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Biochemical Investigations on *Lentinus squarrosulus* (Mont.) Singer by Haemoagglutination Assay with Human Erythrocytes

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Abstract

Biochemical investigations on *Lentinus squarrosulus* (Mont.) Singer by haemoagglutination assay with human erythrocytes.

Haemagglutination was assayed using human type A, B, AB & O erythrocytes and lectin activity was expressed in terms of titre, defined as inverse of highest dilution causing visible haemagglutination.

The results achieved indicated that lectins present in the *L. squarrosulus* mycelium possess the capability to agglutinate all the four human blood groups. This property of mushroom lectins is

of immense significance in pharmaceutical investigations.

It is apparent that *L. squarrosulus* can fit well in the menu of modern calorie conscious society. Its commercial production not only promises a strong food alternative for mushroom lovers with a potential to provide equally potent culinary option as is provided by edible species of *Agaricus*, *Pleurotus*, *Volvariella*, *Lentinula*, etc. but also carries nutraceutical relevance leading to human welfare.

Key words: *Lentinus squarrosulus* (Mont.) Singer, lectins, haemoagglutination assay.

Introduction

Mushrooms being best low calorie food along with their delicious taste and flavor, fit well in the menu of health conscious people of modern society. Because of their growing relevance in our present day life, lot of emphasis is being laid on their identification, characterization, domestication and technological improvement for their bulk production [1]. Out of 7000 species known to possess varying degrees of edibility, about 2000 are medicinal mushrooms with varieties of health attributes [2].

Because of their acceptable culinary and nutraceutical attributes some of specialty mushrooms which are gaining importance include *Pleurotus* spp. (Dhingri), *Volvariella* spp. (Paddy straw mushroom), *Auricularia polytricha* (Mont.) Sacc. (Black ear), *Calocybe indica* P. & C. (Milky mushroom), *Stropharia rugoso-annulata* Farlow & Murrill (Giant mushroom), *Flammulina velutipes* (Curtis) Singer (Winter mushroom), *Pholiota nameko* (T.Ito) S.Ito & Imai (Nameko), *Coprinus lagopus* Fr. (Ink cap), *Tremella fuciformis* Berkeley (Silver ear), *Dictyophora duplicata* (Bamboo sprouts), *Ganoderma lucidum* (Curtis) P. Karst. (Reishi), etc. [3,4]. On the same line highly prized Nigerian edible mushroom *Lentinus squarrosulus* (Mont.) Singer can also be clubbed amongst the category of specialty mushrooms from India point of view as there is hardly any attempt to popularize this mushroom in India, although it is quite common in the Northern and Southern part of India.

Lentinus squarrosulus (Mont.) Singer, on which the present investigation has been undertaken,

belongs to class - Agaricomycetes, order - Polyporales and family – Polyporaceae [5,6]. Keeping in view the nutraceutical importance of *L. squarrosulus* (Mont.) Singer, the present investigation on a sample collected from the rotten stumps of *Juglens regia* from Kotla Barog (H.P.) has been planned.

Material and methods

The material of *L. squarrosulus* (Mont.) Singer was collected from dead stumps of *Juglens regia* at Kotla Barog in Sirmour District of Himachal Pradesh in the month of July in the year 2006. The material was brought to the laboratory, studied for its external and internal morphological details and pure culture was raised and maintained.

Experiments were performed to evaluate the properties of lectins in the mushroom mycelium by haemagglutination assay with human erythrocytes. Yeast glucose medium with pH 4.0 was inoculated with thoroughly homogenized inoculum under aseptic conditions and incubated at $30 \pm 1^{\circ}\text{C}$ for 5, 7, 9 and 12 days. The mycelial mats were harvested by filtration and washed thoroughly with distilled water. Mycelium was homogenized in Phosphate buffered saline (0.1 M, pH 7.2) for 5 minutes in an ice bath in ratio 1 : 1.5 and then grounded in acidified river sand for 30 minutes. Extract was centrifuged at 5000 rpm for 20 minutes at 4°C and supernatant was assayed for haemagglutination activity.

Haemagglutination was assayed using human type A, B, AB & O erythrocytes and lectin activity was expressed in terms of titre, defined as inverse of highest dilution causing visible haemagglutination. For this purpose, 20 μl sample was two fold serially diluted in PBS through the wells of U – bottom microtitre plate. Then 20 μl of erythrocyte suspension (2%, v/v) was added to each well and plates were incubated for 30 minutes at room temperature. The plates were stabilized at 4°C for 1-2 hours and mat formation indicated the presence of haemagglutination activity while button formation indicated the absence of haemagglutination activity

Results and discussion

Lentinus squarrosulus (Mont.) Singer on which the present investigation has been undertaken is an important member of class Agaricomycetes, order Polyporales and family Polyporaceae [5]. In view of this the present investigations on indigenous culture of this mushroom has been successfully attempted with the sole purpose to realize its nutraceutical potential for use in human welfare.

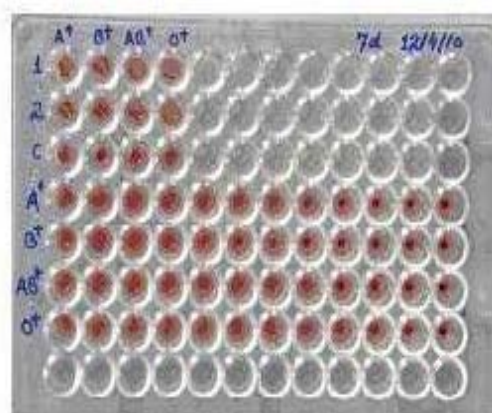
The material was collected from Sirmour District in Himachal Pradesh during July, 2006. It was taxonomically worked out and identified as *L. squarrosulus* (Mont.) Singer. For raising the pure culture of the mushroom a portion of the pileus flesh from the region of confluence between the pileus and stipe of the carpophore was inoculated on Potato dextrose agar under aseptic conditions. Mat formation indicated the presence of haemagglutination activity, while button formation indicated the absence of haemagglutination activity (Figs.-1/A-D). Results are depicted in Table-1 in which haemagglutination activity has been expressed in terms of titre.

Results indicated that lectins present in *L. squarrosulus* mycelium agglutinate all the human blood types. Singh *et. al.* [7] reviewed the role of lectins in host defense system. Mushroom lectins are endowed with antiproliferative, antitumor, mitogenic, hypotensive, vasorelaxing, haemolytic, anti-HIV1 reverse transcriptase, and immunopotentiating activities [8-12] while working on *Agaricus bisporus* observed that besides the effect of lectins on tumor, these also inhibit cell proliferation, a potentially useful property in the treatment of psoriasis.

Hence, *L. squarrosulus* like all other edible specialty mushrooms is suitable for suffering persons to whom low calorie diet is recommended by the doctors and it's nutraceutical relevance can be further explored.

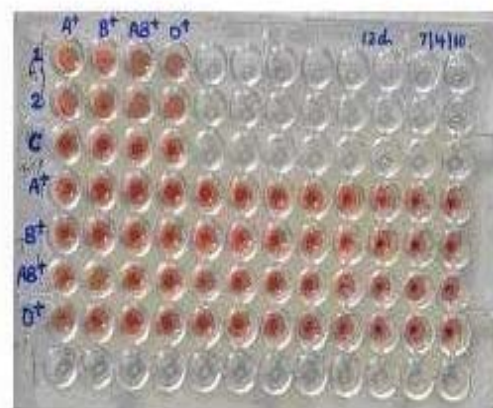
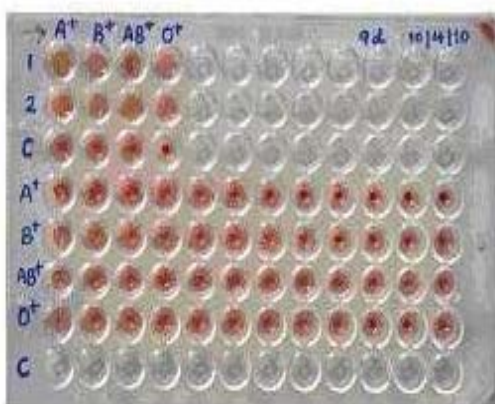
Culture age- 5 days

Culture age- 7 days



A

B



C

D

■ Button ■ Mat

Fig.-1(A to D): Activity of lectins with human erythrocytes

Table-1: Haemagglutination activity of extracts from the mycelium of *L. squarrosulus*

CULTURE AGE (DAYS)	LECTIN ACTIVITY (Titre)			
	A	B	AB	O
5	64	64	256	64
7	64	64	256	128
9	256	128	256	256
12	256	128	256	256

Conclusion

The study attempts to investigate and understand the potential of *L. squarrosulus* (Mont.) Singer. The presence of lectins in its mycelium and their role in haemagglutination activity signifies the nutraceutical and pharmaceutical relevance of this mushroom.

The results achieved indicated that lectins present in the *L. squarrosulus* mycelium possess the capability to agglutinate all the four human blood groups. This property of mushroom lectins is of immense significance in pharmaceutical investigations.

In view of the results achieved during the present investigation it is apparent that *L. squarrosulus* can fit well in the menu of modern calorie conscious society. Its commercial production not only promises a strong food alternative for mushroom lovers with a potential to provide equally potent culinary option as is provided by edible species of *Agaricus*, *Pleurotus*, *Volvariella*, *Lentinula*, etc. but also has nutraceutical significance. It needs the attention of Indian scientists for initiating work for its large scale production and subsequent popularization amongst the consumers.

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References

1. Purkayastha, R. P. and Chandra, A.(1985) *Manual of Indian Edible Mushrooms*. Jagmander Book Agency, New Delhi.
2. Tewari, R. P. (2005) Mushroom, their role in nature and society. In: *Frontiers in Mushroom Biotechnology* (R. D Rai., R. C. Upadhyay and S. R. Sharma, eds.), NRCM, Chambaghat, Solan, pp. 1 - 8.
3. Sharma, S. R. (1997) Scope of specialty mushrooms in India. In: *Advances in Mushroom Biology and Production*. (R. D Rai, B. L. Dhar, and R. N. Verma, eds.) NRCM, Chambaghat, Solan, pp. 193 - 203.
4. Tewari, R. P. (2003) Cultivation of Milky Mushroom (*Calocybe indica*). In: *Summer School on Emerging Areas of Mushroom Research and Production*. (R. D Rai, R. C. Upadhyay and S. R. Sharma eds.), NRCM, Chambaghat, Solan, pp. 127 - 129.
5. Kirk, P. M., Cannon, P. F., Minter, P. F. and Stalpers, J. A. (2008) Ainsworth Bisby's *Dictionary of Fungi* (10th ed.) CAB International Wallingford, Oxon, OX10, 8DE., UK.
6. Abbot, O., Okhuoya, J. A. and Akpaja, E. O. (2008) Growth of *Lentinus squarrosulus* (Mont.) Singer on saw dust of different tropical tree species. *African Journal of Food Science*. 3: 7 - 10.
7. Singh, R. S., Bhari, R. and Kaur, H. P. (2010) Mushroom lectins: Current status and future perspectives. *Critical Reviews in Biotechnology*, 30 (2): 99 – 126.
8. Sharon, N. and Lis, H. Lectins: (1972) Cell-agglutinating and sugar specific proteins. *Science*. **177**: 949 - 959.

9. Goldstein, I. J., Hughes, R. C., (1980) Monsigny, M., Osawa, T. and Sharon, N. What should be called a lectin? *Nature*. 285: 66.
10. Maria, R. C. G. N., Novaes, L. C. G. and Taveira, V. C. (2007) Natural products from Agaricales medicinal mushrooms: Biology, nutritional properties and pharmacological effects on cancer. *Revista Brasileira de Cancerologia*. 53 (4): 411 - 420..
11. Li, Y. R., Liu, Q. H., Wang, H. X. and Ng, T. B. (2008) A novel lectin with potent antitumor, mitogenic and HIV-1 reverse transcriptase inhibitory activities from the edible mushroom *Pleurotus citrinopileatus*. *Biochem. Biophys Acta*. 1780: pp. 51 - 57.
12. Parslew, R., Jones, K. T., Rhodes, J. M. and Sharpe, G. R. (1999) The antiproliferative effect of lectin from edible mushroom (*Agaricus bisporus*) on human keratinocytes: preliminary studies on its use in psoriasis. *Br. J. Dermatol*. 140 (1): 56 - 60.

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