IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 5 Ver. II (Sep - Oct. 2015), PP 44-49
www.iosrjournals.org

Studies on Medicinally Important Ingredients of Dark Mushroom

Dr. C.K. Tiwari, Neelima Shukla,Aviral Asaiya
Department of Plant Pathology,Tropical Forest Research Institute,Gaur Tiraha,Jabalpur(M.P.),India

Abstract: *Ganoderma lucidum is a medicinal mushroom,has a long history of use for promoting health and longevity in China ,Japan and other asian countries.It is a large,dark mushroom with a glossy exterior and and a woody texture.G.lucidum is unique in that its pharmaceutical rather than nutritional value is paramount. G.lucidum products are available in various forms,such as powders ,dietary supplements and tea. G.lucidum produces a combination of enzymes which have medicinal properties.In this study, four types of G.lucidum strains were taken to produce Cellulase ,Endoglucanase Cx and Exoglucanase C1.This study also describes the quantitative and qualitative estimation of these enzymes .*
Key Words: *Ganoderma lucidum,Endoglucanase Cx ,□-1,4 Exoglucanase C1 enzyme.*

I. Introduction

Ganoderma lucidum (leyss) karst are important plant pathogen,causing serious root rot mortality in various tree species in reforested slants not cleared off residual roots and stumps. It belongs to the general category of wood decaying fungi which are well known for their role in destruction of trees and bring about ecological balance in the environment through carbon cycling.

The *G.lucidum* complex known in Chineese "Ling Zhi" and Reishi in Japan the king of herbs,Royal Gano,Chga (in Russia).has long been considered a symbol of good future and prosperity and is the subject of many myths as well as being medicinal herbs in ancient China (Zhao and Zhang 1994).It is widely used as traditional medicine in the orient for more than 2000 years.

In ancient time *G.lucidum* as medicine was considered so auspicious that its medical efficacy has been attested to in the oldest Chinese medical text (presumed to beover 2000 years old). The book which is known in Japan as "Shannon Honskyo" is now accepted as being the original text book of oriental medical science.

Ganoderma has been treated as a herbal medicine first in China and then to be countries of South east Asia which is much credited to the record of Ganoderma and the related application specification in "Sang Song Herbal Classic" compendium of "Materica Medica" in 365 kind of medicine, another 120 are classified as "average" medicine and the remaining 125 are placed in the "fair" category.

The species of *Ganoderma lucidum* is world wide in distribution.It is widely distributed in plain as well as in the hills of India it is a serious root rot pathogenic in nature on many tree species (Bakshi,1976) The fungus cause typical structural root rot and does not attack the heart wood. The fungus posses considerable power of saprophytic survival in which case ,they persists in tree stumps and buried roots that they infect during the parasitic phase. *Ganoderma lucidum* enjoys a wide host range among the conifer and broad leaves tree species.It was noticed that it has been reported on 114 tree species (Tiwari etal.2005).

II. Materials and Methods

(A)COLLECTION OF Ganoderma lucidum

Fruit bodies (sporophore) were collected from Jabalpur and its suburbs on Cassia semia(819),Jacranda montaliis(925),Leucaena leucocephala(924) and Peltophorum pterocartpum(923).When collection of sample it is convenient to place each specimen in a dry paper noting the host on bag. The collection should be sorted out and each specimen given a number. The number, locality date and colour should be written in some sort of diary. (Ryvarden 1980).

(B) DRYING

Small polypores may just be left in or on the paper bag on a windowsill or other place with good ventilation. Larger specimen must be dried more effectively and it is convenient to either place them in a good drier or cut them into radial slices 1-3 cm. thick. (Ryvarden 1980).(C)

Disinfection

Many Polypores, especially of genera Tramates pynopores and Ganoderma are susceptible to infection from insects. There are many methods of disinfection; the simplest is to place one or two methods in each sample (Ryvarden, 1980).

(D)STORING:

Plastic bags with a sealing at top is most effective these bags are very protective and in long run, it may be worth while to use them for specimen from most susceptible genera like Trametes,Pycnopores ,Ganoderma.(Ryvarden,1980).

CULTURE TECHNIQUE OF *G. lucidum*

1. Preparation of sterile conditions: The culture transfers were done in laminar flow clean bench. The plate form was cleaned with a cotton swab moistened in rectified spirit,The U.V. light was put on 15 min. before working in the laminar flow.
2. The PDA medium was poured in sterilized culture tube for making slants.
3 .On solidifying PDA slants were inoculated from the culture of G. lucidum & incubated at 22C After 3-4 days growth of the fungus was observed in the test tubes.
4.Culture characters were studied.

Inoculation Studies:

After 3-4 days, growth of inoculating *G. lucidum* in Petri plates was observed and the following cultural characters were observed.

Cultural characters

1. Growth was observed to be slow (3.4mm./24 hr.)
2. Advancing zone was white to hyaline and evenly apprised.
3. Mat was white to light cream to light yellowish brown exept in crustose area where it was wood brown.
4. Mycelium was cottony wooly below the advancing zone (1st week) gradually changing to farinaceous felty. Flocose felty areas also develop.
5. Crustose layers develop at 1st in small areas (after 2nd week) with with prominent wrinkeles which gradually increase in areas and almost cover the whole mat.(6 th week).
6. Reverse cinnamon to brownish.
7. Odour absent.

Hyphal characters :

Advancing zone :

Hyphae hyline, thin walled & branched with frequent clamp connection 1.5 –3.0 um diameter. Aerial mycelium :

1. Hyphae as in advancing zone,thin to slightly thick walled.
2. Skeletal hyphae, hyaline, slightly thick walled, terminal to intercalary, ellipsoid 8.0-12.0x 6.0um.

Submerged mycelium

With thin walled hyphae & chlamydospore as in aerial mycelium observed. G. lucidum is characterized by its prominent colored crustose areas with wrinkle and presence of chlamydospores, cuticular cells & stag horn hyphae.

BIODEGRADATION OF CELLULOSE

Four Strains of G. lucidum were collected from different tree species from different places. Strains of G. lucidum collected from different host trees were isolated by tissue culture method on Potato Dextrose Agar media and Malt extract & yeast extract agar medium (Bisht & Harsh 1981b) and linear growth of of the test fungus was measyred individually every 24 hr. (in mm.) Garrett (1966, 1970) The ability of different strains of this fungus to utilizes insoluble form of cellulose was investigated by the method of Garrett (1962). Modified by Fergus (1969). & Forbes & Dickinson (1977). Whatman no. 1 filter paper were oven dried at

50.c for 48 hrs. & placed in 125 ml. Conical flask with 25 ml .of Forbes & Dickinson media for 15 min at120.C at 15 lbs pressure.

The flask were sterilized & each flask was inoculated centrally with mycelium disc of uniform size from the growing margin of 7 days old culture of G.lucidum. test fungus in at the 25+2.CPDA medium control flask were prepared by inoculation blank piece of same medium.

The flask were incubated for 4 weeks at the end of incubation period filter paper with growing mycelium .(The mycelium & filter paper not washed.) were removed oven dried & reweighed . This wt.(After substracting the wt. of control).was compared with the initial wt. of filter paper.The final pH of medium was also measured.

Cellulolysis Adequacy Index for each isolate of test fungus was calculated from the wt. of cellulose respiration by the fungus growing on filter paper (%wt.loss) of its linear growth rate in mm./ 24 hrs.

Production of Glucanase

The screening for production by wood decaying fungi has been carried out with 4 white rot fungal strains *G. lucidum*. The basal medium of (Reese &Mandels ,1963) The pH was adjusted 6.0 In each flask of 250 ml. 100 ml of this basal medium was taken & autoclaved. The flasks were then inoculated with uniform discs from growing margins of 7 days old cultures of test fungus& incubated at 27+2.C from 12,13 ,14, 15 weeks.

The cultures were shacked daily 4 times during incubation period. After the desired incubation period(12 to 15 weeks) the broth cultures were centrifuged and the clear supernatant was used for enzymatic activity.

ENDOGLUCANSE ACTIVITY (Cx) and EXOGLUCANASE ACTIVITY (C1) were estimated as described by Mendels and Weber (1969) by measuring reducing sugars as glucose by the Dinitro Salicylic Acid (D.N.S.A.) method (Miller, 1959).

1. ENDOGLUCANSE ACTIVITY (CX): A 0.5 ml. of appropriately diluted enzyme was added to 0.5 ml of 1% CMC in1% 0.05M sodium citrate buffer, pH 4.8 & incubated for 30 min. at 50.C & finally 3 ml. D.N.S.A. reagent was added. The mixture was then heated in a boiling water bath for 5 min. & cooled in a ice bath 7 reducing sugar was determined by absorbance of sample with a systronics 20 colorimeter at 575 nm. Glucose concentration were obtained from a standard prepared by the use of aquas solution of glucose (0.5 mg/ml.)

2. EXOGLUCANASE ACTIVITY(C1): To 50 mg. of absorbent cotton 1.0 ml of 0.05M sodium citrate buffer(pH 4.8) 7 1.0 ml of suitably diluted enzyme were added. The mixture was incubated for 24 hrs. At 50.C & 3 ML. D.N.S.A.reagent was added. Further assay procedure were as described for the Cx component above.

III. Results and Discussion

For estimation of Radial growth of different strains of G. lucidum. Use of 3 basal medium viz. Potato Dextrose Agar, Malt Extract Agar and Yeast Extract Agar. After completion of 6 days incubation, Potato Dextrose agar media shows better performance of Radial growth of test fungus in comparison to other Basal media presented in table 1,2,3 and 4.

The cellulolytic activity of four *G. lucidum* strains tested & their Cellulolysis Adequacy index are presented in table-5

All strains of *G.lucidum* were able to decompose filter paper of cellulose of varying degree. The decomposition was maximum with Ganoderma strain no.925 (24.80) & minimum
923(23.18).

On the basis of their cellulolytic activity, Ganoderma were categorized into 4 different groups (Bisht & Harsh, 1981a).

1.Excellent cellulose decomposer – More than 20%wt. loss
2. Good cellulose decomposer - Between 10-205 wt. loss
3. Moderate cellulose decomposer - Between 10-20%wt. loss.
4. Poor cellulose decomposer - Between 5-105 wt. loss.

In this experiment all the strains of *G. lucidum* decomposing more than 20% cellulose respectively . It was also observed that cellulose decomposition in while rot *G. lucidum* varied greatly in strain to strain with in a species. It was also revealed that cellulose decomposition pattern was irregular among the genera but also between the strains of the same species. The maximum CAI was observed with 925(6.20) strain of *G. lucidum* which also had the highest cellulose decomposition capacity the minimum CAI found with the strain 923(4.12).

It was found that after incubation period, the pH of the medium changed from the initial value 6.0 the pH become low & therefore more acidic.(4.5).As it evident from results, obtained in the present study, All the strains of *G. lucidum* were able to decompose cellulose. The wt. loss in filter paper was considered to be due to cellulolytic activity of test fungus & the loss was attributed to respiratory CO2 since the amount incorporated wt. The mycelium was not assist maximum cellulose decomposition by-

In the present study in accordance with the result obtained by (Harsh and Bisht, 1983& Tiwari *et al*, 2004). Different fungi have varying capacity to decompose cellulose because of different enzymatic machinery

(Hudson, 1972). This was quit evident that cellulose decomposition is different strain of the species.

It is also evident that cellulose activity of fungi under investigation were dependent of their respective growth rate (Garrett,1963). Has stated that the fungi having faster growth rate may not necessary have no enzymatic activity. During cellulose biodegradation, cellobionic acid and gluconic acid were frequently formed the lowering of pH towards acidity after incubation period in the present study was probably due to this reason. After utilization of all these available sugar in the medium these fungi can oxidize the bi product acid with the help oxidases they produce thus resulting the shiftening in pH towards acidic.

The white rot fungi rendered the medium more acidic (Cortwright and Findlay,1950).similar result were obtained by (Birkingshaw *et al*,2004) (Harsh & Bisht,1983 & Tiwari *et a*l, 2004).

Wood decaying fungi including *G. lucidum* are well known cellulolytic fungi for their potential to degrade organic waste(cellulose) .A few studies have been conducted earlier with wood decaying fungi to investigate their cellulolytic ability(Bisht and Harsh 1981a,- Harsh and Tiwari,1998and Tiwari *et.al* 2004)but still ,not much information is available regarding in this genus. Different fungi have different capacities to decompose cellulose because of different enzymatic machinery (Hudson, 1972), Harsh and Tiwari(1990)notice a similar trends with the *Daedalea cubensis,Gloeophyllum striatum,Funalia leonina* and *Oxyporus ravidus* . This was quite evident from the present study seen irregular pattern of cellulose decomposition not only among the different genera but also between different isolates of species of the same genus(Table-5).Variation in the cellulose decomposition among the test fungus occur in different host is also notice.

The quantative estimation of production of glucanase by four strains of G. lucidum presented in table .(6-13)and figure No.1 and 2. .All the test fungi were able to produce glucanase in the basal medium. Maximum endo-glucanase production were found with strain No924(109.79)and minimum with strain No.925(9.23) of G.lucidum. However Exoglucanase production was maximum with strain No 924(85.10)of G.lucidum. and minimum with strain No.923(8.48).Maximum production of Endo and Exo-glucanase by strain No 924 in14th week of incubation Production of exo-glucanaseby strain No. 818 and 923 initially showing similar activity of production

Quantitative variation was observed for the two component of glucanase production during incubation. Highley (1973) pointed out the endo-glucanase production by brown rot fungi is not repressed by monosaccharide, unlike those of white rot fungi which repressed by their high concentration (Eriksson, 1978). This is possibly the reason why cellulase activity was low at the beginning with the white rot test fungus used in present study. In general test fungus *G.lucidum* produces more Endo-glucanase as compared to the other components i.e. Exo-glucanase .

This Study was done on year 2008.The aim of this study was to find out the best

Table -1. Growth of *G. lucidum* strains in PDA

S.No.	No. of Strains	24h in MM	48 h	72 h	96 h	120 hrs	144 hrs	Average growth rate in mm/24h
1	818	5	5	5	6	6	5	5.3
2	923	4	5	5	5	6	5	6.0
3	924	4	5	5	5	5	6	5.0
4	925	5	3	4	4	4	4	4.0
5	Control	0	0	0	0	0	0	0

Table -2. Growth of *G. lucidum* strains in Yeast Extract agar

S.No.	No. of Strains	24h in MM	48 h	72 h	96 h	120 h	144 h	Average growth rate in mm/24h
1	818	5	4	4	4	5	5	4.5
2	923	5	6	6	6	5	5	5.5
3	924	5	5	5	6	4	4	4.8
4	925	5	5	5	5	6	5	5.1
5	Control	0	0	0	0	0	0	0

Table -3. Growth of *G. lucidum* strains in Malt extract agar

S.No.	No. of Strains	24h MM	48 h	72 h	96 h	120 h	144 h	Average growth rate in mm/24h
1	818	5	5	5	5	5	5	5.0
2	923	5	6	6	5	5	5	5.3
3	924	5	5	6	4	4	5	4.8
4	925	5	4	4	4	5	5	4.5
5	Control	0	0	0	0	0	0	0

Table – 4. Growth of *G. Lucidum* strains in different Nutricitional Medium (MM/24h)

S.No.	Name of Strains	PDA	Yeast Extract	Malt Extract
1	818	5.3	4.5	5.0
2	923	6.0	5.5	5.3
3	924	5.0	4.8	4.8
4	925	4.0	5.1	4.5
5	Control	0	0	0

Table - 5 Cellulolytic activity of *G. Lucidum*

S.No.	No. of Strains	Initial pH	Final pH	Growth Rate mm	Wt. Loss	% wt. loss	CAI
1	818	6.0	4.44	5.3	.1356	23.71	4.66
2	923	6.0	4.30	6.0	.1430	24.73	4.12
3	924	6.0	4.15	5.0	.1299	23.18	4.63
4	925	6.0	4.49	4.0	.1429	24.80	6.20
5	Control	6.0	6.0	0	0	0	0

Table – 6 Production of Endoglucanase(12th week)

S.No.	G. lucidum	Initial pH	Final pH	R1	R2	R3	Average
1	818	6.0	3.59	25.81	26.39	26.06	26.08
2	923	6.0	3.51	19.03	19.19	18.86	19.02
3	924	6.0	3.82	9.10	9.92	11.58	10.2
4	925	6.0	4.12	19.8	21.5	18.20	19.83
5	Control	6.0	6.0	0	0	0	0

Table – 7 Production of Endoglucanase(13th week)

S.No.	G. lucidum	Initial pH	Final pH	R1	R2	R3	Average
1	818	6.0	3.77	43.02	42.69	44.18	43.29
2	923	6.0	3.60	28.37	30.69	30.86	29.97
3	924	6.0	3.44	12.08	11.50	11.66	11.74
4	925	6.0	4.11	13.32	14.97	13.98	14.09
5	Control	6.0	6.0	0	0	0	0

Table –8 Production of Endoglucanase(14th week)

S.No.	G. lucidum	Initial pH	Final pH	R1	R2	R3	Average
1	818	6.0	3.72	34.99	32.51	38.47	35.32
2	923	6.0	3.55	25.07	28.87	31.85	28.59
3	924	6.0	3.31	109.38	110.70	109.29	109.79
4	925	6.0	4.05	12.41	9.51	11.66	11.19
5	Control	6.0	6.0	0	0	0	0

Table – 9 Production of Endoglucanase(15th week)

S.No.	G. lucidum	Initial pH	Final pH	R1	R2	R3	Average
1	818	6.0	3.98	9.51	9.51	9.52	9.51
2	923	6.0	3.76	16.54	15.54	12.51	14.86
3	924	6.0	3.89	20.68	21.01	20.68	20.79
4	925	6.0	4.15	9.26	9.10	9.34	9.23
5	Control	6.0		0	0	0	0

Table – 10 Production of Exo-glucanase(12th week)

S.No.	G. lucidum	Initial pH	Final pH	R1	R2	R3	Average
1	818	6.0	3.59	20.85	22.33	23.73	22.30
2	923	6.0	3.51	11.91	12.32	11.50	11.91
3	924	6.0	3.82	17.45	19.11	20.02	18.86
4	925	6.0	4.12	27.46	26.39	28.46	27.43
5	Control	6.0	6.0	0	0	0	0

Table – 11 Production of Exo-glucanase(13th week)

S.No.	G. lucidum	Initial pH	Final pH	R1	R2	R3	Average
1	818	6.0	3.77	24.98	24.73	25.48	25.06
2	923	6.0	3.60	8.68	8.43	8.35	8.48
3	924	6.0	3.44	18.78	18.53	18.20	18.50
4	925	6.0	4.11	26.97	25.98	25.73	26.22
5	Control	6.0	6.0	0	0	0	0

Table –12 Production of Exo-glucanase(14th week)

S.No.	G. lucidum	Initial pH	Final pH	R1	R2	R3	Average
1	818	6.0	3.72	84.89	81.99	84.56	83.81
2	923	6.0	3.55	47.49	50.96	51.87	50.10
3	924	6.0	3.31	85.38	84.80	85.13	85.10
4	925	6.0	4.05	42.69	49.64	47.41	46.58
5	Control	6.0	6.0	0	0	0	0

Table – 13 Production of Exo-glucanase(15th week)

S.No.	G. lucidum	Initial pH	Final pH	R1	R2	R3	Average
1	818	6.0	3.98	14.06	14.39	14.23	14.22
2	923	6.0	3.76	12.49	13.07	13.48	13.01
3	924	6.0	3.89	12.06	13.65	12.57	12.76
4	925	6.0	4.15	64.45	62.30	65.94	64.23
5	Control	6.0	0	0	0	0	0

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