

Promotion of Ganoderma Lucidum Activity on Different Spices Extract of Herbal Plant for the Quantitative & Qualitative Growth

Rajneesh Kumara Sharma¹, Dr.Dushyant Singh Chauhan², Dr.Dharmesh Gupta³

¹Ph.D. Biotechnology, Department of Advanced Science & Technology, Nims University Rajasthan, Jaipur, India.
 ²Guide, Department of Advanced Science & Technology, Nims University Rajasthan, Jaipur, India.
 ³Co- Guide, Department of Advanced Science & Technology, Nims University Rajasthan, Jaipur, India

Abstract

Mushroom cultivation, Not only help nutritional, medicinal requirement but also fill recycle agro waste. Reishi form the complex lignocellulosic & ligninolytic compounds are broke down due to presence of decomposing enzymes by secreting enzyme in wood degradation and plants derive derivatives. The plant waste used as substrate for the production of medicinal edible fungi its effect positively increases in mushroom yield. By using Reishi enzymes increase the rate of mycelium growth herbal spices extract taken to shows there activity as promoting agent. Growth rate of Ganoderma with the addition of spices extracts were examined.

All the extract shows significant activity for the mycelium growth of Reishi. It shows remarkable effect of lignocellulosic on extract spices examine on cup Plate method showing The zone of Exhibition in triplicates. The spice saunth show higher growth than cinnamon than star anise extract. Show maximum size of zone of exhibition at minimum concentration as compare to normal growth parameter. It is observed that Extract of spices shows could be possible source to obtained new raw substrate to act as good growth promoting nutritive substances or nutritive sprays to increase the qualitative &quantitative growth in Ganoderma Lucidum mushroom.

Keywords

Cup Plate Method, Extraction, Ganoderma Lucidium, Reishi, zone of exhibition.

INTRODUCTION

Reishi mushroom is the fungus that is called Immoratily of mushroom having various characteristics a few are seek and different are to be locate out. This mushroom is life saving having various good properties beneficial for today era.reishi mushroom is grown on trees in jungles the spores are spread ed in forest and grown on woody trees.this fungus miraculously cause damage to trees by decaying their lignin and cellulosic[18] part with the help of the enzymes present. [24][27]The enzymes present are ligninolytic and cellulosic these enzymes form web against the wood and decay slowly destroy the whole plant [34].but is not useful for plants. These properties are used in better way in agrowaste products. With cause many types of pollution.

Reishi is grown artificially on waste materials as substrate in benefical way. [32][33] Various approach are used like liquid state fermentation, soild state fermentation etc. in closed conditions which isn't always harming vegetation waste substances convert into top form.[20][22][23]

The fungi is having numerous health medicinal houses which might be utilized by our forefather s in numerous groups in numerous countries The fungi is having numerous health medicinal houses which might be utilized by our forfather s in numerous groups in numerous countries

Reishi is grown on woody materials like woody trees and degarads or decay the [19] trees by their ligninolytic &[2] lignocelluosic [1][16] enzymes

Activity [10] Reishi is artifically grown by solid state fermentation [30][31] for the production lignolytic enzymes [3].[34]these enzymes are used in various industries like paper pulp etc.

Some enzymes with inside the fungal mycelium motive to interrupt down vitamins and convert them into smaller units .an growth with inside the interest of ligninolytic enzymes an enhancement with inside the price of mycelial growth in substrate [4]

METHOD AND MATERIALS

Extraction

The extraction technique used became successive at room temperature with various solvent .The perfect solvent for extracting technique(17) need to have a few conditions, namely: (i) it need to be capable of dissolve the extractive materials, (ii) it need to have comparable boiling degree with the substance, (iii) it need to be inert (it does now no longer react with materials with a purpose to be extracted, (iv) it need to have low boiling factor for smooth evaporation, The extraction is began out through macerating the pattern for twenty-four hours with solvent. The filtrate became filtered with clear out out paper and evaporated to acquire focused hexane extract. The powder of mushroom which has been extracted with suitable solvents The extract yield may be used as a connection with discover the quantity of simplicial had to make a sure quantity of good extracts.

Extraction the usage of solvents includes numerous



methods, together with maceration, percolation and warmth methods, reflux, soxhletation, infusion, igestion, distillation, steam distillation. Various solvents are used in various methods for extractions.

To estimate the spices herbal activity on Reishi mushroom the method used zone of exhibiton. In this method the media is prepare and plates is formed with the strain . the bore or well are formed . the different concentration of the extracted herbal spices is then pour with micropipettes .the plates are incubated with temp for growth activity then the growth is measured with zone reader or bernier caliper

PROCEDURE

Growth media for reishi fungi

The Reishi culture strain is taken from DMR centre solan (H.P) an form subculture tubes for further testing procedures. strains of basidiomycete fungi The strains were cultured on Media Malt Extract powder and Agar Agar type I in petri plates and grown on test tube slants at 27 °C for 24 to 48 hours. The slants were stored at 4 °C and sub-cultured every 4 weeks.

Preparation of media for Mycelial Cultures :

The medium was prepared by dissolving the specified quantity of the Media Malt Extract powder 2gm and Agar Agar type (I) 2gm medium in Distilled water by heating on a water bath and were dispensed in 100 ml volume conical flasks. The conical flasks were closed with cotton plugs and were sterilized by autoclaving at 121°C (15 lb psig) for 15 minutes.

Malt agar and agar agar is taken in proper proportion for plates preparation, distilled water is used to liquefy the media and mix it heat with hot plate to mix uniformly after this Conical flask is plugged with cotton plug put in the autoclave ad autoclaved for 30 mins at 15 lb pressure. Sterilization of petri plates ,forceps, scissors water in conical is steam sterilized with steam sterilization process. The samples and media is formed to normalized room temperature put in laminar air flow.

The media in temperature is maintained [25][23] Use Petri plates sterilized after autoclave filled with 3-4mm depth with nutritive media after mix with reishi strain ,after pouring mix the media properly without any delay than stay for 2hours until it get solidified, after this take a small a stainle steel borer ant get hole to make wells 1234 adjacent to each other remove outcomes of solidified media for making suitable hole or well for putting given amount of active micro organism concentration for check the growth activity of basic nutrient media containing given compound either act as growth enhancer, exhibitor or inhibitor [26]

For mixing of [36] spices extraction solution to the proper Mix in plate by pour plate method

Preparation of petri plates for Mycelial Cultures growth of the Mushroom:

The petridishes were sterilized at $160-170^{\circ}$ C in hot air oven for 30 mins before use. Small sterile borer of uniform size was placed approximately at 10 cm height, having an internal diameter of approximately 6-8 mm and made of stainless steel. Each plate was divided in to four equal portions along the diameter. To each portion one cylindrical cavity was made in medium with the help of sterile borer. Three cavities for test samples and one cavity for the control. The petri dishes were incubated at $27\pm2^{\circ}$ C for 24 to 48 hours. Diameter of the [25] zone of exhibition was measured and the average diameter for each sample was calculated. The diameter obtained by the test sample was compared with that reishi mycelia.

METHOD

Extraction method for spices :

various methods are used for the extraction of varies components like steam distillation extraction, hydro distillation extraction [13] etc [27] cinnamon spices contain high level of phenolic components[5][6] various methods are described phenolic extraction of dalchini spices[7][8][14]the extraction extracted through hot water distillation, steam distillation. [12] with the help of extraction methods various phytochemicals and antioxidants are derived Used in various [29]concentration and poured with the help of micropipettes and stored under optimum condition for observation .after the inoculation the mycelia of Reishi is showing growth on petri plates and measured properly and observation are recorded. To estimate the spices herbal activity on Ganoderma lucidum or Reishi mushroom the method used zone of exhibiton.by using cup plate or agarwell diffusion method.

DATA ANALYSIS

Collection of Data:-

Table shows the growth of reishi as zone of exhibition (mean and standard deviation) of Reishi on wells with the different concentration versus spices extracts .the plates are used in triplicates. Data is determined as below.

			ZOE(mm)20µ	ZOE(mm)30µ	
sno.	Sample	ZOE(mm)10µ1	1	1	ZOE(mm)40µl
1	control	20.00	21.5	22.00	24.00
2	Star anise	21.50	23.00	24.80	25.80
3	Cinnamon	22.00	24.00	25.40	26.60
4	Saunth	24.00	25.00	26.70	28.40



Analysis of extraction spices and their effect on Ganoderma lucidum



Analysis of extraction spices (20 $\mu l)$ and their effect on Ganoderma lucidum



Analysis of extraction spices (30µl) and their effect on ganoderma lucidum



Analysis of extraction spices (10 $\mu l)$ and their effect on Ganoderma lucidum



Analysis of extraction spices (40µl) and their effect on Ganoderma lucidum



DISCUSSION & RESULTS

The spices used cinnamon .star anise .saunth is having antioxidant properties used on reishi mushroom or lucidum . the lignocellulosic activity Ganoderma on extracted spices examine on cup Plate method showing The zone of Exhibition in triplicates the growth activity of saunth. The spice saunth show higher growth than cinnamon than star anise extract. Show maximum size of zone of exhibition at minimum concentration as compare this indicates the saunth is act as more growth inducer or growth enhancer then cinnamon them star anise. the spices extracts are used as growth promotor ,their sole sprays are increasing the growth mycelia in on substrate and giving good yields. the combination of spices extract sole and reishi fungi fruit in modern era form new nutrient products having both the quality observed and benefited in future . The data observation that the saunth play a very good role in mycelia growth than cinnamon and then star anises these spices agrowaste are used in various proportion with substrate to form good yield of mushroom.

REFERENCES

- Seon-Hwa Lim, Yun-Hae Lee, and Hee-Wan Kang Efficient Recovery of Lignocellulolytic Enzymes of Spent Mushroom Compost from Oyster Mushrooms, Pleurotus spp., and Potential Usein Dye Decolorization Published online 2013 Dec 19. doi: 10.5941/MYCO.2013.41.4.214Mycobiology. 2013 Dec; 41(4): 214–220.
- [2] Production of a cellulolytic enzyme system in mixed-culture solid-state fermentation of soybean hulls supplemented with wheat bran Process Biochemistry Volume 45, Issue 1, 2010, pp. 120-128
- [3] Production of ligninolytic enzymes by solid state fermentation using Pleurotus ostreatus Annals of Agrarian Science, Volume 15, Issue 2, 2017, pp. 273-277
- [4] nr.curvetto,D.figlas,r.devalis at el ,growth and prodctivity of different pleutrotus oestrous strains on sunflower seed hulls supplemented with NNH4 Bioresource Tech 84(2)(2002) 171-176,doi:10.1016/S0960-8524(02)00013-5.
- [5] WangYH,AvulaB,NanayakkaraNPD,ZhaoJ,Khan IA,cassia cinnamon asasource of coumarin in cinnamon flavored food and suppliments in the united states 61: 4470-4476(2013)

[6] Eva Dvorackova, Marie Snobolova, Lucie Chromcova, and Petr hrdlicka Effect of extraction methods on the phenolic compounds contents and antioxidants capacities of cinnamon extracts publisjed onaug 31 2015.

hnoarete

-ISSN: 2583-1968

- [7] PrzygodkaM,ZielinskD,ciesarova at el comparison of methods for evaluation of the antioxidant capacity and phenolic compounds in common pices LWT –food sciences technology 58:321-326(2014)
- [8] Nandan SS Prakesh DVS,vangalapati M, optimization of physico-chemical parameters for the extraction of phenolic components from cinnamon spices J.Acad.Indus Res 1: 183-185 (2012)
- [9] Sasidhara R. and T. Thirunalasundari Lignolytic and lignocellulosic enzymes of Ganoderma lucidum in liquid medium Department of Industrial Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India Euro. J. Exp. Bio., 2014, 4(2):375-379 Pelagia Research Library
- [10] Fan, L., Pandey, A., Mohan, R., Soccol, C.R., 2000. Use of various coffee industry residues for the cultivation of Pleurotus ostreatus in solid state fermentation. Acta Biotechnolica 20(1), 41-52. DOI: 10.1002/abio.370200108.
- [11] Tushar Dhanani,Sonal Shah NA Gajbhiye ,Satyanshu Kumar Effect of extraction methods on yield phytochemical constituents and antioxidants activity of Withania somnifera, 5 mach 2013
- [12] Spingo,G De,FDM,2009 Microwave assited extraction of tea phenols a phenomenon study J Food Eng 93 210,217
- [13] Farnsworth NR. Biological approaches to the screening and evaluation of natural products. In: Rasoanaivo P, Ratsimamanga-Urverg S(Eds) Biological Evaluation of Plants with Reference to the Malagasy Flora, Madagascar, 1993; pp. 35-43.
- [14] Laufenberg, G., Kunz, B., Nystroem, M., 2003. Transformation of vegetable waste into value added products:
 (A) the upgrading concept; (B) practical implementations. Bioresource Technology 87, 167-198.
- [15] (Zhang, 2008).Zhang, Y.H.P., 2008. Reviving the Carbohydrate economy via multi-product lignocellulose bioefineries. Journal of Industrial Microbiology and Biotechnology 35, 367-375.
- [16] Reddy KS, Reddy CS, Ganapaty S. Psychopharmacological Studies of Hydro Alcoholic Extractof Whole Plant of Marsilea quadrifolia. Journal of Scientific Research. 2012; 4 (1), 279-285.
- [17] Zhang, Y.H.P., 2008. Reviving the Carbohydrate economy via multi-product lignocellulose bioefineries. Journal of Industrial Microbiology and Biotechnology 35, 367-375.
- [18] Laufenberg, G., Kunz, B., Nystroem, M., 2003. Transformation of vegetable waste into value added products:
 (A) the upgrading concept; (B) practical implementations. Bioresource Technology 87, 167-198.
- [19] Fan, L., Pandey, A., Mohan, R., Soccol, C.R., 2000. Use of various coffee industry residues for the cultivation of Pleurotus ostreatus in solid state fermentation. Acta Biotechnolica 20(1), 41-52. DOI: 10.1002/abio.370200108.

- [20] Dubey, S.C., 1999. Effect of different substrates and amendments on yield of Pleurotus species. Journal of Mycology and Plant Pathology 29(2), 209-213.
- [21] Croan, S.C., 2004. Conversion of conifer wastes into edible and medicinal mushrooms. Forest Product Journal 54, 68-76. Delmas, J., Mamoun, M., 1983. Pleurotus cornucopiae, a mushroom which can now be grown in France. Ravue Horticole 240, 39-46.
- [22] Balazs, S., Szabo I., 1979. Temperature requirement studies of some new mushroom species in culture. Mushroom Science 10(2), 421-427.
- [23] Roberts E. C., Snell E. E. 1946; An improved medium for microbiological assay with Lactobacillus casei. J. biol. Chem 163:499
- [24] Cuthbertson W. F. J., Lloyd Joan T., Pegler H. F. 1951; The assay of vitamin B12. Part III. Microbiological estimation with Lactobacillus lactis Domer by the plate method. Analyst 76:133
- [25] Bacharach A. L., Cuthbertson W. F. J. 1948; The cup-plate method in microbiological assay with special reference to riboflavine and aneurine. Analyst 73:334
- [26] Fungal biodegradation and enzymatic modification of lignin Mehdi Dashtban,Heidi Schraft, Tarannum A. Syed, and Wensheng Qin
- [27] Detection of Extracellular Enzyme Activities in Ganoderma neo-japonicum Woo-Sik Jo, Ha-Na Park, Doo-Hyun Cho, Young-Bok Yoo, and Seung-Chun Park
- [28] Lignolytic and lignocellulosic enzymes of Ganoderma lucidum in liquid medium Sasidhara R. and T. Thirunalasundari Department of Industrial Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India
- [29] Kim S, Dale BE. Global potential bioethanol production from wasted crops and crop residues. Biomass and Bioenergy. 2004;26:361–375.
- [30] Sanchez C. Lignocellulosic residues: biodegradation and bioconversion by fungi. Biotechnology Adv.2009;27:185–194.
- [31] Fackler K, Gradinger C, Hinterstoisser B, Mess-ner K, Schwanninger M. Lignin degradation by white rot fungi on spruce wood shavings during short-time solid-state fermentations monitored by near infrared spectroscopy. Enzyme and Microbial Technology. 2006;39:1476–1483.
- [32] Wesenberg D, Kyriakides I, Agathos SN. White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnol Adv. 2003;22:161–187.
- [33] Ahlawat, O.P., et al., Profile of the extracellular lignocellulolytic enzymes activities as a tool to select the promising strains of Volvariella volvacea (Bull. ex Fr.) sing. Indian Journal of Microbiology, 2008. 48(3): p. 389-396
- [34] Pointing, S.B., Qualitative methods for the determination of lignocellulolytic enzyme production by tropical fungi. Fungal Diversity 1999. 2: p. 17-33.
- [35] O Sagic AG karahan, M Ozcan and G o zkans effect of some spicesextracts on bacterial inhibition.