WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 8, 2257-2264. Research Article ISSN 2277– 7105

# SCREENING FOR BIOACTIVE POTENTIAL OF EDIBLE MUSHROOM EXTRACTS AGAINST TOOTH DECAY BACTERIA

**Gurusamy Chelladurai[1*] and Venkad Uma[2]**

[1]Department of Zoology, G.Venkataswamy Naidu College, Kovilpatti, Tamilnadu, India.
[2]Department of Plant Biology and Plant Biotechnology, G.Venkataswamy Naidu College, Kovilpatti, Tamilnadu, India.

Article Received on
18 June 2017,
Revised on 09 July 2017,
Accepted on 30 July 2017
DOI: 10.20959/wjpr20178-9127

***Corresponding Author**
**Gurusamy Chelladurai**
Department of Zoology, G.Venkataswamy Naidu College, Kovilpatti, Tamilnadu, India.

## ABSTRACT

The present study aimed to evaluate the antibacterial and anti-mitotic assay of four medicinally important mushroom ethanolic extracts of *Pleuroutus sajor-caju*, *Agaricus bisporus*, *Calocybe indica* and *Ganoderma lucidum* against *Streptococcus* mutans isolated from tooth decay using Invitro screening methodology. Among the four mushroom tested, the *G. lucidum* extract showed maximum inhibitory activity recorded (16 ± 0.58 mm) and the minimum was recorded (10 ± 1.23 mm) in *C. indica* which was statistically significant ($p<0.05$). The methanolic extract of *G. lucidum* showed highest anti-mitotic activity at the concentration of 30 µL with a mitotic index 90.4%. Based on the results, it is concluded that mushrooms extract act as a good antibacterial agent against the bacterial pathogen isolated from tooth decay.

**KEY WORDS**: Medicinal Mushroom Extracts, S. Mutans, Antibacterial Activity, Anti-Mitotic Activity.

## INTRODUCTION

Dental caries is a microbial disease of the calcified tissues of the teeth characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth.[1] The mushroom are the macro fungi with fleshy, that bear their fertile surface either on lamella or lining, opening out by means of pores. More than 200 species of fungi are reported as edible throughout the world and about 283 are reported to be available in India. The medicines of fungal origin have been used from time immemorial. Mushrooms such as *A. bisporus, L. edodes, A. Auricular* and many *Pleurotus* species have been shown to posse's

antagonistic effects against bacteria, fungi, viruses and cancer.[2] The dietary mushroom provides a large variety of bioactive properties and they are effective against certain life threatening disease. The major medicinal properties attributed to mushrooms include anti-cancer, anti-biotic, anti-diabetic, anti-viral activity, immunity and blood control effects.[3] *Pleurotus sajor-caju* has anti-hypertensive effect through its active ingredients which affects the rennin angiotensin system.[4] *Ganoderma lucidum* has antibacterial activity against many drug resistant microorganisms that poses a serious threat to the treatment of infection disease.[5] *Calocybe inida* is a good source of immunostimulant and antioxidant materials for human and other living organisms.[6] Mushrooms have long been valued as highly tasty and nutritional foods by many cultures throughout the world and its containing all the essential amino acids but limited in sulphur containing amino acids like cystine and methionine.[7] Some of the edible mushrooms have also gained importance in modern medicine for their various pharmacological values. *Agaricus campestris*, *Flammulina mellae* and *F. odilpis* found to be active against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* etc. The crude extracts derived from some Portuguese wild mushrooms including *Cantharellus cibarius, Hypholoma fasciculare*, and *Ramaria botrytis* showed antibacterial activity against Gram-positive bacteria.[8] The present study was to evaluate the *invitro* screening of four medicinally important mushrooms extract against tooth decayed pathogen.

## MATERIALS AND METHODS

### Collection and Extraction of Medicinal Mushrooms

The fresh wild edible mushroom, *P. sajor-caju, A. bisporus, C. indica* and *G. lucidum* were collected from Tuticorin local market, Tamilnadu, India. The collected mushrooms were then washed thoroughly under tap water and shade dried at room temperature. The dried mushroom was crushed and powdered by mortar-pestle and collected in air-tight plastic jars. The dried samples were extracted with ethanol by Soxhlet apparatus until the colourless solution. The extract was then filtered with whatmann no1. Filter paper and then concentrated by using rotary evaporator at low temperature and pressure. The resultant residues were stored at 4 °C for further antibacterial screening.[9]

### Collection and identification of Test Pathogen

The decayed tooth samples were collected from Tuticorin Medical College and Hospital, Tuticorin, Tamil Nadu, India and they were brought to the laboratory with the help of Transport media. The collected bacterial pathogen was identified and confirmed by using

conventional microbiological and biochemical procedure, from Department of Microbiology, Kamaraj College, Tuticorin. The hydrolysis of starch test, lipid test, casein test, carbohydrate fermentation test, indole, methyl red test, voges proskauer test, citrate and catalase test were used for the confirmation of the test pathogens followed by Bergey's Manual of Bacteriology.[10]

**Inhibitory activity of Mushroom extracts against Tooth Decay Pathogen**

The *S. mutans* isolated from tooth decayed sample were inoculated into 10 ml of sterile nutrient broth and incubated at 37ºC for 24 hours. Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile Mueller- Hinton agar plates. Agar wells were prepared with the help of sterile cork borer with 10mm diameter. Different concentrations of mushrooms extracts (10µL, 20µL and 30µL) were added to 5 mm wells in the plate by using a micropipette, the plates were incubated in an upright position at 37ºC for 24 hours. Positive control well containing 50 µL of tetracycline (1 mg/ mL) and negative control containing 50 µL of ethanol were used. The result was calculated by measuring the zone of inhibition in millimetres. The diameter of inhibition zones was measured in mm and the results were recorded.[11]

**Anti-mitotic actitvity**

The anti-mitotic activity was determined by *Allium cepa* root meristematic cells. The *A. cepa* bulbs were sprouted in tap water for 48 hrs at room temperature. The bulbs that developed uniform roots were used for the experiment. These roots (three roots per concentration) were treated with extract at concentration of (10µL, 20µL and 30µL). The water was used for dilution as well as a blank and methotrexate was used as a standard for study. After 3 hrs the tips were fixed in the fixing solution of dil HCL and then stained with acetocarmine. The mitotic index was calculated by following formula.

$$\text{Mitotic Index} = \frac{\text{Number of Dividing cells}}{\text{Total number of Cells observed}} \times 100$$

**Statistical analysis**

All data were express as the mean ± SD. The group means were compared by one way analysis of variance (ANOVA) followed by Duncan multiple range test $p<0.05$ was considering significant.

## RESULTS

### Inhibitory activity of Mushroom extracts against Tooth Decay Pathogen

The antibacterial activities of ethanolic extract of mushrooms (A. bisporus, C. indica, P.sajor-caju, G. lucidum) were tabulated in (Table 1).

**Table. 1: Antibacterial activity of mushrooms extract against *S. mutans* (Mean ± SD).**

| S. No | Mushrooms Extract | Zone of Inhibition (mm) | | |
|---|---|---|---|---|
| 1 | *Ganoderma lucidum* | 10µL | 20µL | 30µL |
| | | 12 ± 1.08[a] | 14 ± 0.57[a] | 16 ± 0.58[a] |
| 2 | *Agaricus bisporus* | 9 ±1.32[a] | 10 ± 0.79[b] | 12 ± 1.65[a] |
| 3 | *Pleurotus sajor-caju* | 8 ± 1.53[a] | 9 ±1.03[a] | 11 ± 0.98[b] |
| 4 | *Calocybe indica* | 6 ± 0.89[a] | 9 ±1.10[a] | 10 ±1.23[a] |

[a,b] Values (Mean ± Standard Deviation) in the same row sharing the same superscript are not significantly different (P<0.05).

Among the antibacterial activity the ethanolic extract of *G. lucidum* showed significantly (p<0.05) maximum inhibitory activity against test pathogen (16 ± 0.58 mm) followed by *A. bisporus* (12 ± 1.08 mm), *P. sajor-caju* (11 ± 0.98 mm) and *C. indica* (10 ± 0.79 mm). The positive control tetracycline (26 ± 0.5 mm) was active against test bacterial strain. The highest inhibition activity of *G.lucidum* was taken to further analysis of anti-mitotic activity.

### Anti-mitotic activity

The anti-mitotic activity of ethanolic extract of *G.lucidum* showed highest activity at the concentration of 20µL (90.4 % Mitotic index) followed by 10µL (89.3 % Mitotic index), 30µL (87.3 % Mitotic index) and control (78.5 % Mitotic index) (Table 2).

**Table. 2: Anti-mitotic activity of ethanolic extract of *G.lucidum.***

| S. No | Concentration of Extract (µL) | Total no. of cells | No. of dividing cells | Mitotic index (%) |
|---|---|---|---|---|
| 1 | Control | 281 ± 1.0[a] | 220 ± 1.73[a] | 78.5 |
| 2 | 10 | 358 ± 1.15[a] | 320 ± 1.33[a] | 89.3 |
| 3 | 20 | 410 ± 1.15[b] | 358 ± 1.52[a] | 90.4 |
| 4 | 30 | 420 ± 0.57[a] | 380 ± 0.85[a] | 87.3 |

[a,b] Values (Mean ± Standard Deviation) in the same row sharing the same superscript are not significantly different (P<0.05).

The comparative analysis of normal cell division of *A. cepa* without treated extract and treated with ethanolic extract of *G. lucidum* is shown in (Plate 1 and 2).

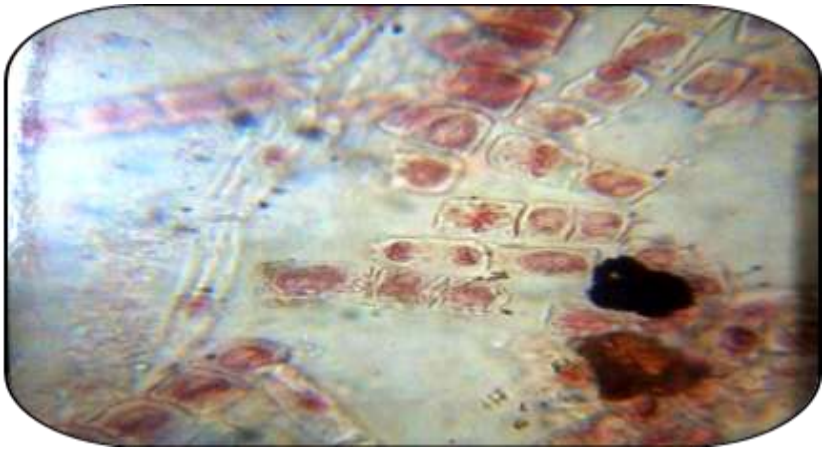

**Plate. 1: Normal cell division of *Allium cepa* root.**

**Plate. 2: Cell division of *Allium cepa* with ethanolic extract of *Ganoderma lucidum.***

## DISCUSSION

The mushrooms are protein rich, providing balanced diet and a precious vegetable that would solve the problem of malnutrition. In recent years *Ganoderma* species were the most preferable because its acts as a rich immunostimulant and antibacterial drug against disease causing pathogens.[12, 13] In the present study, among the four ethanolic extracts of mushroom tested, the maximum activity was observed in *G. lucidum* (16 mm) and minimum activity was observed in *C. indica* (10 mm) against *S. mutans*.[14] Similarly the antimicrobial activity of four wild edible mushrooms extract against human isolated pathogenic bacteria and fungi was reported. Reported that the aqueous extract of *G. lucidum* exhibited least antibacterial activity than the organic solvents. In the present study, anti-mitotic activity was screened using *A. cepa* root meristematic cell. The ethanolic extract of *G.lucidum* shows maximum activity was 20µL (90.4%) of mitotic index and minimum of 30µL (87.3%) of mitotix index.[15] Similar results were reported about the anti-mitotic activity and cytotoxicity effects of *Mussaenda*

*queensirkit*, the maximum mitotic index in 2000µL and minimum 100µL. The mitotic index does not increase considerably in the case of the extract when compared to the reference compound. Higher plant such as *A. cepa* can be used as genetic models to evaluate genotoxic effects such as chromosome aberrations and disturbances in the mitotic cycle.[16] Recently, a glucosylceramide isolated from *Pleurotus citrinopileatus* was found to be active against *Escherichia coli* and *S. aureus* with LC50values of 275.1 µM and 323.2 µM, respectively.[17] *Allium cepa* root chromosomal aberration assay has been frequently used to determine the cytotoxic, mutagenic and genotoxic effects of several substances.[18] The antibacterial activity of crude extract of *P. citrinopileatus* showed strong inhibition against *E. coli* and *S. aureus* was reported.[19] Bioactive compounds found in edible mushroom are known to play a vital role in promoting health. The presence of essential nutrients and minerals in the wild edible mushroom plays an important role in improvement of human health.[20]

## CONCLUSION

The present study revealed that the methanolic extract of *G. lucidium* exhibits better antibacterial activity when compared to other mushrooms. This study strongly suggests that wild edible mushrooms can be used as antibacterial agent in the development of new drug candidate for the new therapeutic intervention of tooth decay infection. Further work is under progress to identify the bioactive principles and to elucidate their mechanism of pharmacological action.

## ACKNOWLEDGEMENT

The first author thankful to the CMLRE-Project (Grant No. MoES/10-MLR/01/12), Ministry of Earth Sciences (MoEs), Government of India, for providing the financial assistance.

## REFERENCES

1. Jayakumar J, Ramesh E, Geraldine P. Antioxidant activity of the oyster mushroom, Pleurotus ostreatus, on CCl4-induced liver injury in rats. Food Chem Toxicol. 2006; 44: 189-199.
2. Tochikura TS, Nakashima H, Ohashi Y, Yamamoto P. Inhibition (in vitro) of Replication and of the Cytopathic effect of Human immunodeficiency virus by an extract of the culture medium of Lentinus edodes mycelia. Med Microbiol Immm. 1998; 177: 235-244.
3. Nayanajose K, Janardhanan K. Antioxidant and antitumor activity of Pleurotus florida, Current Science. 2000; 79: 94 1-943.
4. Chang R. Functional properties of edible mushrooms. Nutr Re. 1996; 54(11): 91-93.

5. Sadaf Quereshi AK, Pandey S, Sandh S. Evaluation of antibacterial activity of different Ganoderma lucidum extracts. Jour Scien Rese. 2010; 3: 9-13.
6. Chang ST. Global impact edible and medicinal mushrooms on human welfare in the 21st century: Non green evolution. Int. J. Med. Mushr. 1999; 1: 1-7.
7. Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira I. Wild and commercial. Mushrooms as source of nutrients and nutraceuticals. Food Chem Toxicol. 2008; 46: 2742-2747.
8. Suresh M, Arularasan S, Srikumaran S. Screening on antimicrobial activity of Marine gastropods Babylonia zeylanica (bruguière, 1789) and Harpa conoidalis (Lamarck, 1822) from Mudasalodai, Southeast coast of India. Inter Jour Pharma Scie 2012; 4: 1-5.
9. Buchanan RE, Gibbons NE. Bergey's Manual of Determinative Bacteriology (8 ed.) The Williams and Wilkins Company USA. 1974; 1268.
10. Mohanraj R. Anti HIV-I, Anti-microbial activity of the leaf extract of Calotropis procera. Intl J Green Pharm. 2010; 4: 242-246.
11. Grant WF. Chromosome aberration assays in Allium cepa. Mutation Research. 1982; 99: 273-291.
12. Gao Y, Zhous S, Huang M, Xu A. Antibacterial and antiviral value of the genus Ganoderma P. Karst. Species (Aphyllophoro mycetidea). A rev Inter Jour Med mush. 2003; 5: 235-246.
13. Ayodele SM, Idoko ME. Antimicrobial activity of four wild edible mushrooms in Nigeria. Int Jou Sci nature. 2011; 2: 55-58.
14. Vidyalakshmi KS, Charles AI, Vasanthi R. Anti-mitotic and cytotoxic effect of Mussaenda queensirkit. Jour pharm tox, 2007; 2: 660-665.
15. Kwan Yuet Ping, Ibrahim Darah, Umi Kalsom Yusuf, Chen Yeng, Sreenivasan Sasidharan. Genotoxicity of Euphorbia hirta: An Allium cepa.Assay Molecules. 2012; 17: 7782-7791.
16. Meng TX, Ishikawa H, Shimizu K, Ohga S, Kondo R. A glucosylceramide with antimicrobial activity from the edible mushroom Pleurotus citrinopileatus. J Wood Sci. 2012; 58: 81-86.
17. Fernandes TCC, Mazzeo DEC, Arin-Morales MA. Mech-anism of Micronuclei formation in polyploidizated cells of Allium cepa exposed to Trifluralin herbicide Pestic. Biochem Phys.2007; 88: 252-259.
18. Porntep Chomcheon, Bancha Kheawkum, Pornsinee Sriwiset, Suphak Dulsamphan, Chalermkieat Dulsamphan. Antibacterial activity of crude extracts from edible

mushrooms Pleurotus citrinopileatus and Tricholoma crassum. Berk Thai J Pharm Sci. 2003; 37: 107-111.

19. Mehmet Akyuz1, Ayse Nilay Onganer, Pmar Erecevit, Sevda Kirbag. Antimicrobial activity of some Edible Mushrooms in the Eastern and Southeast Anatolia Region of Turkey, Gazi University. Journal of Science. 2010; 23: 125-130.
20. Ogbe AO, Obekan AD. Proximate, Mineral and Anti-Nutrient Composition of Wild Ganoderma lucidum: Implication on Its Utilization in Poultry Production. Journal of Applied Animal Science, 2013; 3: 161-166.