ANTIBACTERIAL ACTIVITY OF FRUITING BODY EXTRACTS FROM *Pycnoporus sanguineus* MUSHROOM

Tran Duc Tuong[1*], Pham Ha Thanh Nguyen[1], and Pham Van Hiep[2]

[1]Faculty of Natural Sciences Teacher Education, Dong Thap University

[2]Students Affairs Office, Dong Thap University

[*]Corresponding author: tdtuong@dthu.edu.vn

Article history

Received: 07/5/2021; Received in revised form: 17/6/2021; Accepted: 19/7/2021

Abstract

This study aims to provide an in vitro evidence for the potential antibacterial activity of the ethanolic and aqueous extracts from fruiting bodies of the Pycnoporus sanguineus (L.: Fr.) Murrill mushroom via agar well diffusion method (concentration of extracts 250 mg/mL) and determination of the antibacterial activity and minimum inhibitory concentrations (MIC) of ethanolic and aqueous extracts from the fruiting bodies of this mushroom by agar dilution method (concentration range of 25; 12.5; 6.25; 3.125 and 1.5625 mg/mL). Ampicillin was used as a positive control for these assays. The results shows that the ethanolic and aqueous extracts from fruiting bodies of the P. sanguineus mushroom created the antibacterial halo ring (6 - 11 mm) on five bacteria strains (E. coli, P. aeruginosa, S. faecalis, S. aureus, and K. pneumoniae). The ampicillin created a fairly large antibacterial halo ring (19 - 34 mm) on three strains of E. coli, S. faecalis and S. aureus. The MIC values of two types of extracts from the fruiting bodies of the this mushroom on the five tested strains of bacteria were all ≤ 25 mg/mL. However, the MIC value of the aqueous extract (≤ 6.25 mg/mL) was lower than that of the ethanolic extract (≤ 25 mg/mL). The obtained results indicate that both ethanolic and aqueous extracts from fruiting bodies of the P. sanguineus were able to fight against the five bacteria strains. The P. sanguineus mushroom is potentially used as a natural herbal in antibacterial activity.

Keywords: *Antibacterial activity, antibacterial halo ring, Pycnoporus sanguineus mushroom.*

DOI: https://doi.org/10.52714/dthu.11.5.2022.986

Cite: Tran Duc Tuong, Pham Ha Thanh Nguyen, and Pham Van Hiep. (2022). Antibacterial activity of fruiting body extracts from *Pycnoporus sanguineus* mushroom. *Dong Thap University Journal of Science, 11*(5), 104-111.

HOẠT TÍNH KHÁNG KHUẨN CỦA CAO CHIẾT QUẢ THỂ NẤM VÂN CHI ĐỎ (*Pycnoporus sanguineus*)

Trần Đức Tường[1*], Phạm Hà Thanh Nguyên[1] và Phạm Văn Hiệp[2]

[1]Khoa Sư phạm Khoa học tự nhiên, Trường Đại học Đồng Tháp

[2]Phòng Công tác sinh viên, Trường Đại học Đồng Tháp

[*]Tác giả liên hệ: tdtuong@dthu.edu.vn

Lịch sử bài báo

Ngày nhận: 07/5/2021; Ngày nhận chỉnh sửa: 17/6/2021; Ngày duyệt đăng: 19/7/2021

Tóm tắt

Mục tiêu của nghiên cứu này là cung cấp bằng chứng in vitro về tiềm năng kháng khuẩn của cao chiết ethanol và cao chiết nước quả thể nấm vân chi đỏ (Pycnoporus sanguineus) qua phương pháp khuếch tán chất thử (nồng độ cao chiết 250 mg/mL) qua giếng thạch và xác định nồng độ tối thiểu ức chế vi khuẩn (MIC) của cao chiết ethanol và cao chiết nước quả thể nấm bằng phương pháp pha loãng chất thử trong thạch ở các nồng độ giảm theo cấp số mũ (25; 12,5; 6,25; 3,125 và 1,5625 mg/mL). Ampicillin được sử dụng làm chất đối chiếu cho các thử nghiệm này. Kết quả cho thấy cao chiết ethanol và cao chiết nước quả thể nấm vân chi đỏ tạo được các vòng kháng khuẩn (6 - 11 mm) trên 5 chủng vi khuẩn (E. coli, P. aeruginosa, S. faecalis, S. aureus và K. pneumoniae). Giá trị MIC của cả 2 loại cao chiết trên 5 chủng vi khuẩn thử nghiệm đều ≤ 25 mg/mL. Tuy nhiên, giá trị MIC của cao chiết nước (≤ 6,25 mg/mL) thấp hơn so với cao chiết ethanol (≤ 25 mg/mL). Từ những kết quả thử nghiệm trên cho phép kết luận cao chiết ethanol và cao chiết nước quả thể nấm vân chi đỏ đều có thể chống lại 5 chủng vi khuẩn gây bệnh. Nấm vân chi đỏ có tiềm năng được sử dụng như một loại thảo dược tự nhiên có khả năng kháng khuẩn.

Từ khoá: *Hoạt tính kháng khuẩn, nấm vân chi đỏ, vòng kháng khuẩn.*

1. Introduction

Pycnoporus sanguineus (L.: Fr.) Murrill mushroom has been considered as one of the 25 major medicinal macrofungi worldwide (Boa, 2004). This mushroom is known to be rich in various bioactive substances with antibacterial, antifungal, antiviral, antiparasitic, antioxidant, antiinflammatory, antiproliferative, anticancer, antitumour, cytotoxic, anti-HIV, hypocholesterolemic, antidiabetic, anticoagulant, hepatoprotective, and more other activities (Wasser and Weis, 1999; Ajith and Janardhanan, 2017; Tuong *et al*., 2018). Qualitative phytochemical analysis of the extracts from fruiting bodies of the *P. sanguineus* revealed the presence of flavonoids, saponins, tannins, and terpenoids (Tuong *et al*., 2018). *P. sanguineus* mushroom has also been successfully cultivated on various agricultural by-products such as corn cobs, melaleuca bark and rice husk (Tuong *et al*., 2017; Tuong *et al*., 2019).

In the world, especially developing countries, the problem of antimicrobial (Drug) resistance has become alarming. The burden of treatment costs caused by bacterial infections is quite large due to the replacement of old antibiotics with new, expensive ones. Medicinal herbs and mushrooms are increasingly demonstrating their important roles in the pharmaceutical industry as a biosafety alternative to synthetic chemical drugs (Mahesh and Satish, 2008; Nguyen Thanh Hai and Bui Thi Tho, 2013). The *Pycnoporus sanguineus* mushroom has long been used by indigenous peoples of the tribes of Africa and the Americas to treat a number of diseases and skin lesions (Alibert, 1944; Fidalgo, 1965; Fidalgo and Hirata, 1979; Pérez-Silva *et al*., 1988). Nowadays, the increased level of antibiotics resistance of pathogenic microorganisms requires an attempt to search for alternative drugs from medicinal plants with less adverse effects than that of existing drugs. Therefore, natural medicinal mushroom sources containing compounds with antibacterial effects are prioritized.

2. Materials and methods

2.1. Materials

Pycnoporus sp. mushroom was collected from Tay Ninh province, Vietnam. Based on the DNA sequencing the 606 bp ITS region in combination with morphological characterization of the trimitic hyphal system and basidiocarb, this mushroom was identified as *Pycnoporus sanguineus* (L.:Fr) Murill, and the accession number supplied by NCBI was MH225776. This mushroom was planted on the formula of compost consisting of 50% corn cobs and 50% rubber sawdust at the Biotechnology Research and Development Institute, Can Tho University, Vietnam.

2.2. Chemicals and reagents

Mueller-Hinton agar (Merck, Germany), nutrient broth (Merck, Germany), tryptic soy broth (Merck, Germany), tryptic soy agar (Merck, Germany), ampicillin 500 mg (Mekophar, Vietnam). *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Klebsiella pneumoniae* ATCC 35657 were provided by Saigon Center for Pharmaceutical Science and Technology, Ho Chi Minh University of Medicine and Pharmacy.

2.3. Methods

2.3.1. Extraction

After being crushed, the fruiting bodies of *P. sanguineus* were soaked in solvents (ethanol 96% v/v at room temperature for 72 hours and water at 80ºC for 7 hours) at the ratio of 1:15. Soaking solution was filtered through filter paper. The filtrate was concentrated to remove solvents with a vacuum rotary evaporator (IKA RV 05 Basic, Germany), yielding total ethanolic and aqueous extracts (Nguyen Thi Thu Huong and Nguyen Thi Ngoc Hang, 2010; Nguyen Thi Minh Tu, 2009).

2.3.2. In vitro assay of antibacterial activity of ethanolic and aqueous extracts from the P. sanguineus mushroom by agar well diffusion method

The experiment was performed by agar well diffusion method (concentration 250 mg/mL) described by Andrews (2001). Testing on bacteria included *E. coli, P. aeruginosa, S. faecalis, S. aureus* and *K. pneumoniae*. Evaluating size of antibacterial halo ring (mm) appeared in the agar well diffusion of extracts from the *P. sanguineus* mushroom.

2.3.3. Determination of MIC of ethanolic and aqueous extracts from the fruiting bodies of P. sanguineus mushroom by agar dilution method

The experiment was performed by agar dilution method, whereby exponentially decreasing concentrations of the extracts from the *P. sanguineus* mushroom (concentration range of 25; 12.5; 6.25; 3.125 and 1.5625 mg/mL) and determining minimum inhibitory concentrations of ethanolic and aqueous extracts from the fruiting bodies of *P. sanguineus* mushroom.

3. Results and discussion

3.1. Antibacterial activity of ethanolic and aqueous extracts from the fruiting bodies of the *Pycnoporus sanguineus* mushroom

As shown in Table 1 and Figure 1, ethanolic and aqueous extracts from fruiting bodies of the *P. sanguineus* created the antibacterial halo ring (6 - 11 mm) on five bacteria strains.

Table 1. The diameter of the antibacterial halo ring (mm)

Samples	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*S. faecalis* ATCC 29212	*S. aureus* ATCC 29213	*K. pneumoniae* ATCC 35657
Ethanolic extract	9	8	8	11	6
Aqueous extract	7	6	8	8	9
Control	-	-	-	-	-
Ampicillin	34	-	19	26	-

Notes: Samples (-) without antibacterial activity; the diameter of the antibacterial halo ring includes the diameter of the agar well of 4 mm.

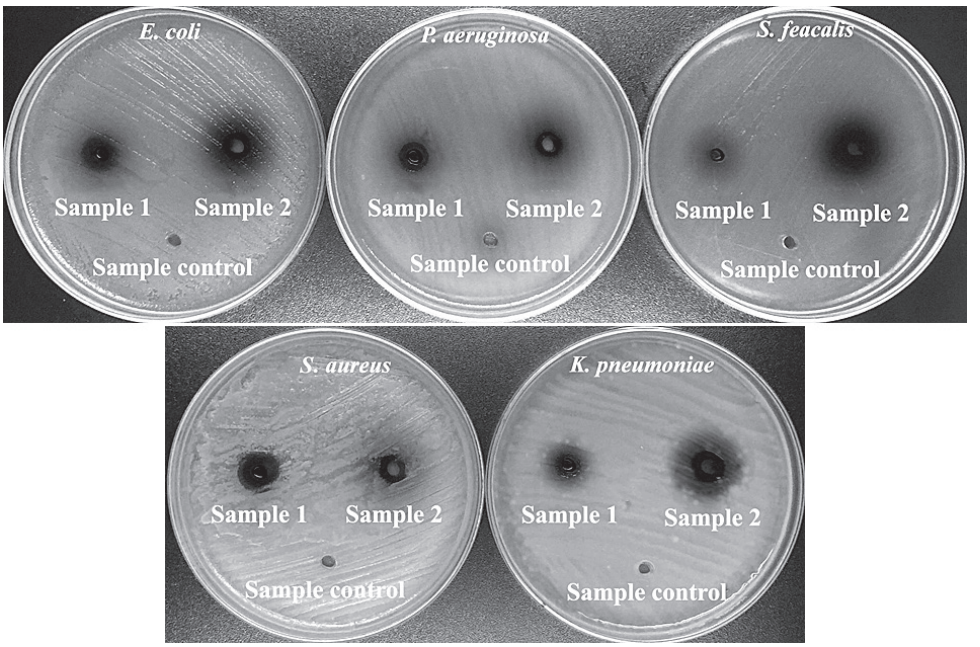

Figure 1. The antibacterial halo ring appeared on assay of antibacterial activity of ethanolic and aqueous extracts from the fruiting bodies of the *P. sanguineus* mushroom

Notes: Sample 1 (ethanolic extract); Sample 2 (aqueous extract); Sample control (distilled water).

The antibacterial activity of the *P. sanguineus* related to biological compounds such as polyphenols, saponins, tannins and triterpenoids has been determined (Tran Duc Tuong *et al*., 2018). Polyphenols become antibiotics due to their ability to complexize with proteins that inactivates the function of that protein in pathogenic bacteria. Polyphenols can also break down bacterial membranes by interacting with membrane lipids (Cushnie and Lamb, 2005). Saponins are considered as natural antibiotic compounds in the protective systems of plants and mushrooms (Hassan *et al*., 2010). Tannins and triterpenoids have a strong antibacterial activity (James *et al*., 2006; Rabi and Bishayee, 2009; Wagner and Elmadfa, 2003).

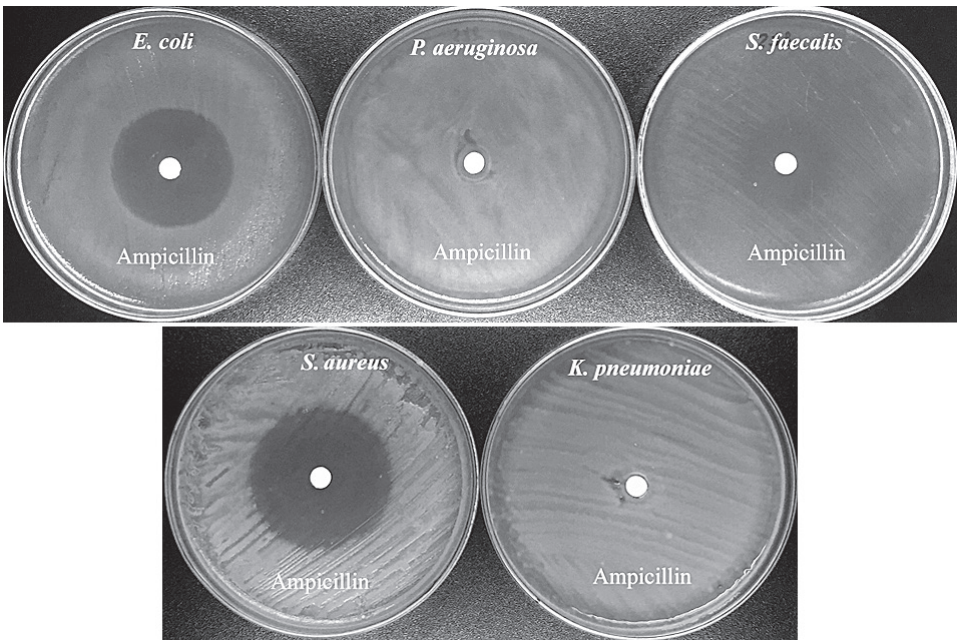

Figure 2. The antibacterial halo ring appeared on assay of ampicillin's antibacterial activity

3.2. Minimum inhibitory concentration (MIC) values of ethanolic and aqueous extracts from the fruiting bodies of the *Pycnoporus sanguineus* mushroom

As seen in Table 2, Figure 3 and Figure 4 show that the minimum bacterial inhibitory concentration (MIC) values of the two types of extracts from the fruiting bodies of the *P. sanguineus* mushroom on five tested strains of bacteria were all ≤ 25 mg/mL. However, the MIC value of the aqueous extract (≤ 6.25 mg/mL) was lower than that of the ethanolic extract (≤ 25 mg/mL). The results of this study are consistent with that of Deka *et al*. (2017) on studying the antibacterial activity of ethanolic extract from the fruiting bodies of three types of mushrooms on the tested bacterial strains (*E. coli, P. aeruginosa, S. aureus*...) with variable MIC values in the concentration range (12.5 - 25 mg/mL for *P. sanguineus, T. versicolor*) and (12.5 - 50 mg/mL for *T. elegans*). The antibacterial activity of ethanolic extract (MIC ≤ 25 mg/mL) and aqueous extract (MIC ≤ 6.25 mg / mL) from *P. sanguineus* are similar to ethanolic extract (MIC = 20 - 30 mg/mL) from *Allium schoenoprasum* on bacteria strains (*E. coli, Pseudomonas sp., S. aureus*) (Le Thi Huong Ha *et al*., 2013), but are higher than that of ethanolic extracts (MIC = 15.63 - 125 mg/mL) and aqueous extract (MIC = 62.5 - 125 mg/mL) from the fruit of *Lycoperdon perlatum* mushroom (Akpi *et al*., 2017).

Table 2. MIC values (mg/mL) of ethanolic and aqueous extracts from the fruiting bodies of the *P. sanguineus* mushroom and ampicillin

Samples	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*S. faecalis* ATCC 29212	*S. aureus* ATCC 29213	*K. pneumoniae* ATCC 35657
Ethanolic extract	12,5	12.5	25	3.125	< 1.5625
Aqueous extract	6.25	6.25	3.125	< 1.5625	< 1.5625
Ampicillin	< 0.000125	-	0.001	< 0.000125	-

Note: Non-determination of MIC (-).

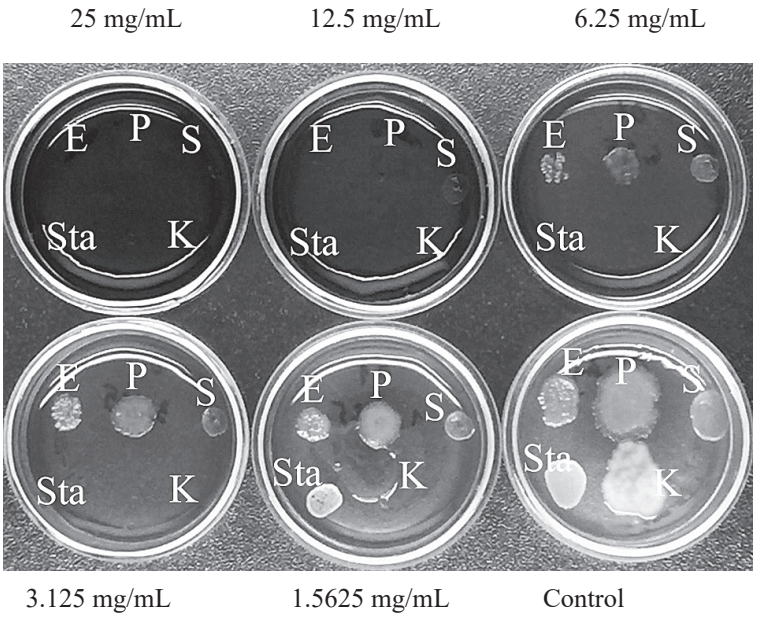

Figure 3. Image of Minimum inhibitory concentration (MIC) test of ethanolic extract

Notes: E. coli (E), P. aeruginosa (P), S. faecalis (S), S. aureus (Sta), K. pneumoniae (K).

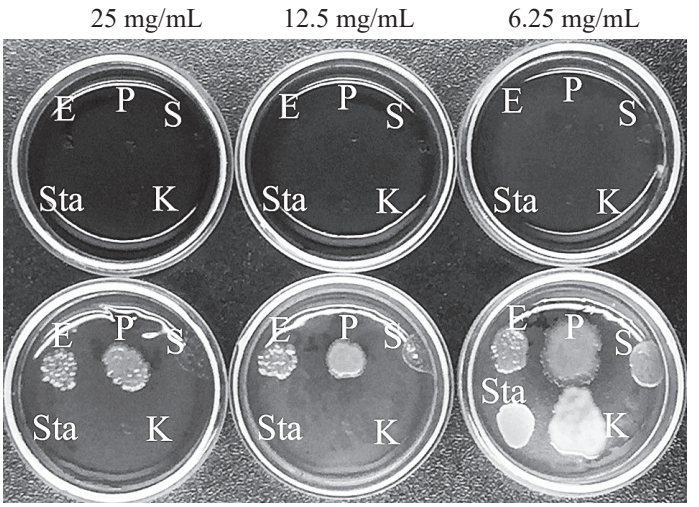

Figure 4. Image of Minimum inhibitory concentration (MIC) test of aqueous extract

Notes: E. coli (E), P. aeruginosa (P), S. faecalis (S), S. aureus (Sta), K. pneumoniae (K).

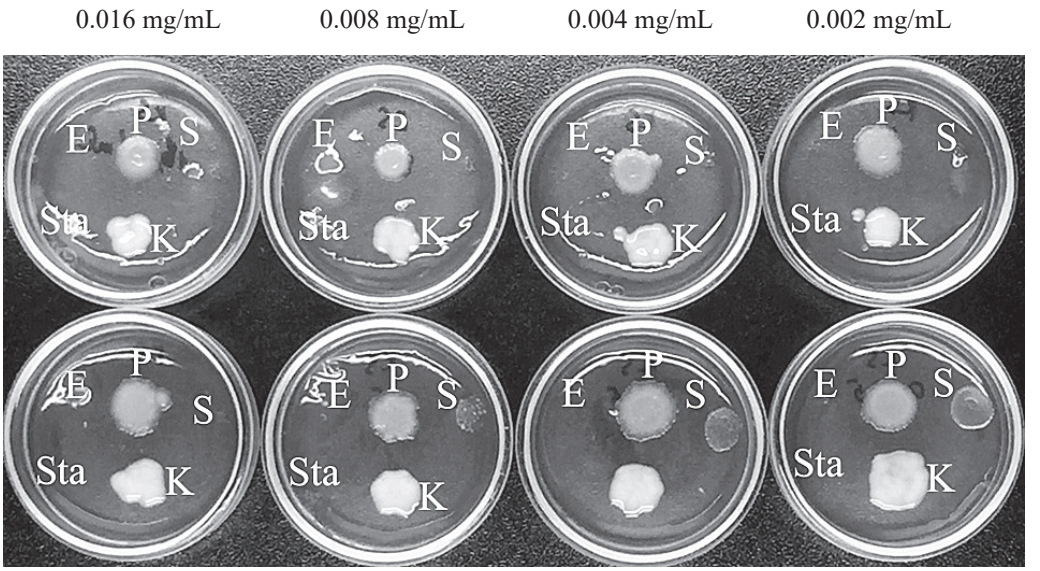

Figure 5. Image of Minimum inhibitory concentration (MIC) test of ampicillin

Notes: E. coli (E), P. aeruginosa (P), S. faecalis (S), S. aureus (Sta), K. pneumoniae (K).

4. Conclusion

Pycnoporus sanguineus (Trametes sanguinea) shows the potential to be used as a natural source of antibacterial compounds. The results prove that both ethanolic and aqueous extracts from fruiting bodies of the *P. sanguineus* are able to fight against five bacteria strains (*E. coli, P. aeruginosa, S. faecalis, S. aureus and K. pneumoniae*). Hence, it could be of great importance to develop further studies addressing the issues such as the purification and identification of these compounds responsible for antibacterial activity of *P. sanguineus* mushroom.

Acknowledgments

This research is supported by the project SPD2020.01.08 from Dong Thap University in 2020./.

References

Ajith, T.A. and Janardhanan, K.K. (2007). Indian medicinal mushrooms as a source of antioxidant and antitumor agents. *Journal of Clinical Biochemistry and Nutrition, 40*, 157-162.

Akpi, U.K., Odoh, C.K., Ideh, E.E., and Adobu, U.S. (2017). Antimicrobial activity of *Lycoperdon perlatum* whole fruit body on common pathogenic bacteria and fungi. *African Journal of Clinical and Experimental Microbiology, 18*(2), 79-85.

Alibert, H. (1944). Note sar les champignons poussant dans le bas Dahomey et sur deux agaricinees estimdes des indig/~nes de cette meme region. *Notes Africaines, 22*, 11-12.

Andrews, J.M. (2001). BSAC standardized dics susceptibility testing method. *Journal of Antimicrobial Chemotherapy, 48*, 43-57.

Boa, E. (2004). Wild edible fungi: A global overview of their use and importance to people (Non-wood forest products series No. 17). Forestry Department, Rome, Italy: FAO, 147 pp.

Cushnie, T.P.T. and Lamb, A.J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents, 26*, 343-356.

Deka, A.C., Indrani, S., Sneha, D., and Sarma, T.C. (2017). Antimicrobial properties and phytochemical screening of some wild macrofungi of Rani - Garbhanga Reserve forest area of Assam, India. *Advances in Applied Science Research Journal, 8*(3), 17-22.

Fidalgo, O. (1965). Conhecimento micolegico dos indios brasileiros. *Rickia, 2*, 1-10.

Fidalgo, O. and Hirata, J.M. (1979). Etnomicologia caiabi, txicffo e txucarrarnffe. *Rickia, 8*, 1-5.

Hassan, S.M., Haq, A.U., Byrd, J.A., Berhowd,

M.A., Cartwrightb, A.L., and Bailey, C.A. (2010). Haemolytic and antimicrobial activities of saponin-rich extracts from guar meal. *Food Chemistry, 119*, 600-605.

James, T.Y. *et al*. (2006). Reconstructing the early evolution of fungi using a six-gene phylogeny. *Nature, 443*(7113), 818-822.

Le Thi Huong Ha, Pham Thu Thuy, and Vu Ngoc Boi. (2013). Study extraction and investigation of antibacterial, antioxidant activity of *Allium schoenoprasum. Journal of Fisheries Science and Technology, 4*, 88-94.

Mahesh, B. and Satish, S. (2008). Antimicrobial activity of some important medicinal plants against animal and human pathogens. *World Journal of Agricultural Sciences, 4*(5), 839-843.

Nguyen Thanh Hai and Bui Thi Tho. (2013). Study on *in vitro* antimicrobial activity of garlic extract (*Allium sativum* L.) on pathogenic *E. coli* and ampicillin-resistant *E. coli*, kanamycin. *Science and Technology Development Journal, 11*(6), 804-808.

Nguyen Thi Minh Tu. (2009). The process of extracting bioactive substances from *Ganoderma lucidum. Journal of Science and Technology, 47*(1), 45-53.

Nguyen Thi Thu Huong and Nguyen Thi Ngoc Hang. (2010). Study on antioxidant activity towards liver protection of *Ganoderma lucidum. Ho Chi Minh City Medical Journal, Specialist in Traditional Medicine, 14*(2), 129-134.

Pérez-Silva, E., Aguirre-Acosta, E., and Perez-Amador, C. (1988). Aspectos sobre el uso y distribucion de *Pycnoporus sanguineus* (Polyporaceae) en Mexico. *Revista Mexicana de Micologia, 4*, 137-144.

Rabi, T. and Bishayee, A. (2009). Terpenoids and breast cancer chemoprevention. *Breast Cancer Res Treat, 115*, 223-239.

Tran Duc Tuong, Duong Xuan Chu, and Bui Thi Minh Dieu. (2018). Hypoglycemic activity of fruiting body extracts from *Pycnoporus sanguineus* (L.: Fr.) Murrill mushroom. *Academia Journal of Biology, 40*(3), 37-44. DOI: 10.15625/2615-9023/v40n3.13146.

Tran Duc Tuong, Duong Xuan Chu, and Bui Thi Minh Dieu. (2017). Effect of the replacement of rubber sawdust by corn cobs on culturing mushroom *Pycnoporus sanguineus, Journal of Vietnam Agricultural Science and Technology, 85*(12), 98-103.

Tran Duc Tuong, Vo Thi Thu Duyen, Duong Xuan Chu, and Bui Thi Minh Dieu (2019). Effects of the replacement of rubber sawdust by melaleuca bark for *Pycnoporus sanguineus* (L.: Fr.) Murr. mushroom cultivation, *Can Tho University Journal of Science, 55*(2), 186-190.

Tran Duc Tuong. (2019). Production of *Pycnoporus sanguineus* on substrates of corn cobs and rice hulls, *Science and Technology Journal of Agriculture and Rural Development, 19*(370), 29-35.

Wagner, K.H. and Elmadfa, I. (2003). Biological relevance of terpenoids: Overview focusing on mono-di and tetraterpenes. *Annals of Nutrition and Metabolism, 47*, 95-106.

Wasser, S.P., Weis, A.L. (1999). Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives (review). *International Journal of Medicinal Mushrooms, 1*, 31-62.