*International Journal of Pharmacological Research*
ISSN: 2277-3312
www.ssjournals.com
Journal DOI:10.7439/ijpr

# Structural elucidation of novel bioactive compound (2-chloro-1-(2-chlorocyclopropyl)-2-(4-nitrophenyl)ethanone) from basidiomycetous fungus *Lentinus squarrosulus*

**N. Usha**[1], S. Shashikanth[2] and G. R. Janardhana[1*]

[1]*Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore 570006, Karnataka, India.*
[2]*Department of Studies in Organic Chemistry, University of Mysore, Manasagangotri, Mysore 570006, Karnataka, India.*

## Corresponding author*
Dr. G. R. Janardhana,
Department of Studies in Botany
University of Mysore, Manasagangotri,
Mysore 570006, Karnataka, India.
E-mail: grjbelur@gmail.com

## Abstract
While screening for bioactive compounds from macrofungi of Western Ghats of Karnataka, *Lentinus squarrosulus* a member of polyporaceae was collected and mycelium of the fungus was obtained on potato dextrose agar medium and cultured in a liquid medium (containing 2% glucose, 1% peptone and 2% yeast extract) for 20 days on a rotary shaker for the production of secondary metabolites. The cell free culture fluid (100ml) was subjected to solvent extraction with dichloromethane (100ml) and the extract was evaluated for antimicrobial activity by agar diffusion method and cytotoxic activity of the extract was also determined by MTT and SRB assay on MCF7 cell line. The results revealed that the dichloromethane extract was inhibitory against *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus* and *Bacillus subtilus* and also fungi such as *Aspergillus niger, Fusarium solani* and *Trichoderma harzianum*. The extract showed 13-27% cell inhibition at 63-1000 µg/ml concentration by MTT and SRB assays on MCF7 cell line. The structural elucidation of the compound by spectroscopic NMR ($^{1}H$ and $C^{13}$), IR and mass spectrometric analysis confirmed the presence of 2-chloro-1-(2-chlorocyclopropyl)-2-(4-nitrophenyl)ethanone responsible for antimicrobial and cytotoxic activity.
**Keywords:** Antimicrobial activity, Cytotoxicity, *Lentinus squarrosulus*, 2-chloro-1-(2-chlorocyclopropyl)-2-(4-nitrophenyl)ethanone

## 1. Introduction
*Lentinus squarrosulus* is one of the important macrofungus belongs to the family Polyporaceae. It is an edible mushroom widely distributed throughout Thailand, southern part of Nigeria, equatorial region of Africa, South-East Asia, Pacific islands, Australia and Northern and Southern India [1-4]. The fruiting bodies contain 22.82% crude protein, 7.52% ash, 2.76% moisture, 6.29% crude fat and 60.65% soluble carbohydrate on a dry weight basis [5]. It grows on dead leaves and wood logs producing characteristic tough carpophores having gills with serrated margins [6]. The analysis of dried *L. edodes* led to the identification of an antifungal polysulfide lentionine (1,2,3,5,6-pentathiepane) and related compounds [7]. Further, isolation of low molecular weight secondary metabolites from cell cultures such as hirsutane sesquiterpenes from *L. crinitus* DR-5 [8] and *L. connatus* BCC 8996 [9], chromanones from *L. crinitus* [10], striguerollide A, striguellol A, and striguellone A from *L. striguellus* [11] and 2-methoxy-5-methyl-1,4-benzoquinone from *L. adhaerens* [12] indicates its potential application. In the present investigation, a novel bioactive compound 2-chloro-1-(2-chlorocyclopropyl)-2-(4-nitrophenyl)ethanone with antimicrobial and cytotoxic activity was elucidated from submerged culture of *L. squarrosulus*.

## 2. Materials and Methods
### 2.1 Collection of *Lentinus* and Identification
*Lentinus squarrosulus* growing on dead woods in Western Ghats of Karnataka (India) was collected and deposited in the Museum collections, Department of Studies in Botany, University of Mysore (UOMMGB 201297). Further, fruiting bodies were examined for their size, shape, colour of the stipe**,** pileus, position of the gills, lamella colour, attachment, presence and absence of annulus and volva. The size, shape, ornamentation and colour of the basidiospores and their dimensions were also determined [13,14]. Fungal tissues (5mm) from the pileus region of the fruiting body where the lamellae joins the stipe was inoculated aseptically on Petri plate containing sterilized Potato Dextrose Agar (PDA) medium and incubated at 28±2ºC for 7 days [15].

For molecular identification, the genomic DNA from fungal mycelium was isolated using gDNA isolation kit

following manufacturer's instructions and the isolated DNA was used for polymerase chain reaction (PCR) (Applied Biosystems, U.S.A) employing universal primers DR [5'-GGTCCGTGTTTCAAGACGG-3'] and DF [5'-ACCCGCTGAACTTAAGC-3'] and the amplified PCR product was separated on 1.2% agarose gel containing 1% ethidium bromide. The amplified 28S-rDNA was subsequently sequenced by an automated sequencer (3730 xl DNA analyzer, Applied Biosystems, U.S.A). The sequence obtained was subjected to nBLAST analysis to identify the sequence homology and the fungus taxonomic information. A phylogenetic tree was constructed using the Neighbor-Joining method [16].

**2.2 Isolation of bioactive compound**

A sterilized 100 ml of liquid medium containing 2g glucose, 1g peptone and 2g yeast extract was inoculated with 5mm agar disks containing actively growing *L. squarrosulus* in 250ml Erlenmeyer flasks. The flasks were incubated at 25ºC at 150 rpm on a rotary shaker for 20 days. The culture fluid was separated from mycelium by filtration and the filtrate (100ml) was extracted with 300ml of dichloromethane three times and solvent was evaporated to complete dryness and used for antimicrobial, cytotoxic and structural elucidation [17-19].

**2.3 Antimicrobial assay**

The dichloromethane extract was further screened for antimicrobial activity against bacterial cultures (*Escherichia coli* (MTCC 729), *Enterobacter aerogenes* (MTCC 2829), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilus* (MTCC 121)) and also antifungal activity (*Aspergillus niger* (MTCC 872), *Fusarium solani* (MTCC 2935) and *Trichoderma harzianum* (MTCC 3172)) respectively. All the test organisms used in this study were obtained from the Microbial Type Culture Collections (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

For antibacterial studies, seeded nutrient agar (NA) plates were prepared with test bacteria and aseptically, sterile filter paper disc (6mm) containing 50µl of the dichloromethane extract was placed on the surface of NA plates and incubated at 37ºC for 24 hours. The filter disk with 50 µl of dichloromethane served as control and the antibiotic Tetracycline (10µg) served as positive control. The inhibitory activity against test bacteria was determined by measuring the zone of inhibition in a millimeter scale. Similarly, for antifungal activity, seeded potato dextrose agar (PDA) plates were prepared with the respective fungal cultures. The sterile filter disc containing 50 µl of the dichloromethane extract was placed in the center of PDA plates and incubated at 27ºC for 48-72 hours. The disk containing only solvent served as control and the Nystatin (10µg) served as positive control. The antifungal activity of the extract was determined by measuring the zone of inhibition in a millimeter scale. The mean values obtained were subjected to statistical analyses [20].

**2.4 MTT and SRB assay on MCF7 cell lines**

A monolayer cell culture (MCF7) was trypsinized and the cell count was adjusted to 1.0 x $10^5$ cells/ml using Dulbecco's Modified Eagle's Medium (DMEM) containing 10% Fetal Bovine Serum (FBS). To each well of 96 well Microtitre plate, 0.1 ml of the diluted cell suspension (10,000 cells) was added. After 24 hours of incubation, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs (62.5, 125, 250, 500, 1000µg/ml) were added on to the partial monolayer. The plates were then incubated at 37$^o$ C for 3 days in 5% $CO_2$ atmosphere, and microscopic examination was carried out and observations were made on every 24 h intervals. After 72 hours, in MTT assay, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 hours at 37$^o$ C in 5% $CO_2$ atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microtitreplate reader at a wavelength of 540 nm [21].

In SRB assay, after 72 h, 25 µl of 50% Trichloroacetic Acid (TCA) was added to the wells gently in such a way that it forms a thin layer over the extract to form an overall concentration of 10%. The plates were incubated at 4$^o$ C for 1 hour. The plates were flicked and washed five times with water to remove traces of medium, extract and serum, air-dried and stained with SRB for 30 min. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried. Tris base (10 mM, 100 µl) was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 min. The absorbance was measured at a wavelength of 540 nm. The percentage growth inhibition both in MTT and SRB assay was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% ($IC_{50}$) values was generated from the dose-response curves for cell line [22].

$$\text{Growth Inhibition (\%)} = 100 - \left( \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \text{ X } 100 \right)$$

**2.5 Statistical analysis:** The data obtained was analysed statistically by Schiff's Post Hoc Test using 7.5 version of SPSS computer software.

**2.6 Separation and structural elucidation of the compound**

The extract was separated by thin layer chromatography using Silica Gel 60 $UV_{254}$. The aliquots (10µl) of extract was charged on the plate and separated in a solvent system (butanol-acetic acid-water V/V 2:1:1) and visualized under UV light (245nm) and the $R_f$ value of the separated compound was calculated. The compound was collected and subjected to NMR ($^1H$ and $C^{13}$), IR and mass spectrometry for structural elucidation [11,18]. NMR ($^1H$ and $C^{13}$): The Nuclear Magnetic Spectroscopic (NMR) analysis was recorded on a Bruker DRX 500 NMR instrument operating at 500 MHz. The mass spectrum was obtained using a Q-TOF Waters Ultima instrument (Q-TOF GAA 082, Waters, Manchester, UK) fitted with an electron spray ionisation source for accurate molecular weight. Infrared spectrum was obtained using Fourier Transform Infra-Red (Thermo Nicolet Nexus 670, USA).

## 3. Results

Based on micro-morphometry and cultural parameters the macrofungus from the Western Ghats of Karnataka was identified as *Lentinus squarrosulus* (Mont). Singer. Sporophores were 12-16 cm in length, pileus 8-12 cm in diameter, funnel shaped, white becoming pale straw color to pale light brown, entire with varying pale brownish concentric zones of brownish squarrose scales on the surface, attachment of lemellae decurrent. Stipe was more or less excentric, subcylindrical, solid with appressed scales on the surface shown in figure 1A,B. Basidiospores 4, hyaline, thin walled, subcylindric, 7.5 x 2.8 µm shown in figure 1F. Colony on PDA was cottony white and becoming yellow ocher, growth initially slow, later moderately rapid. Mycelium was hyaline, highly branched with prominent clamp connections shown in figure 1C, D, E. Mycelia formed the characteristic white to yellowish round balls of 10-20mm diameter. The PCR results showed the amplification of 555bp 28S rDNA and the sequence shared 96% homology with *Lentinus squarrosulus* when subjected to GenBlast analysis. The representative sequence was deposited in GeneBank (Acc. No. KF155516) [23].

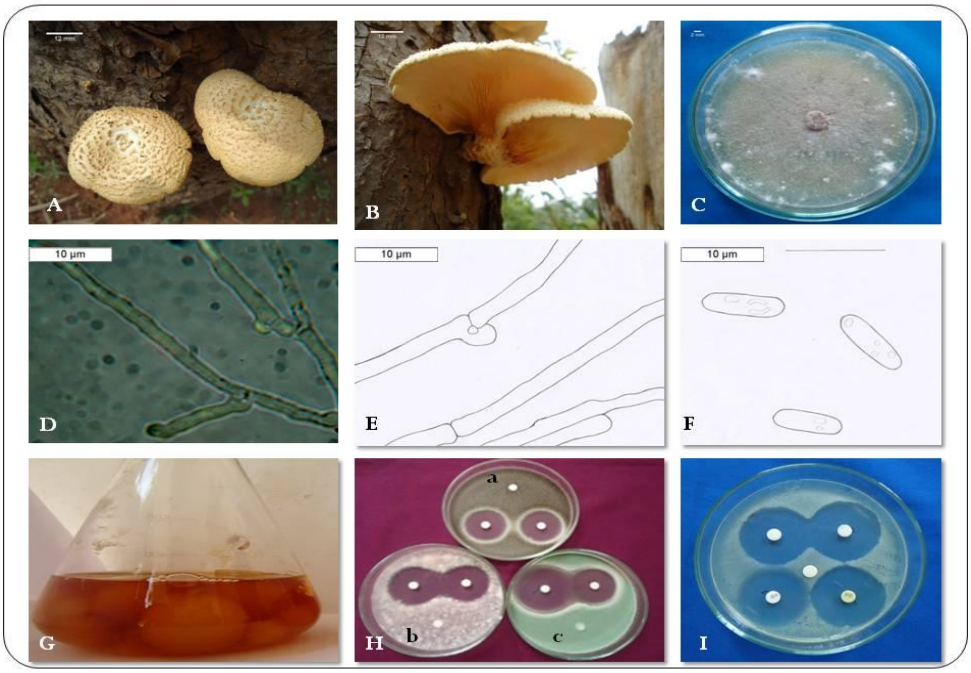


**Figure 1:** A and B- Sporophore of *L. squarrosulus* on rotten wood; C-. Mycelia growing on PDA; D- mycelium under compound microscope (450X) with clamp connection; E- Camera lucida drawings showing the clamp connection; F- basiodiospores; G- Mycelial balls formed after incubation on a rotary shaker; H and I- Antifungal activity of against a- *A. niger*; b-*F. solani*; c -*T. harzianum* and antibacterial activity against *E. coli* by disc diffusion method.

Dichloromethane extract of the macrofungus showed antibacterial activity against *Staphylococcus aureus* (28mm), *Escherichia coli* (27mm), *Bacillus subtilus* (27mm) followed by antifungal activity against *Aspergillus niger, Fusarium solani* and *Trichoderma harzianum* (20mm) respectively. The compound was less inhibitory against *Enterobacter aerogenes* (8mm) shown in figure 1H, I and table 1.

**Table 1: Antimicrobial activity of the dichloromethane extract obtained from *L. squarrosulus.***

| | *Zone of Inhibition (mm) | | |
|---|---|---|---|
| **Test organisms** | **Dicholoromethane extract** | **Tetracycline (10µg)** | **Nystatin (10µg)** |
| ***E. coli*** | 27.66±0.57 | 23.33±0.52 | ND |
| ***E. aerogenes*** | 08.33±0.57 | 23.66±0.57 | ND |
| ***B. subtilis*** | 27.33±0.57 | 27.66±0.56 | ND |
| ***S. aureus*** | 28.66±0.57 | 27.66±0.57 | ND |
| ***A. niger*** | 20.00±0.57 | ND | 21.53±0.50 |
| ***F. oxysporum*** | 20.00±0.57 | ND | 21.45±0.51 |
| ***T. harzianum*** | 20.00±1.00 | ND | 23.88±0.52 |

* Values are mean ± SD of the three independent experiments; ND-Not Done

Antimicrobial activity results showed that dichloromethane extract dependently decreased viability of cancer cells. The numbers of cells were reduced by 13% at 62.5 µg/ml, 16% at 125 µg/ml, 18% at 250 µg/ml, 22% at 500 µg/ml and 27% at 1000 µg/ml concentration in MTT assay. The reduction was 14% at 62.5 µg/ml, 17% at 125 µg/ml, 21% at 250 µg/ml, 25% at 500 µg/ml and 27% at 1000 µg/ml concentration in SRB assay. An increase in concentration of the dichloromethane extract reduced the number of cells proportionately and the $IC_{50}$ values for MCF7 cell line was >1000 µg/ml in both MTT and SRB assays shown in table 2 & 3.

**Table 2: Effect of dichloromethane extract of *L. squarrosulus* on MCF 7 cell line by MTT assay**

| Dichloromethane extract (µg/ml) | Absorbance at 540nm | Inhibition of MCF-7 (%) |
|---|---|---|
| **1000** | $0.66\pm0.00^{a}$ | $26.58\pm0.11^{e}$ |
| **500** | $0.69\pm0.00^{b}$ | $22.39\pm0.46^{d}$ |
| **250** | $0.73\pm0.00^{c}$ | $17.90\pm0.44^{c}$ |
| **125** | $0.75\pm0.00^{d}$ | $15.68\pm0.29^{b}$ |
| **62.5** | $0.77\pm0.00^{e}$ | $13.42\pm0.35^{a}$ |
| **Control** | $0.89\pm0.06^{f}$ | - |

*Values followed by different superscript letters differ significantly through column ($p<0.05$) by Schiff's post hoc test.

**Table 3: Effect of dichloromethane extract of *L. squarrosulus* on MCF 7 cell line by SRB assay**

| Dichloromethane extract (µg/ml) | Absorbance at 540nm | Inhibition of MCF-7 (%) |
|---|---|---|
| **1000** | $0.53\pm0.01^{a}$ | $26.62\pm1.67^{e}$ |
| **500** | $0.54\pm0.00^{b}$ | $25.38\pm1.16^{d}$ |
| **250** | $0.57\pm0.00^{c}$ | $21.18\pm0.55^{c}$ |
| **125** | $0.60\pm0.00^{d}$ | $17.17\pm0.78^{b}$ |
| **62.5** | $0.62\pm0.01^{e}$ | $14.06\pm0.55^{a}$ |
| **Control** | $0.72\pm0.06^{f}$ | - |

*Values followed by different superscript letters differ significantly through column ($p<0.05$) by Schiff's post hoc test.

Thin layer chromatographic studies revealed the presence of single fluorescent band having $R_f$ value of 0.8. Structure of the isolated compound has been arrived at by the study of IR, $^{1}H$ and $C^{13}$ NMR and mass spectrometry. Molecular formula of the compound was found to be $C_{11}H_{11}Cl_2O_3N$ (Molecular weight 276). IR stretching frequency of C=O group observed at $1684cm^{-1}$. Hydroxyl OH group at $3531cm^{-1}$. C-Cl stretching frequency observed at $503cm^{-1}$ and $552cm^{-1}$. $^{1}H$ NMR: δ 3.6(m, 1H), 3.8 (m, 1H), 4.13 (m, 1H), 5.15(s, 1H), 6.2(s, 1H, NH), 7.65(d, 2H, Ar-H), 8.2(d, 2H, Ar-H). $^{13}C$ NMR: 58.49, 62.23, 67.38, 71.32, 124.15, 128.33, 148.61, 151.60, 166.55. Mass Spectrum: $276(m^{+})$. The IR, $^{1}H$ and $C^{13}$ NMR and mass spectrometry data led to the structure of 2-chloro-1-(2-chlorocyclopropyl)-2-(4-nitrophenyl)ethanone shown in figure 2.

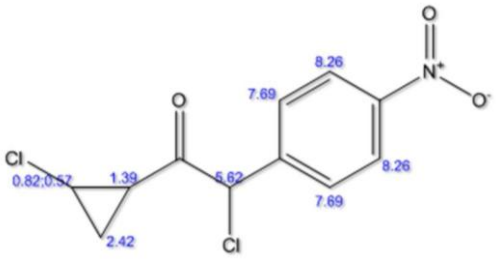


**Figure 2: Structure of the bioactive compound 2-chloro-1-(2-chlorocyclopropyl)-2-(4-nitrophenyl) ethanone isolated from *L. squarrosulus***

## 4. Discussion

The genus *Lentinus* includes the wood decaying species, characterized by decurrent lamellae, hyaline elliptical spores and includes about 40 species which have a widespread distribution, especially in subtropical regions [24]. Importantly a number of *Lentinus* species are widely used as food and possess therapeutic properties. Although *Lentinus* species are reported as edible and have medicinal properties [3,25], limited studies on isolation of antimicrobial compound from this macrofungus under submerged fermentation. Many attempts have been made to obtain useful and potent cellular or extracellular substances from submerged mycelial culture for application as nutraceutical or pharmaceutical industry. The status of edibility and medicinal value of some *Lentinus* species has been reported by many researchers [26]. Watling [27], reported *Lentinus tuber-regium* (Fr.) Fr. and *Lentinus squarrosulus* Mont. is edible and also a sources of food throughout central Africa. In India, *L. squarrosulus* has been reported from South, Tamil Nadu and Kerala [28][23] as well as from the North India, Himachal Pradesh [28]. Nine species of *Lentinus* including *L. squarrosulus* have been recorded from Kerala [29]. It has been also reported from Africa, South-East Asia, the Pacific Islands and Australia. The fungus reported as *L. critinus* from southern India (Tamil Nadu) represents *L. squarrosulus* [30].

In the present investigation, *in vitro* assays showed that dichloromethane extract of *L. squarrosulus* was able to induce significant inhibition of test bacteria, fungi and cancer cells. These findings are in agreement with recent reports, heteroglycan, isolated from *L. squarrosulus* (Mont.), consisting of fucose, galactose, and glucose shows macrophage as well as strong splenocyte and thymocyte activation [5]. A new tetrahydrobenzofuran derivative, (6S,7S)-6,7-dihydroxy-3,6-dimethyl-2-isovaleroyl-4,5,6,7- tetrahydrobenzofuran, together with the known 1,2 dihydroxymintlactone was isolated from the fermentation broth of the edible mushroom fungus *Lentinus squarrosulus* BCC 22366 [31]. An intracellular lectin of 55 kDa with highest specificity for mucin and asialofetuin has been purified from *Lentinus squarrosulus* [4]. Two antibiotic compounds from *L. squarrosulus*, purification and study of the antibiotic substances produced by *L. squarrosulus* have been carried out although their structures have not been elucidated [25][31].This is first attempt to isolate 2-chloro-1-(2-chlorocyclopropyl)-2-(4-nitrophenyl)ethanone having antimicrobial and anticancer activity with potential application in pharmaceutical industry.

## 5. Conclusion

This investigation adds to the present information available from the genus *Lentinus* and suggests that the bioactive compound 2-chloro-1-(2-chlorocyclopropyl)-2-(4-nitrophenyl)ethanone isolated from *L. squarrosulus* collected from Western Ghats of Karnataka has potential to be used as natural source of antibiotics with antimicrobial and cytotoxic activity and clinical trials would assure the right conclusions.

## Acknowledgments

Authors gratefully thank the Institution of Excellence (IOE), University of Mysore for financial support (No. UOM/IOE/Research/1/2010-11 Dated 22.04.2010) for the investigations.

## References

[1] Pukahuta C, Suwanarit P, Shianagawa E *et al*. Combination of laccase, xylanase and cellulase in lignocellulose degradation by white-rot fungi, *Lentinus polychorus* Lev. and *L. squarrosulus* Mont. Kasetsart J. *Natural Science* 38: 2004: 65–78.

[2] Kirk PM, Cannon P, David JC *et al*. *Ainsworth and Bisby's Dictionary of the fungi* Wallingford, Oxon, UK: CAB International: 2008.

[3] Isikhuemhen OS, Mikiashvili NA, Adenipekun CO *et al*. The tropical white rot fungus, *Lentinus squarrosulus* Mont.: lignocellulolytic enzymes activities and sugar release from cornstalks under solid state fermentation. *World Journal of Microbiology and Biotechnology* 28; 2012: 1961-1966.

[4] Singh RS, Kaur HP, Kumar P *et al*. Purification and characterization of a thermostable mycelial lectin from basidiomycete *Lentinus squarrosulus. Biologia* 8(6): 2013:1034-1040.

[5] Bhunia SK, Dey B, Maity KK *et al*. Structural characterization of an immunoenhancing heteroglycan isolated from an aqueous extract of an edible mushroom *Lentinus squarrosulus* (Mont.) Singer. *Carbohydrate Research* 345: 2010:2542-2549.

[6] Singer R. The Agaricales of Modern Taxonomy. Koeltz Scientific Books. Koenigestein i.m. Taunus, Germany: 1986.

[7] Takazawa HF, Tajima Miyashita C. An antifungal compound from shiitake (*Lentinus edodes*). *Yakugaku Zasshi* 102: 1982:489-491.

[8] Abate D, Abraham WR. Antimicrobial metabolites from *Lentinus crinitus*. *Journal of Antibiotics* 47: 1994:1348-1350.

[9] Rukachaisirikul V, Tansakul C, Saithong S *et al*. Hirsutane sesquiterpenes from the fungus *Lentinus connatus* BCC8996. *Journal of Natural Production* 68: 2005:1674-1676.
[10] Abraham WR, Abate D. Chromanones from *Lentinus crinitus* (Basidiomycetes). *Z Naturforsch* 50(c): 1995:748-750.
[11] Zheng Y, Zhao B, Lu C *et al*. Isolation, structure elucidation and apoptosis-inducing activity of new compounds from the edible fungus *Lentinus striguellus*. *Natural Products Communication* 4(4): 2009:501-506.
[12] Lauer U, Anke T, Hansskke F. Antibiotics from basidiomycetes. XXXVIII. 2-Methoxy-5-methyl-1,4-benzoquinone, a thromboxane A2 receptor antagonist from *Lentinus adhaerens*. *Journal of Antibiotics* 44(1): 1991:59-65.
[13] Christensen CM. Edible Mushrooms. University of Minnesota Press, Minneapolis: 1981.
[14] Laessoe T. Mushrooms. A Dorling Kindersley Limited, London; 2000.
[15] Nobbels MK. Identification of cultures of wood-inhabiting Hymenomycetes. *Canadian Journal of Botany* 43: 1965:1097-1139.
[16] Das SK, Mandal A, Datta AK. Nucleotide Sequencing and Identification of Some Wild Mushrooms. *The Scientific World Journal*: 2013:1-7.
[17] Hatvani N. Antibacterial effect of the culture fluid of *Lentinus edodes* mycelium grown in submerged liquid culture. *International Journal of Antimicrobial Agents* 17; 2001:71–74.
[18] Yamac M, Bilgili F. Antimicrobial activities of fruit bodies and/or mycelial cultures of some mushrooms isolates. *Pharmaceutical Biology* 2006; 44(9): 660-667.
[19] Halpern GM. Mushroom cultivation. *In G. M. Halpern (Ed.), Healing mushrooms: Effective treatments for today's illnesses*. Garden City Park, New York; 2007.
[20] Bauer AW, Kirby WM, Sherris JC *et al*. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 1966; 45(4): 493–496.
[21] Francis D, Rita L. Rapid colorometric assay for cell growth and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *Journal of Immunological Methods* 1986; 89: 271-277.
[22] Philip S, Rista S, Dominic S *et al*. New colorimetric cytotoxic assay for anti cancer drug screening, *Journal of National Cancer Institute*, 1990; 82: 1107-1112.
[23] Manimohan P, Divya N, Kumar TKA. The genus *Lentinus* in Kerala State, India. *Mycotaxon* 2004; 90(2): 311-318.
[24] Gulati A, Atri NS, Sharma SK *et al.* Nutritional Studies on five wild Lentinus species from North-West India. *World Journal of Dairy & Food Sciences*, 2011; 6(2): 140–145.
[25] Sudirman LI, Lefebvre G, Kiffer E *et al*. Purification of antibiotics produced by *Lentinus squarrosulus* and preliminary characterization of a compound active against *Rigidoporus lignosus.Current Microbiology* 1994; 29: 1-6.
[26] Abdullah S. Diversity of Malaysian *Lentinus* fr. and chemical analysis of antioxidative extract from mycelia of *Lentinus squarrosulus* mont. / Sumaiyah Abdullah. PhD thesis, University of Malaya: 2014.
[27] Watling R. Comparison of the macromycete boitas in selected tropical areas of Africa and Australia. *In aspects of tropical mycology*. Cambridge University Press, UK; 1995.
[28] Castillo G, Nihoul A, Demoulin V. Correlation between in vitro growth response to temperature and the habitat of some lignicolous fungi from Papua New Guinea coastal forests. *Mycologia* 2004; 25: 57-81.
[29] Sharma SK, Atri NS. Study on Mycelial Growth Pattern of Five Wild *Lentinus* Species. European Journal of Applied Science 2013; 5(3): 71-75.
[30] Natarajan K, Manjula M Studies on *Lentinus polychorus* Lév., *squarrosulus* Mont. *Indian Mushroom Science* 1978; 1: 451–453.
[31] Isaka M, Sappan M, Rachtawee P *et al*. A tetrahydrobenzofuran derivative from the fermentation broth of *Lentinus squarrosulus* BCC 22366. *Phytochemistry letters* 2011; 4(2): 106-108.