*Available online*www.ijpras.com

International Journal of Pharmaceutical Research&Allied Sciences, 2018, 7(2):1-7

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Prelude to Successful Cultivation of Hericium in the Philippines: Understanding its Mycelial Growth Response on Different Culture Media and its Antibacterial Activity

Arianne V. Julian[1*], Christopher A. Wright[2,3] and Renato G. Reyes[1,4,5]

[1]Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines
[2]Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, Michigan, USA
[3]Easygrow Mushrooms, Inc. Farmington Hills, Michigan, USA
[4]Flora and Fauna Analytical and Diagnostic Laboratory, Nueva Ecija, Philippines
[5]Center for Tropical Mushroom Research and Development, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines
**Corresponding Author*

ABSTRACT

Hericium mushrooms are edible fungi belonging to the Hericiaceae family with a long history of usage in traditional medicine in China. In the Philippines, however, information on its production, cultivation or nutraceutical properties is relatively unknown. In this study, four strains of Hericium spp. including H. americanum, H. erinaceus, H. coralloides and Hericium sp. were evaluated for their nutritional requirements using various commercially available culture media and their antibacterial activity against Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 in two dilutions (60 ml and 80 ml sterile distilled water + 12-hour culture - bacterial suspension) on Mueller Hinton Agar. Highest mycelial growth response of H. americanum and H. coralloides was observed on Sabouraud Dextrose Agar while H. erinaceus and Hericium sp. grew best on Potato Dextrose Agar. The ability of H. americanum mycelia to inhibit bacterial growth at 12 and 24 hours after inoculation against E. coli was reported. Meanwhile, both H. americanum and H. erinaceus demonstrated antibacterial activity against S. aureus ATCC 25923 in both dilutions.

Key words: *Hericium Spp., Antibacterial Activity, Mycelial Growth.*

INTRODUCTION

Hericium species are edible tooth fungi belonging to the Phylum Basidiomycota, Class Agaricomycetes, Order Russulales, Family Hericiaceae [1]. These saprophytes generally have short stalks and form a whitish cluster of downward cascading spines which can commonly be found on dead woods or living trees in Asia, Europe, and North America [2, 3].

Among the *Hericium* spp., *Hericium erinaceus* is the most extensively documented and known for various pharmacological properties such as antioxidant, hepatoprotective [4], gastroprotective [5], hypoglycemic, [6], hypolipidimic [7], anti-tumor [8] and anti-fatigue [9]. Recent studies also show that fungal protein from *H. erinaceus* exhibited immunomodulatory activities which can be an adjunct drug for immunotherapy [10]. Several reports also demonstrated its stimulating activity to the synthesis of nerve growth factor which might have a preventive and ameliorative effect in age-related neurological dysfunctions such as Alzheimer's disease and Parkinson's disease [11,12, 13]. It is also reported that the polysaccharide in the fruiting body may have a positive influence on immune function which can be beneficial against stomach, esophageal, and skin cancer [14]. Similarly, compounds isolated

from *Hericium coralloides* induced nerve growth factor and exhibit antiproliferative activity against certain human cancer cell lines [15].

Mushrooms contain various bioactive compounds with pharmacological importance [16]. It is reported that there are over 60 antimicrobial compounds which have already been isolated from mushrooms but presently, only the microscopic fungi compounds are used as antibiotics available on the market [17]. Cases of multi-drug resistant bacteria have increased for the past years which can be attributed to the development and indiscriminate use of several antimicrobial medicines [18, 19]. Consequently, there has been an increasing interest to discover the potential of mushrooms for their antibacterial property [20].

To our knowledge, there is little or no known work or information on the cultivation, consumption or medicinal properties of *Hericium* in the Philippines. Thus, this study can be a pioneer to elucidate the potential of *Hericium* mushrooms as functional food in the country. The present study aimed to determine the optimum growth condition among commercially available culture media and the antibacterial activity of *H. americanum, H. erinaceus, H. coralloides* and *Hericium* sp.

MATERIALS AND METHODS

Source and Revival of Pure Culture

Pure cultures of *H. americanum, H. erinaceus, H. coralloides* and *Hericium* sp. were obtained from the Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, Michigan, USA. An approximate 10-mm agar block per strain was aseptically transferred into a sterilized Potato Dextrose Agar (PDA) culture plates, and incubated at room temperature until full mycelial colonization.

Influence of Different Commercial Culture Media on Mycelial Growth

Four commercially available culture media (potato dextrose agar, sabouraud dextrose agar, malt extract agar and mycological agar) were used to evaluate the nutritional requirements of *H. americanum, H. erinaceus, H. coralloides* and *Hericium* sp. based on their influence on secondary mycelial growth. The media were sterilized at 15 psi, 121 °C for 30 min and dispensed in sterile Petri plates with three replicates per treatment. Ten mm mycelial disc from each mushroom strain was aseptically inoculated in sterile culture plates, sealed with parafilm and incubated at 28–30°C. Mycelial growth diameter was measured every 24 h using a digital Vernier caliper for ten days. Mycelial density was described as very thin (+), thin (++), thick (+++), very thick or cottony (++++).

Evaluation of Antibacterial Activity

The antibacterial property of *H. americanum, H. erinaceus, H. coralloides* and *Hericium* sp. was tested against Gram-positive *Staphylococcus aureus* ATCC 25923 and Gram-negative *Escherichia coli ATCC 25922*. The following dilutions of the bacterial test organisms were used: 60 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth and 80 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth. For 60 ml and 80 ml dilutions of *S. aureus* ATCC 25923, the bacterial counts were 1.67×10^6 cfu/ml and 1.24×10^6 cfu/ml, respectively. In *E. coli*, the bacterial count for 60 ml dilution was 1.26×10^7 cfu/ml while 80 ml dilution had 7.4×10^6 cfu/ml. To determine the antimicrobial efficacy of the mushrooms, aliquots of each dilution of test cultures (0.1 ml) were evenly spread over the surface of plated Mueller Hinton Agar using a sterile bacterial cell spreader. Ten mm of mycelial discs from every strain were aseptically inoculated at the center of the plated medium with each concentration of bacterial suspension. Meanwhile, sterile paper discs measuring 6.6 mm diameter were saturated with Ampicillin and Streptomycin then placed over the culture plates of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, respectively, which served as control for each test bacterium. These plates were incubated at 32°C for 24 h. The diameter of zone of inhibition was initially measured after 12 h using a caliper.

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) in one-way classification analysis using SPSS software version 20 and significant differences of mean values were compared using Tukey's test at 95% least significant difference ($p < 0.05$).

RESULTS & DISCUSSION

Mycelial Growth Performance

Determination of the most favorable culture media for mycelial growth is the first critical stage for successful mushroom production. Hence, maintenance of mushroom species in optimum media condition is important for both research and industrial use [21]. This study evaluated the optimum nutritional requirements of *H. americanum, H. erinaceus, H. coralloides* and *Hericium* sp. on four commercially available culture media. Table 1 presents the mycelial growth and density of *Hericium* spp. after ten days of incubation. *H. americanum* showed significant differences in mycelial growth on various growing agar media but grew best on sabouraud dextrose agar (SDA) with a mean mycelial diameter of 35.97 mm and very thick (++++) mycelial density (Fig. 1). For *H. erinaceus*, no statistical difference was noted among the media but using potato dextrose agar (PDA) resulted to highest mycelial diameter (56.95 mm). Meanwhile, *H. coralloides* mycelial response was the best in SDA (62.33 mm). For *Hericium* sp., both PDA and mycological agar (MA) were statistically comparable in terms of diameter (90 mm and 84.17 mm, respectively) but luxuriance of growth was noted very thin in MA and very thick or cottony in PDA (Fig. 1). Thus, PDA was the best option for *Hericium* sp. Noticeably, *Hericium* sp. showed the fastest mycelial growth, completely colonizing the culture plates in 10 days. On the other hand, the slowest mycelial response was recorded in *H. americanum*.

Table 1. Mycelial growth diameter and mycelial density of *H. americanum, H. erinaceus, H. coralloides* and *Hericium* sp. on different culture media after 10 days of incubation

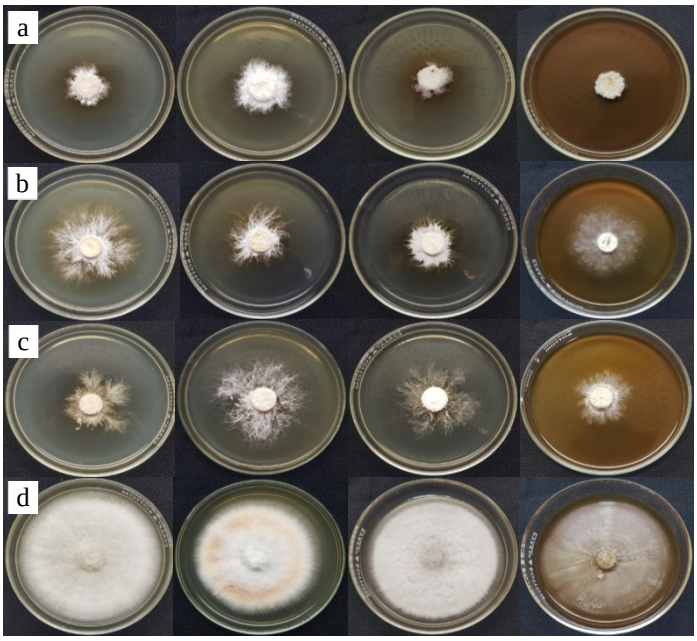
Hericium strains	Culture Media	Mycelial growth (mm)	Mycelial density
H. americanum	PDA	27.62 ± 3.43^{b}	++++
	SDA	35.97 ± 0.35^{a}	++++
	MA	20.82 ± 2.86^{c}	+++
	MEA	18.67 ± 0.87^{c}	+++
H. erinaceus	PDA	56.95 ± 1.60^{a}	++++
	SDA	53.17 ± 6.01^{a}	++++
	MA	40.20 ± 3.48^{a}	++
	MEA	47.68 ± 3.84^{ab}	++++
H. coralloides	PDA	47.65 ± 7.68^{b}	+++
	SDA	62.33 ± 1.54^{a}	++++
	MA	52.30 ± 2.92^{ab}	+++
	MEA	35.30 ± 4.73^{c}	+++
Hericium sp.	PDA	90.00 ± 0.00^{a}	++++
	SDA	72.95 ± 2.52^{b}	++++
	MA	84.17 ± 1.27^{a}	+
	MEA	72.18 ± 8.39^{b}	++++

Values presented are means and SD

Treatment means in each column with the same letter of superscript are not significantly different from each other at 5% level of significance using Tukey's test. Mycelial density column: very thin (+), thin (++), thick (+++), very thick or cottony (++++)

Abbreviations: PDA – potato dextrose agar, SDA - sabouraud dextrose agar, MA – mycological agar, and MEA – malt extract agar

Among the commercially available culture media, PDA and SDA demonstrated the best options for mycelial growth on the tested *Hericium* strains. PDA is one of the most commonly used medium for fungal growth, which contains dextrose, potato extract and agar. Meanwhile, the composition of SDA includes 1% (w:v) peptone, 4% (w:v) glucose and 1·5–2·0% (w:v) agar [22]. This result implies that different strains of *Hericium* require different nutritional requirement. Similarly, [23] reported that four strains of *H. erinaceus* grow most favorably on PDA.



PDA SDA MA MEA

Figure 1. Culture plates of (a) *H. americanum*, (b) *H. erinaceus*, (c) *H. coralloides* and (d) *Hericium* sp. on different media after 10 days of incubation

Antibacterial Activity of *Hericium* spp.

The antibacterial activity of *H. americanum, H. erinaceus, H. coralloides* and *Hericium* sp. mycelia was quantitatively evaluated by measuring the presence of clear zone signifying strong inhibition as presented in Table 2. Among the *Hericium* strains, only *H. americanum* showed ability to inhibit bacterial growth in both dilutions of *E. coli* ATCC 25922 after 12 h and 24 h (Fig. 2). Meanwhile, both *H. americanum* and *H. erinaceus* exhibited antibacterial activity against *S. aureus* ATCC 25923 in both concentrations (Fig. 3). The extent of these inhibitions against *S. aureus* is comparable to *Collybia reinakeana* RGR-FE–NSC strain, a Philippine endemic edible mushroom, as reported by [24]. Similarly, 12-22 mm zone of inhibition was also documented for aqueous and organic solvents extract of *Agaricus bisporus* and *Pleurotus sajor caju* against *S. aureus* and *E. coli.* [25, 26] also reported that freeze-dried fruitbody extract of *H. erinaceus* exhibited clear inhibition zones against *S. aureus*, while fresh fruibody, oven-dried and mycelium extracts produced hazy zones against *S. aureus* and *E. coli*. Isolated compounds of *H. erinaceus* also exhibited antimicrobial activity against *Bacillus subtilis, Saccharomyces cerevisiae, Verticillium dahliae* and *Aspergillus niger* [27].

Table 2. Antibacterial activity of *Hericium* spp. Mycelia against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 (zone of inhibition of growth in mm)

	12 h		24 h	
E. coli ATCC 25922	A	B	A	B
Streptomycin	23.80	24.16	23.58	23.33
H. americanum	13.33	15.85	13.08	12.30
H. erinaceus	NI	NI	NI	NI
H. coralloides	NI	NI	NI	NI
Hericium sp.	NI	NI	NI	NI
S. aureus ATCC 25923				
Ampicillin	43.43	44.55	41.58	49.60
H. americanum	14.10	15.60	13.13	16.10
H. erinaceus	14.33	13.93	10.98	12.95
H. coralloides	NI	NI	NI	NI
Hericium sp.	NI	NI	NI	NI

A (60 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth)
B (80 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth)
NI- no inhibition

Out of the four strains of *Hericium* used in this study, only one showed antibacterial activity against *E. coli* ATCC 25922 while two strains demonstrated inhibition on bacterial growth of *S. aureus* ATCC 25923. Generally, Gram-negative bacteria are more resistant to antimicrobial agents since this type of bacteria possesses an outer membrane which is not present in Gram-positive bacteria [28]. These outer membranes serve as a barrier of the cell wall against the penetration of various substances which make Gram-negative bacteria less susceptible to antibiotics than Gram-positive bacteria [29, 30].

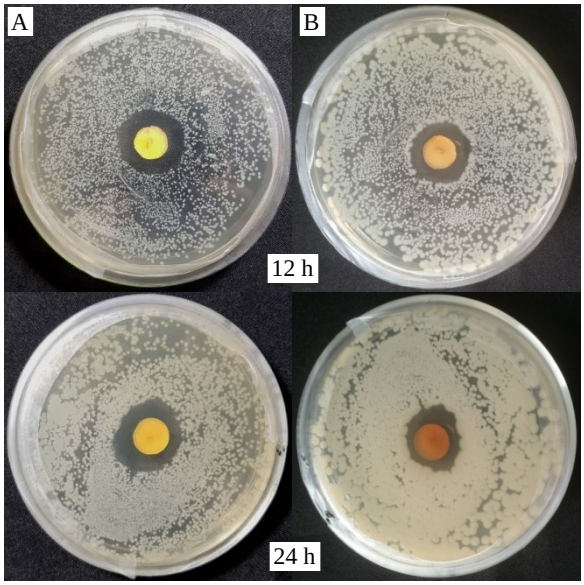
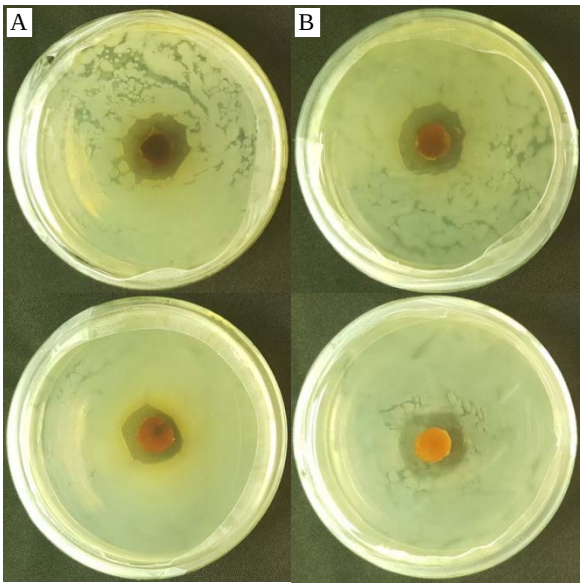


Figure 2. Zone of inhibition of H. americanum in A and B dilutions after 12 h and 24 h against E. coli ATCC 25922
A (60 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth)
B (80 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth)



(a) (b)

Figure 3. Zone of inhibition of (a) H. americanum and (b) H. erinaceus in A and B dilutions after 24 h against S. aureus ATCC 25923
A (60 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth)
B (80 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth)

CONCLUSION

This investigation determined that the optimum nutritional requirement for mycelial growth of H. americanum and H. coralloides was observed on Sabouraud Dextrose Agar while in H. erinaceus and Hericium sp., highest mycelial

growth and density were recorded in Potato Dextrose Agar. Inhibition on bacterial growth of E. coli ATCC 25922 and S. aureus ATCC 25923 was demonstrated by H. americanum. H. erinaceus also showed antibacterial potential against S. aureus ATCC 25923.

ACKNOWLEDGEMENT

The authors acknowledge the CLSU Tuklas Lunas Center for the use of its laboratory facilities.

REFERENCES

1. Sokół, S., Golak-Siwulska, I., Sobieralski, K., Siwulski, M., Górka, K., Biology, cultivation, and medicinal functions of the mushroom Hericium erinaceum, Acta Mycologica, 2015; 50(2) :1-18.
2. Ginns, J., *Hericium* in North America: cultural characteristics and mating behavior, Canadian Journal of Botany, 1985; 63:1551-1563.
3. Grumezescu, A.M., Holban, A.M. Therapeutic Foods Handbook of Food Bioengineering Volume 8. San Diego: Elsevier Science Publishing Co Inc; 2017, 154-156.
4. Zhang, Z., Lv, G., Pan, H., Pandey, A., He, W., Fan, L., Antioxidant and hepatoprotective potential of endopolysaccharides from *Hericium erinaceus* grown on tofu whey, International Journal of Biological Macromolecules, 2012; 51 (5):1140-1146.
5. Wong, J.Y., Abdulla, M.A., Raman, J., Phan, C.W., Kuppusamy, U.R., Golbabapour, S., Sabaratnam, V., Gastroprotective Effects of Lion's Mane Mushroom *Hericium erinaceus* (Bull.:Fr.) Pers. (Aphyllophoromycetideae) Extract against Ethanol-Induced Ulcer in Rats, Evidence-Based Complementary and Alternative Medicine, 2013; vol. 2013, Article ID 492976, 9 pages.
6. Wang, J.C., Hu, SH., Wang, J.T., Chen, K.S., Chia, Y.C., Hypoglycemic effect of extract of Hericium erinaceus, Journal of the Science of Food and Agriculture, 2005; 85(4):641-646.
7. Yang, B.K., Park, J.B., Song, C.H., Hypolipidemic Effect of an Exo-biopolymer Produced from a Submerged Mycelial Culture of *Hericium erinaceus*, Bioscience, Biotechnology, and Biochemistry, 2003; 67(6):1292-1298.
8. Wang, J.C., Hu, S.H., Su, C.H., Lee, T.M., Antitumor and immunoenhancing activities of polysaccharide from culture broth of *Hericium* spp., The Kaohsiung Journal of Medical Sciences, 2001; 17(9):461-467.
9. Liu, J., Du, C., Wang, Y., Yu, Z., Anti-fatigue activities of polysaccharides extracted from *Hericium erinaceus*, Experimental and Therapeutic Medicine, 2015; 9(2):483-487.
10. Diling, C., Chaoqun, Z., Jian, Y., Jian, L., Jiyan, S., Yizhen, X., Guoxiao L., Immunomodulatory Activities of a Fungal Protein Extracted from *Hericium erinaceus* through Regulating the Gut Microbiota, Froniers in Immunology, 2017; 8:666.
11. Kawagishi, H., Ando, M., Sakamoto, H., Furukawa, S., Hericenones C, D and E, stimulators of nerve growth factor(NGF)-synthesis, from the mushroom *Hericium erinaceum*, Tetrahedron Letters 1991; 32(35):4561–4564.
12. Phan, C.W., David, P., Naidu, M., Wong, K.H., Sabaratnam, V., Therapeutic potential of culinary-medicinal mushrooms for the management of neurodegenerative diseases: diversity, metabolite, and mechanism, Critical Reviews in Biotechnology, 2015; 35:355–68.
13. Mori, K., Obara, Y., Moriya, T., Inatomi, S., Nakahata, N., Effects of *Hericium erinaceus* on amyloid beta (25–35) peptide-induced learning and memory deficits in mice, Biomedical Research, 2011; 32(1):67–72.
14. Mizuno, T., Bioactive substances in *Hericium erinaceus* (Bull.:Fr.) Pers. (Yamabushitake), and its medicinal utilization, International Journal of Medicinal Mushrooms, 1999; 1:105–19.
15. Wittstein, K., Rascher, M., Rupcic, Z., Löwen, E.,Winter, B., Köster, R.W., Stadler, M., Corallocins A–C, Nerve Growth and Brain-Derived Neurotrophic Factor Inducing Metabolites from the Mushroom *Hericium coralloides*, Journal of Natural Products, 2016; 79(9):2264-2269.
16. Flegg, P.B., Maw, G., Mushrooms and their possible contribution to the world, Mushroom Journal, 1997; 48:395–403.
17. Lindequist, U., Niedermeyer, T.H.J., Jülich, W.D., The pharmacological potential of mushrooms, Evidence-Based Complementary and Alternative Medicine, 2005; 2:285–299.
18. Smânia, A., Monache, F.D., Loguercio-Leite, C., Albino Smânia, E.F., Lehmkuhl Gerber, A., Antimicrobial activity of basidiomycetes, International Journal of Medicinal Mushrooms, 2001; 3:87.

19. Anke, T., Oberwinkler, F., Steglich, W., Höfle, G., The striatins – New antibiotics from the basidiomycete *Cyathus striatus* (Huds. ex Pers.) Willd., The Journal of Antibiotics (Tokyo), 1977; 30:221-225.
20. Kim, D.M., Pyun, C.W., Ko, H.G., Park, W.M., Isolation of antimicrobial substances from *Hericium erinaceum*, Mycobiology, 2000; 28:33–38.
21. Bhatt, P., R.P. Singh, Sati, S.C., Evaluation of different *Pleurotus* hybrids for their growth requirements in-vitro, Indian Phytopathology, 2010; 63:424-426.
22. Odds, F.C., Sabouraud('s) agar. Journal of Medical and Veterinary Mycology, 2009; 29(6):355-359.
23. Imtiaj, A., Jayasinghe, C., Lee, G.W., et al., Vegetative Growth of Four Strains of *Hericium erinaceus* Collected from Different Habitats, Mycobiology, 2008; 36(2):88-92.
24. Reyes, R.G., Del Rosario, M.G., Padua, J.G., Malonzo, M.C. et al., The first report on the mycelial growth performance and antibacterial activity of *Collybia reinakeana* RGR-FE –NSC strain, a Philippine endemic edible mushroom, International Journal of Pharmaceutical Research & Allied Sciences, 2017; 6(1):113-119.
25. Tambekar, D.H., Sonar, T.P., Khodke, M.V., Khante, B.S., The Novel Antibacterials from Two Edible Mushrooms: *Agaricus bisporus* and *Pleurotus sajor caju*, International Journal of Pharmacology, 2006; 2(5):584-587.
26. Wong, K.H., Sabaratnam, V., Abdullah, N., Kuppusamy, U.R., Naidu, M., Effects of Cultivation Techniques and Processing on Antimicrobial and Antioxidant Activities of *Hericium erinaceus* (Bull.:Fr.) Pers. Extracts, Food Technology and Biotechnology, 2009; 47(1):47–55.
27. Okamoto, K., Shimada, A., Shirai, R., Sakamoto, H., Yoshida, S., Ojima, F., Ishiguro, Y., Sakai, T., Kawagishi, H., Antimicrobial chlorinated orcinol derivatives from mycelia of *Hericium erinaceum*. Phytochemistry, 1993; 34:1445–1446.
28. Basile, A., Sorbo, S., Giordano, S., Lavitola, A., Castaldo Cobianchi, R., Antibacterial activity in *Pleurochaete squarrosa* extract (Bryophyta), International Journal of Antimicrobial Agents, 1998; 10:169–172.
29. Duffy, C.F., Power, R.F., Antioxidant and antimicrobial properties of some Chinese plant extracts, International Journal of Antimicrobial Agents, 2001; 17:527–529.
30. Nostro, A., Germanò, M.P., D'Angelo, V., Marino, A., Cannatelli, M.A., Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity, Letters in Applied Microbiology, 2000; 30:379–384.