the expanded portion of the carpophore (fruit body) which may be thick, fleshy, membranous or corky. On the underside of the pileus, gills are situated. These gills bear spores on their surface and exhibit a change in colour corresponding to that of the spores.

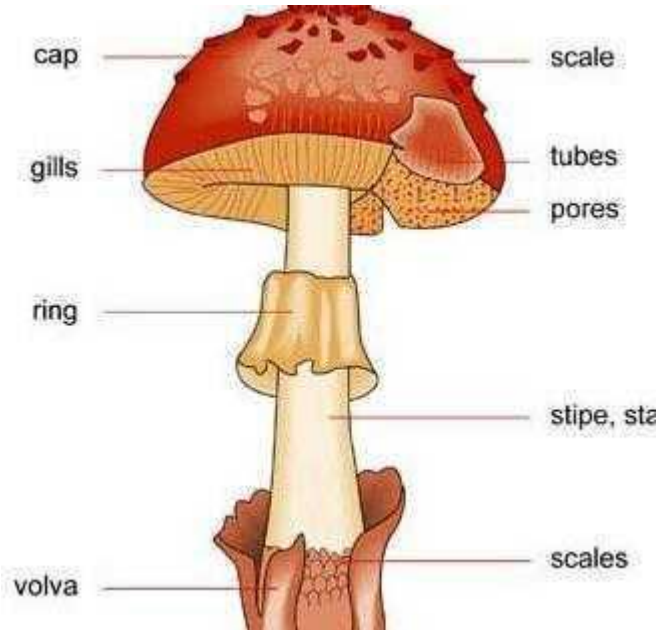


Figure-1

Cultivation process involves four major steps
a. Preparation of compost
b. Spawning of compost
c. Casing (Covering the spawned compost)
d. Cropping and crop management

Preparation of compost
Unlike other traditional crops soil is not the appropriate substrate for mushroom cultivation.
Rather, the substrate for mushroom called compost, is prepared from agro wastes like straw, stem, shoot, apices etc. with organic manure. Mushroom substrate may be simply defined as a lingocellulosic material that supports the growth, development and fruiting of mushroom mycelium.
This compost is pasteurized by various micro-organisms and at appropriate temperature range.

Methodology for compost preparation
Compost is an artificially prepared growth medium from which mushroom is able to derive important nutrients required for growth and fructification. Cemented floors are required for making good quality compost. There are two main methods for compost preparation:

1. Long method of composting
This is an outdoor process and takes around 28 days in its completion with a total of seven turnings.
Before making compost, wheat straw is spread on cemented floor and is turned many times with water being spread at regular intervals.
Day 0: At the stage, there should be around 75% humidity content in the wheat straw, to which wheat bran, calcium ammonium nitrate, urea, murate of potash, and super phosphate are mixed thoroughly and evenly. The material is then piled 1.5m thick x1.25m high with the help of wooden rectangular block. The blocks are removed. Once the entire material has been stacked up or piled up. Water is sprayed twice or thrice to keep the substrate moist. Temperature should be 0 in the range of 70-75 C.

1 turning Day 6: On the sixth day first turning is given to the stack. The purpose of turning is that every portion of the pile should get equal amount of aeration and water. If the turnings are not given, then anaerobic condition may prevail which may lead to the formation of non- selective compost. In the stack, the central zone is fermenting at its peak and has maximum temperature rest of the portion is either not at all fermented or ferments improperly. The correct method of turning is as: Removing about 15cm of compost from the top and spread it on one side of the floor, the rest part of compost on the other side of the floor. Now turning is done by shaking the outer (top most) part and the inner part of the compost, first separately and then missing them altogether thoroughly with the help of wooden buckets.

2 turning (Day 10): On the tenth day, again the top most part and the inner part of the compost is separated, water is sprayed on the top part. Again the two parts are piled up together in such a way that now the top part is inside and the inner part is on the top of the stack.

3 turning (day 13): it is also done in the same way as described earlier. Gypsum and furadan are mixed at this stage.

4 turning (day 16): The same process of turning is followed.

5 turning (day 19): The compost is turned in the same manner and B.H.C. is added.

6 turning (day 22): The same process of turning is followed.

7 turning (day 25): if no ammonia persists in the compost, spawning is done.

2. Short method of composting
Compost prepared by short method composting is superior in production quality and the chances of infection and disease is quite low.
This method is accomplished in two phases:
Phase I- Outdoor composting
Wheat straw mixed with chicken manure is sprayed with water and a 45cm high pile is made on the fourth day and first turning is made. On 7 day, wheat bran, gypsum and urea is mixed thoroughly and piled up to 1.25-1.50

m height with a width ranging from 1.25 -1.5 m. The internal temperature of the compost should be maintained at 70-75 C within 24hr. Second turning is done on this day where as third turning is done on 8 day with subsequent mixing of gypsum. On the 10 day, the compost is transferred to the pasteurization tunnel. Compost is filled in the pasteurization tunnel to a height of 7‟‟. Filling height depends upon the size of the tunnel.

Phase II- Indoor composting

This is the pasteurization procedure which is done in a closed environment. Pasteurization has got many purposes.

i) If the temperature during composting has been low and the compost is heterogeneous, many parasites (nematodes, pathogens, flies and mites etc.) will survive in the compost mass, therefore, pasteurization is the best means with which these parasites can be destroyed.

ii) To end fermentation and to convert compost into a chemical and biologica state favourable to the development of the mycelium and unfavourable to moulds.

iii) Conversion of ammonia into microbial protein. Compost is filled in the pasteurization tunnel and as soon as the compost in the tunnel is completely filled the doors and fresh air damper are properly closed and blower is put on for recirculation of air 150-250 cubic metre/ 1000 kg compost/ hour. The phase II process is completed in three stages:

i) **Pre-peak heat stage:** After about 12-15 hours of compost filling, the temperature of 0 compost starts rising and once 48-50 C is obtained, it should be maintained for 36-40 hours with ventilation system. Normally such temperature is achieved by self generation of heat by the compost mass without steam injection.

ii) **Peak heat stage:** raise the temperature of compost to 57-58 C by self generation of heat from microbial activity if it is not obtained.injecting the live steam in the bulk chamber and maintain for 8 hours in order to ensure effective pasteurization. Fresh air introduced by opening of the fresh air damper to 1/6 or 1/4 of its capacity and air outlet too is opened to the same extent.

iii) **Post- peak heat stage:** lower down the temperature gradually to 48-52 C and maintain till no traces of ammonia are detected in compost. This may take 3-4 days in a balanced formulation. When the compost is free from ammonia, full fresh air is introduced by opening the damper to its maximum capacity and cool down the compost to around 25 C which is considered as the favourable temperature for spawning.

Spawning

The process of mixing of the spawn in the compost is known as spawning. Spawn is thoroughly mixed in the compost at the rate of 600-750 gm per 100 kg of compost (0.6 - 0.75%). The spawned compost is filled in tray or polypropylene bags covered with formalin treated news papers. In case of bags, they should be folded at the top and covered up. After spawning,temperature and humidity of crop room should be maintained at 18-22 C and 85-90%, respectively. Water should be sprayed over the covered news papers, walls and floors of the crop room. After 12-14 days of spawning white mycelial growth is seen running the entire length of the tray/bag. This is then covered with casing soil on the surface.

Casing soil

The significance of casing soil is to maintain the moisture content and exchange of gases within the surface of the compost which helps in the proper growth of the mycelium. The pH of the casing soil should be 7.5-7.8 and must be free from any infection or disease. **Pasteurization of casing**

soil

The casing soil is piled on cemented floor and is treated with 4% formalin solution. Thorough turning of the soil is done and it is covered with polythene sheet for the next 3-4 days.Pasteurization of casing soil at 65 C for 6-8 hours is found to be much more effective.

Using the casing soil

3-4cm thick layer of casing soil is being spread uniformly on the compost when the surface has been covered by white mycelium of the fungus. Formalin solution (0.5%) is then being sprayed. Temperature and humidity of the crop room should be maintained at 14-18 C and 80-85%, respectively. Proper ventilation should be arranged with water being sprayed once or twice a day.

Harvesting of crop

Pin head initiation takes place after 10-12 days of casing and the fruiting bodies of the mushroom can be harvested for around 50-60 days. The crops should be harvested before the gills open as this may decrease its quality and market value.

Productivity

From 100 kg compost prepared by long method of composting 14-18 kg of mushroom can be obtained. Similarly, 18-20 kg mushroom can be obtained from pasteurized compost (Short Method Compost).44

Agaricus bisporus is an edibe basidiomycete mushroom native to grassand in India, Europe & North America. Agaricus bisporus constitute 90% of the total mushroom consumed in the united states,it is one of the most widely cultivated mushroom in the world. The original wild form bore a brownish cap & dark brown gills but more familiar is the current variant with white cap, stalk & flesh & brown gills.42 It is the most important economic & commercial mushroom that is widely cultivated in most coutries of the world. Currently, these are more than 70 varieties of Agaricus bisporus, Major ones include, crimini mushroom, baby partobella, baby bella, mini bella, portabellini, Roman mushroom, Italian mushroom & brown mushroom. While the diversity of gene pool is an important prerequisite for any breeding, the commercial cultivars of Agaricus bisporus are unable to bear a broad genetic diversity, which is mainly due to unusual life cycle of Agaricus bisporus as a secondary homothallic fungus.45 It is specified the most amount of cultivation & consumption among edible mushrooms are harvested 2-3 day period in a 7-10 day cycle called flushes or breaks, Based on existing statistics, The worldwide production of edible mushroom was about 6 million tons in 1997 & increased to 22 millions tons in 2009, from which more than 37% related to edible Agaricus bisporus button mushroom. The stips of mushroom is usually considered waste & is used to feed animals. More than 3500 million tons waste & less valued remains are produced thought the world.46 Agaricus bisporus have been reported to have hypoglycemic effects(reduction of blood glucose levels) & antihyperglycemic effects. It is low calorie foods for diabetic patients since they contain very low amount of fats & cholesterol, low levels of carbohydrates, high content of proteins, vitamins & mineral.

III. RESEARCH ENVISAGED

AIM – Preparation and evaluation studies on FORMULATION DEVELOPMENT OFMUSHROOM (AGARICUS BISPORUS) FOR ANTI-DIABETIC ACTIVITY ALONG WITH PHARMACEUTICAL EVALUATION

Scientific Classification:-

Kingdom:- Fungi
Division:- Basidiomycota
Class :- Agaricomycetes
Order :- Agaricales
Family :- Agaricaceae
Genus :- Agaricus
Species :- A. bisporus

Taxonomic Name

Agaricus bisporus

Synonyms

Psalliota hortensis f. bispora

Common Names :-

Common mushroom
Button mushroom
White mushroom
Portobello mushroom
Table mushroom
Champignon mushroom
Swiss brown mushroom
Crimini mushroom
Brown cap mushroom
Chestnut mushroom

PLAN OF WORK

Collection & authentication of sample materials.

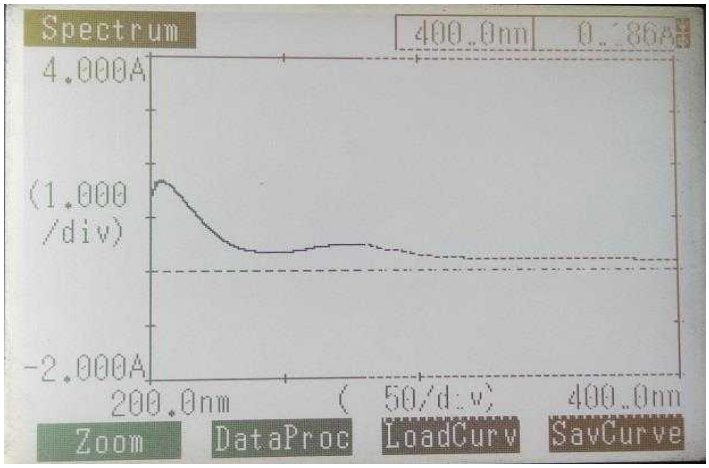
Drying & size reduction of sample.

- Successive extraction with methanol.
- Preliminary Phytochemical screening.
- Development of different type of herbal formulation dosage form viz.
- Conventional tablet
- Suspension
- Evaluation & stability studies of above dosage form.
- Establishment of pharmacological activity (Antidiabetic activity).

Analytical Methods Used

Scanning & Development of Maximum wave length (λmax)-

In order to ascertain the wavelength of maximum absorption of the extract, solution of the extract in methnol was scanned using spectrophotometer with in the wavelength range of 200- 400nm against black.The resulting spectrum is show in figure-1 and the absorption maximum at 271nm for extract.



(Figure- 2)

Prepration of stock solution:-

Standard stock solution of extract was prepared by dissolving 100 mg of powder of Agaricus in 100 ml methnol in 100 ml volumetric flask & filterd.

Preparation of working standard solution and concentration of standard graph:-

To conctruct Beer‟s law plot for extract, the stock solution was further used to prepare working standard of concentrations ranging from 2 to 10µg/ml different aliquots of working standard solution of extract was transferred separately into a series of 10ml volumetric flask and diluted to 10ml using methnol. The absorbances were measured at λmax 271nm against methnol as blank. The standard graph for extract was plotted by taking concentration of the extract on x-axis & yaxis at 271nm. The resulting graph show in figure-2.

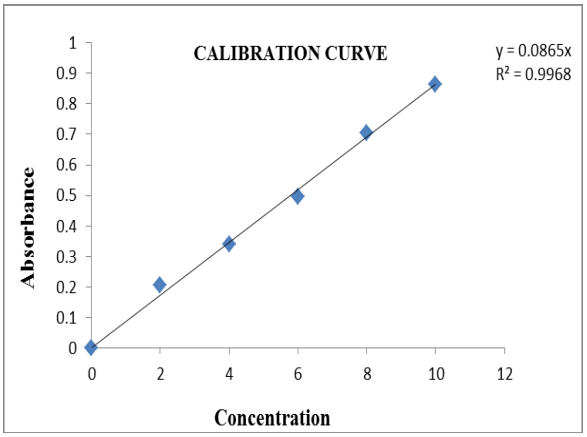


Figure-3

The linear relationship between the concentration of extract and the corresponding absorbance values was shown by- Y= 0.0865x. A positive correlation between the concentration of extract and corresponding absorbance values was observed (correlation coefficient, r=0.996). The amount of extract in all the formulation was calculated using the linear relationship as given above or directoly from the standard graph as showin Fig.3.

Extraction

The preliminary phytochemical screening of the sample involves extraction of the material and identification of the active constituent.

- Agaricus bisporus were collected from local market

 Agaricus bisporus were then subjected to shade drying for 5 days
 After that the Agaricus bisporus were cut into small into small pieces & finally grinded with the help of electronic grinder.

Method for extraction- Continous hot process by using soxhlet apparatus. Following materials are used-
1. Soxhlet apparatus
2. Methnol
3. Distilled water
4. Shade dried coarse powder of Agaricus bisporus.

Dried powder of Agaricus bisporus was taken in conical flask filterd with condenser and methanol was added in the ratio of (1:10). This mixture was heated on waterbath for 4 hours and extract was filtered through Whatman No. filter paper. This extract was cooled at room temperature and then allowed to stand overnight foe methanol to evapotate.29 The resulting phytochemical screening of the sample is show in table-1

Table -1 Phytochemical analysis of Agaricus bisporus Phyto-constituents Biochemical Test Result
Mayer‟s Test+
Wagner‟s Test +
Molisch‟s Test +
Fehling‟s Test -
Bendict‟s Test +
Legal‟s Test +
Keller-Killani Test +
Protien Millon‟s Test+
Flavanoids Alkaline Reagent+
Saponins Forthing Test+
Phenolic Lead acetate Test+
Steroids Salkowski Test+
Triterpenoids Salkowski Test-
Note * (+) Presence & (-) Absence

IV. RESULTS AND DISCUSSION

The phytochemical compositions observed in this study have show the presence of some vital phytochemicals, viz alkaloids, carbohydrate, glycoside, protein flavonoids, saponis, phenolic steroids. Bioactive compounds found in edible mushroom are known to play a vital role in promoting health & good source of some natural anti-diabetic properties. Saponins for instant comprise a large family of structurally related compound containing a steroid. They are reported to have a wide range of pharmaceutical properties, such as Anti-diabetic effects. Thus these sample can be used in the mangment of diabetes or for antidiabetic activities.

It was found that the anti-diabetic activity of Agaricus bisporus formulation in dosage form at dose of 200mg/kg of body weight and its effective on wistar albino rats and also compared with standard drug glibenclamide.It was found that the formulation at the dose of 200mg/kg of body weight in one day after single dose administration was more effective or significant compared to standard drug. we have demonstrated that the dose of Agaricus bisporus mushroom have significant antihyperglycemic activities. It did lower of glucose level in diabetic rats.

V. CONCLUSION

Based on the result obtained from the present study it can be concluded Agaricus bisporus have the anti diabetic activity. The result of preliminary phytochemical analysis are identify the bioactive compound viz alkaloids, carbohydrate, glycoside,protein, flavonoids,saponin, phenolic, steroids and they are reported to have a wide range of pharmaceutical properties, such as antidiabetic effects. Oral herbal dosage forms of Agaricus bisporus like tablet & suspension showed good elegance. the herbal tablets were prepared by direct compression method. Tablets were prepared using Methyl cellulose & Lactose was used as a binder in varying concentration & magnesium stearate as lubricant. Seven batches of the tablets were prepared & micromeritic, properties were determined for all physical mixture of Agaricus bisporus. .The physical properties of all tablets was determined & the results of the Uniformity of weight, Hardness, Friability, Disintigration time was found acceptable. According to the dissolution rate study and physical properties of all formulation the batch 7 are the optimized. Herbal suspension was prepared & stability parameters were evaluated. World health organization guidelines & parameters are now very essential for developing herbal products for various diseases. Moreover pharmaceutical formulation in the form of suspensions many require preservatives, coloring, flavouring agents & other similar

additives.Therefore, the necessity of adding a preservative at the desired level as well as its physical & chemical compatibility with other constituents of the medicinal product must be demonstrated. Sugar free gold(zydus wellness) was selected as a sweetning agent & Tween 80 is polysorbate used as surfactant & also used to increase bioavalibility in oral suspension & due to non- ionic nature it does not change pH of he suspension. Carboxymethyl cellulose improves viscosity & stability of suspension. Lemon oil was used as a flavouring agent in suspension. Sodium benzoate is used as a preservative. Its relatively non- toxic & least harmful preservative. The prepared suspension formulation were found to have redispersibility property with sedimentation studies showed that the sedimentation volume of formulation F3, which indicates that the formulation were optimum & acceptable.All stability parameter are optimum stable & acceptable at variable tempreture. There was no significant change observed in physicochemical & organoleptic behaviour.

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