OVERVIEW OF MORPHOLOGY, DISTRIBUTION, CHEMICAL COMPOSITION, BIOLOGICAL ACTIVITIES AND CULTURE MEDIUM OF *Xylaria nigripes*

Nguyen Thi Ngoc Nhi(1), Tran Minh Trung(1)
(1) Thu Dau Mot university
Corresponding author: nhintn@tdmu.edu.vn

DOI: 10.37550/tdmu.EJS/2025.02.659

Article Info

Volume: 7
Issue: 2
Jun: 2025
Received: May. 19th, 2025
Accepted: May. 28th, 2025
Page No: 610-617

Abstract

Xylaria nigripes is a rare medicinal mushroom in the Xylariaceae family, which has long been used in traditional medicine to aid in treating conditions such as insomnia, neurasthenia, and inflammation. This fungus usually grows in an environment characterized by termite nests. Recent studies have shown that X. nigripes contains many valuable biological compounds such as polysaccharides, nucleosides, and sterols, which provide important biological effects, such as antioxidants, liver protection, immune system regulation, and diabetes treatment. In addition to pharmacological potential, many research works have focused on developing X. nigripes biomass kernel techniques under artificial culture conditions, in order to optimize growth and accumulation of active ingredients. These results not only contribute to clarifying the application potential of this mushroom in the pharmaceutical field but also create a scientific foundation for the sustainable exploitation of this rare medicinal resource.

Keywords: biological activity, biological compounds, chemical composition, culture medium, *Xylaria nigripes*

1. Introduction

Xylaria nigripes is a valuable medicinal mushroom in the Xylariaceae family. In traditional medicine, *X. nigripes* are used to treat insomnia and trauma, *X. nigripes* is also used as a nerve tonic (Lin et al., 2013; Divate et al., 2017; Lai et al., 2021; Mouffouk et al., 2023; Ngo et al., 2024). Aqueous extract and 70% ethanol extract of *X. nigripes* showed an effective protective effect on PC12 cells against H2O2 causing cell damage by inhibiting lactate dehydrogenase release, reducing DNA damage, restoring mitochondrial membrane potential, and preventing abnormal apoptosis through the regulation of increased Bcl-2 and decreasing regulation of Bax and caspase 3. Ethanol extract of Oleander mushroom, in addition to better neuroprotective effects, also has higher antioxidant activity by removing 2,2-Diphenyl-1-picrylhydrazyl radicals, inhibiting lipid oxidation, and reducing energy (Zhao et al., 2014; Chen et al., 2014; Peng et al., 2015; Huang et al., 2015; Divate et al., 2017; Jeong et al., 2021).

Analyzing the active ingredient in *X. nigripes*, two new compounds were detected, xylariamino acid A and phenethyl ester. acid A and phenethyl ester. These compounds have neuroprotective effects against PC12 cell damage caused by hypoxia and glucose. Furthermore, phenethyl ester compounds can significantly reduce the rate of programmed cell death (Long et al., 2021; Li et al., 2021). In the world, pharmacological studies of *X. nigripes* do not only stop at chemical composition and classification but focus on discovering new active ingredients that fight cancer, anti-inflammatory, protect the liver and kidneys. In Vietnam, research on *X. nigripes* is still limited, there are very few studies on biomass culture, and isolation of compounds for application in medicinal materials. Therefore, it is necessary to have an overview of the morphology, distribution, chemical composition, biological activity, and culture environment of *X. nigripes*.

2. Morphology and distribution of *X. nigripes*

The morphology and distribution of the Euphorbia mushroom is a topic of interest in mycological research, especially the ecological significance and diversity in the genus *Xylaria*. Angiosperms are classified according to the subgenus *Pseudoxylaria*, which has been recorded in various ecological niches, especially in relation to termite nests and soil environments. A study conducted in northeastern Thailand highlighted the morphological characteristics of the *X. nigripes*, highlighting the distinct characteristics that distinguish it from other species in the *Xylaria complex* (Niwana Wangsawat et al., 2021). The morphological diversity of the Oleander mushroom manifests different variations, possibly due to being influenced by environmental factors (Karun et al., 2015). The distribution of the Oleander mushroom is not limited to a specific geographical area, it has been reported in various regions, including Southwest India and some regions of Northeast India (Karun et al., 2015; Ramesh Veluchamy, 2012).

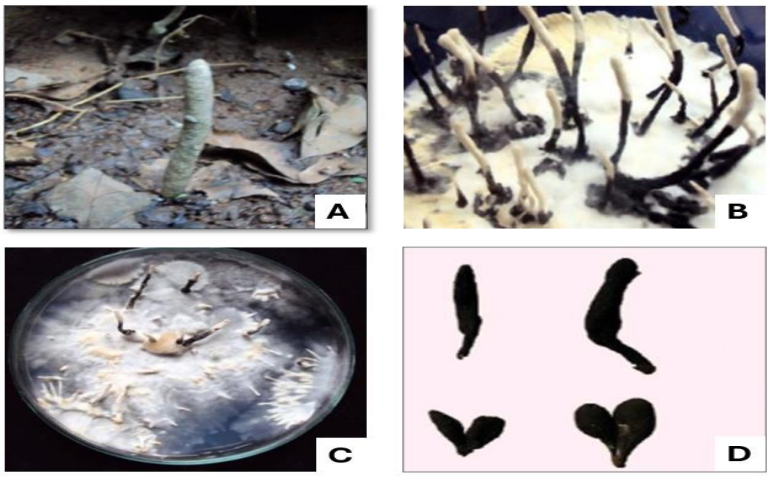

Figure 1. Macro and micro characteristics of X. nigripes (A. Natural habitat of fruiting bodies; B. Liquid culture morphology (PDB) in a 500ml triangle; C. Culture morphology on PDA plates (diameter 9cm) after 2 weeks of incubation; D. Morphological transformation of fruiting bodies). (Ramesh et al., 2012)

Oyster mushrooms belong to a large group of species in the genus Xylaria, which are capable of expressing similar morphological characteristics, reflecting complex evolutionary relationships between species in the genus (Okane et al., 2025). The global distribution of species *Xylaria* suggests that X. *nigripes* has a wider ecological range

than previously recorded (Zhu et al., 2024). Morphological studies have shown that Oleander mushroom exhibits characteristic morphological characteristics, which play an important role in identifying species in the genus Xylaria. These traits have been systematically described in taxonomic studies, demonstrating the usefulness of traditional morphology in establishing species identity. However, due to the presence of many morphological similarities between species in the genus, the application of phylogenetic analyses based on molecular data has become an essential aid tool, helping to elucidate intrinsic evolutionary relationships and determine species boundaries more accurately (Okane et al., 2025). Thanks to a combination of morphological methods and modern molecular biology techniques, the taxonomic position of the *X. nigripes* has been clearly established, thereby confirming its specificity and differentiation from closely related species (Latha et al., 2015).

3. Chemical composition in *X. nigripes*

The chemical composition of the Oleander mushroom includes many bioactive compounds, which contributes to the pharmacological properties of this mushroom. Recent studies have identified several major compounds, such as polysaccharide, ergosterol, cholesterol, along with various nucleosides including adenosine, adenine, uridine, and guanosine (Long et al., 2023; Yu et al., 2023). These compounds are believed to play an essential role in bringing about the therapeutic effects of X. nigripes.

In 2016, author Juan Xiong et al. published nineteen compounds found in X. nigripes, mainly consisting of pyrrole-containing alkaloids and phytosterols isolated from EtOH extract of X. nigripes. On the basis of spectroscopic methods, the structure of the new natural compounds was identified as (4S)-3,4-dihydro-4-(4-hydroxybenzyl)-3-oxo-1Hpyrrolo[2,1-c][1,4]oxazine-6-carbaldehyde;methyl(2S)-2-[2-formyl-5(hydroxymethyl)-1H-pyrrol-1-yl]-3-(4hydroxyphenyl)propanoate and 3-{4-[(2R)-(2,3-dihydroxy-3-methylbutoxy]phenyl}-7-hydroxy-4H-chromen-4-1.

Polysaccharide is one of the most studied components of X. nigripes, which has immunomodulatory and anti-inflammatory properties. Studies have shown that polysaccharides extracted from X. nigripes can enhance the immune response and anti-inflammatory protective effects (Divate et al., 2017; Song et al., 2021). The specific structure and mechanism of action of these polysaccharides is still being studied, but their presence is an important factor in the medicinal efficacy of mushrooms.

In addition to polysaccharides, X. nigripes also contains significant levels of fatty acids and compounds belonging to the sterol group, typically ergosterol and cholesterol. In particular, ergosterol is known as a biological precursor of vitamin D_2, and has been shown to have pronounced antifungal properties (Yu et al., 2023). The presence of these components not only enriches the chemical profile of the species, but also opens up the potential for application in the healthcare field, especially in the prevention and treatment of neurological disorders. Specifically, sesquiterpenoids belonging to the brasilane group isolated from Oleander mushrooms have shown significant neuroprotective activity in preclinical research models (Long et al., 2024).

4. Biological activity of *X. nigripes*

The biological activities of the Oleander mushroom play an important role in establishing the medicinal value of this mushroom. Numerous studies have shed light on the

outstanding pharmacological properties of *X. nigripes*, including antioxidant, anti-inflammatory, and neuroprotective activity. Specifically, extracts from this mushroom have been shown to effectively protect cells against the effects of oxidative stress on various cell lines, typically PC12 neurons, through the mechanism of inhibiting apoptosis and enhancing cell survival (Divate and cs, 2017; Long et al., 2024). In addition, Oo hyphae also showed significant anti-inflammatory effects, as shown by the ability to inhibit the production of pro-inflammatory cytokines such as TNF-α and IL-6 in LPS-stimulated macrophage models (Divate et al., 2017; Jen et al., 2021). These findings reinforce the potential application of *X. nigripes* in the prevention and treatment of diseases related to oxidative stress and inflammation.

X. nigripes have been noted to possess powerful antioxidant properties. In a comparative study, Ko and colleagues evaluated the antioxidant activity and free radical clearance of cultured olive mycelium extracts compared to a commercial product derived from this mushroom (XNP). The results showed that the cultured mycelium extract exhibited significantly higher overall antioxidant efficacy, superior to Trolox and vitamin C in in vitro model systems. The authors suggest that this activity is mainly related to the high polyphenol content, rather than due to polysaccharides. In addition to these findings, Hung and colleagues isolated and evaluated the activity of intracellular polysaccharides (XnIPS-1) from Olive mycelium, showing that the compound had the most potent reducibility among the antioxidant components analyzed.

Divate et al. (2017) noted the potential to enhance antioxidant activity in fermented cereal products with *X. nigripes*, in which fermented red beans showed the highest biological efficiency. Antioxidant activity is evaluated through indices including DPPH free radical clearance, lipid peroxidation inhibition, and reduction potential.

In addition to the neuroprotective effects, the hepatoprotective effect of *X. nigripes* has also been highly determined. Song et al. (2010) demonstrated that extracts from this fungus can significantly reduce liver damage caused by carbon tetrachloride (CCl_4) in experimental mouse models. This hepatoprotective effect is thought to be related to the presence of polyphenol compounds in the chemical composition of the mushroom, suggesting the potential application of *X. nigripes* as a biological agent in aiding the treatment of liver damage caused by oxidative stress.

In addition to its liver-protective properties, *X. nigripes* has been shown to have an immunomodulatory effect. Author Ko et al. (2011) studied the effects of water and ethanol extracts of Oleander on peritoneal macrophage cells in Balb/c mice, showing that these extracts can modulate the immune response, especially in the context of lipopolysaccharide (LPS) stimulation. This suggests that *X. nigripes* can enhance immune function, making it a candidate for further exploration in immunotherapy.

Recent studies have shown that Oleander mushroom possesses significant neuroprotective potential, especially in the context of cognitive impairment associated with sleep deprivation and REM sleep disorders. Zhao et al. (2014) demonstrated that Oleander mushroom extract has the ability to improve memory impairment in a sleep-deprived mouse model, thereby showing the potential role of this fungus in regulating cognitive function and protecting the central nervous system.

In 2021, Li et al olated and identified a series of naphthalenone derivatives from *X. nigripes*. These compounds exhibit pronounced neuroprotective activity on the PC12 neuronal strain through an antioxidant mechanism, suggesting the ability to reduce

neuronal damage caused by oxidative agents. These results contribute to elucidating the biological role of secondary compounds in *X. nigripes*, and strengthen the application potential of this mushroom in supporting the treatment of neurological disorders associated with cognitive impairment.

Recent pharmacological studies have clarified the neuroprotective potential of Oleander mushrooms through the isolation and evaluation of the biological activity of natural compounds. Li et al. (2021) successfully isolated five pairs of optical isomers belonging to the naphthalenone derivatives group from *X. nigripes*. Among them, xylarinaps A, B, D, and E compounds showed the ability to significantly improve the survival rate of PC12 cells damaged by oxygen and glucose deprivation (OGD). At the same time, these compounds significantly reduce levels of malondialdehyde (MDA) – an indicator of lipid peroxidation damage – and increase the activity of endogenous antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). These results suggest the potential neuroprotective role of naphthalenone derivatives in the model of neuronal damage due to oxidative stress.

Long et al. (2024) isolated five sesquiterpenoids belonging to the brasilane structural framework from *X. nigripes*. These compounds exhibit significant protective effects on the PC12 cell line under glutamate toxicity, which is manifested in increasing cell survival and strongly inhibiting apoptosis. In addition, these sesquiterpenoids reduce the concentration of oxidative stress markers and limit the accumulation of reactive oxygen species (ROS). The above findings show the potential application of compounds isolated from X. nigripes in the research and development of therapies for neuropathy associated with oxidative stress and neurodegeneration.

Divate authors also confirm the neuroprotective potential is enhanced in grains fermented from Olives, with red beans exhibiting the highest bioactivity. Neuroprotective activity is assessed by the PC12 protective test. In addition, the hypoglycemic effects of Oleander mushrooms have been studied, especially in the context of insulin resistance. (Chen et al., 2015) have reported that water extract of Oleander significantly reduces plasma glucose levels in steroid-induced insulin-resistant mice, suggesting a potential role in the control of diabetes and metabolic disorders.

The anti-inflammatory activity of *X. nigripes* has been extensively studied. In 2011, Huey-Jiun Ko and colleagues discovered the immunomodulatory compounds of water extract (XN-H) and ethanol (XN-E) in peritoneal macrophages induced by lipopolysaccharide (LPS). The results indicated that both XN-H and XN-E inhibited the production of inflammatory mediators such as nitric oxide (NO), prostaglandin E2 (PGE2), and cytokines (IL-1β, IL-6, TNF-α, and IFN-γ). XN-H exhibits stronger potency, possibly due to its higher β-glucan content.

Recent studies have provided further evidence of the anti-inflammatory activity of *X. nigripes* in in vitro and in vivo models. Divate et al. demonstrated that Olive mycelium extract, including hot water extract and 70% ethanol extract, is capable of effectively inhibiting the production of inflammatory mediators such as nitric oxide (NO), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) in the RAW264.7 macrophage cell line stimulated by lipopolysaccharide (LPS). At the same time, these extracts also promote phagocytic activity and increase the secretion of interleukin-10 (IL-10), a cytokine that has anti-inflammatory effects.

In another study, Ching-Fu Chen and colleagues compared the anti-inflammatory efficacy of water-insoluble polysaccharides isolated from two cultured strains of *X. nigripes*. The results showed that both strains inhibited the production of inflammatory mediators, with the TXNP strain showing significantly stronger efficacy.

Chang et al. (2017) isolated two compounds with high anti-inflammatory activity from Oleander mushrooms, nigriperne C and alcoho fomannoxin. Both compounds showed potent inhibition of the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), and reduced NO production in BV-2 microglia cells, a line of glial cells isolated from mouse brains.

Divate and colleagues also confirmed that fermented cereal products with *X. nigripes*, especially red beans, exhibit enhanced anti-inflammatory activity. This effect was evaluated through the measurement of NO concentrations in RAW264.7 cells after treatment with LPS, and through inhibition of COX-2 enzyme activity. These results show the potential application of Oleander mushrooms in developing functional products and therapeutic agents for inflammatory diseases.

Li et al. (2015) isolated and determined the structure of several novel alkaloids from the *X. nigripes*, and demonstrated that these compounds are directly related to the beneficial biological effects of this mushroom. These findings play an important role in clarifying the chemical basis of previously documented therapeutic effects. In addition to the above research direction, Chen et al. (2018) conducted an evaluation of the physicochemical characteristics and anti-inflammatory activity of water-insoluble polysaccharides, isolated from different strains of *X. nigripes*. The results suggest that differences in the structural composition of polysaccharides between strains can lead to significant changes in anti-inflammatory potency. This finding emphasizes the importance of selecting appropriate fungal strains in the research and development of highly biologically active preparations, especially in the context of their therapeutic application for diseases related to inflammation and immune disorders.

5. Studies on the fruiting environment and biomass increase of *X. nigripes*

X. nigripes have received significant interest in research due to its potential applications in traditional medicine and functional medicine. In particular, optimizing culture conditions to increase biomass and fruiting is an important approach to exploit bioactive compounds from this fungus.

One of the methods of culture of interest is submersible culture in a liquid medium. This technique allows for flexible control of growth parameters and has been proven to be effective in improving mycelium biomass yield. Dulay et al. (2021) showed that adjusting physical factors (such as shaking speed) and nutrient composition of the culture medium can optimize the growth of *X. nigripes*'s mycelium, thereby improving the efficiency of accumulating bioactive substances for research and application.

In addition to submersible cultures, solid culture methods, typically dextrose potato jelly (PDA), have also been used to isolate and conserve mycelium from natural fruiting bodies. Compounds extracted from cultured mycelium under these conditions have shown significant antimicrobial activity (Long et al., 2023). Therefore, it is essential to develop an effective culture process in a variety of environments, not only to serve the research goal but also to expand the possibilidty of commercial application, especially in the production of medicinal preparations from *X. nigripes*.

6. Conclusion

X. nigripes is a rare medicinal mushroom with biological potential and wide application in traditional and modern medicine. Current studies show that this mushroom contains many valuable bioactive compounds such as polysaccharides, nucleosides, sterols, and rare alkaloid derivatives, which contribute to outstanding bioactive properties such as antioxidant, anti-inflammatory, neuroprotective, immunomodulatory, and diabetes treatment. In particular, the results of the neuroprotective activity of *X. nigripes* in cell damage and cognitive impairment models open up potential applications in the treatment of neurological diseases. In addition, the optimization of biomass and fungal fruiting conditions in an artificial environment has created a premise for the sustainable exploitation of this rare medicinal resource, and at the same time promoted the possibility of large-scale production for the pharmaceutical industry. However, more in-depth research is still needed to clarify the mechanism of action, treatment effectiveness on clinical models and standardize bioactive compounds. These research directions will not only improve the application value of *X. nigripes* but also contribute to expanding the treasure of medicinal herbs from mushrooms in current medicine.

References

Chaima Mouffouk, S., Mouffouk, S., Mouffouk, S., & Haba, H. (2023). Traditional use, phytochemistry and pharmacological properties of the genus *Onopordum*. *Current Chemical Biology*, *17*(2), 124-139.

Chang, J. C., Hsiao, G., Lin, R. K., Kuo, Y. H., Ju, Y. M., & Lee, T. H. (2017). Bioactive constituents from the termite nest-derived medicinal fungus *Xylaria nigripes*. *Journal of Natural Products*, *80*(1), 38-44.

Chen, C. F., Su, C. H., Lai, M. N., & Ng, L. T. (2018). Differences in water soluble non-digestible polysaccharides and anti-inflammatory activities of fruiting bodies from two cultivated *Xylaria nigripes* strains.

Chen, J. Z., Lo, H. C., Lin, F. Y., Chang, S. L., Hsieh, C. W., Liang, Z. C., & Hsu, T. H. (2014). Effects of medium components and culture conditions on mycelial biomass and the production of bioactive ingredients in submerged culture of *Xylaria nigripes* (Ascomycetes), a Chinese medicinal fungus.

Chen, Y. I., Tzeng, C. Y., Cheng, Y. W., Hsu, T. H., Ho, W. J., Liang, Z. C., & Chang, S. L. (2015). The involvement of serotonin in the hypoglycemic effects produced by administration of the aqueous extract of *Xylaria nigripes* with steroid-induced insulin-resistant rats. *Phytotherapy Research*, *29*(5), 770-776.

Divate, R. D., Wang, C. C., Chou, S. T., Chang, C. T., Wang, P. M., & Chung, Y. C. (2017). Using wheat bran and soybean meal as solid-state fermentation substances for the production of *Xylaria nigripes* with bioactivities. *Journal of the Taiwan Institute of Chemical Engineers*, 70, 127-133.

Divate, R. D., Wang, P. M., Wang, C. C., Chou, S. T., Chang, C. T., & Chung, Y. C. (2017). Protective effect of medicinal fungus *Xylaria nigripes* mycelia extracts against hydrogen peroxide-induced apoptosis in PC12 cells. *International Journal of Immunopathology and Pharmacology*, *30*(1), 105-112.

Dulay, R. M. R., Cabrera, E. C., Kalaw, S. P., & Reyes, R. G. (2021). Optimization of submerged culture conditions for mycelial biomass production of fourteen *Lentinus* isolates from Luzon Island, Philippines. *Biocatalysis and Agricultural Biotechnology*, 38, 102226.

Huang, C., Lin, Y., Su, H., & Ye, D. (2015). Forsythiaside protects against hydrogen peroxide-induced oxidative stress and apoptosis in PC12 cells. *Neurochemical Research*, 40, 27-35.

Hung, M. C., Tsai, C. C., Hsu, T. H., Liang, Z. C., Lin, F. Y., Chang, S. L., & Hsieh, C. W. (2015). Biological activities of the polysaccharides produced from different sources of *Xylaria nigripes* (Ascomycetes), a Chinese medicinal fungus. *International Journal of Medicinal Mushrooms*, *17*(2).

Izumi Okane, H. M. H., Yu-Ming Ju, C. R. L., Chun-Yun Huang, I. C. K. (2025). *Xylaria iriomotensis* sp. nov. from termite nests and notes on *X. angulosa*. *Botanical Studies*, 66.

Jen, C. I., Su, C. H., Lu, M. K., Lai, M. N., & Ng, L. T. (2021). Synergistic anti-inflammatory effects of different polysaccharide components from *Xylaria nigripes*. *Journal of Food Biochemistry*, *45*(4), e13694.

Ko, H. J., Song, A., Lai, M. N., & Ng, L. T. (2009). Antioxidant and antiradical activities of Wu Ling Shen in a cell-free system. *The American Journal of Chinese Medicine*, *37*(4), 815-828.

Lai, M. N., Hsu, H. C., & Ng, L. T. (2021). Safety assessment of the standardized aqueous extract from solid-state cultured *Xylaria nigripes* (Wuling Shen) in rats. *Clinical Phytoscience*, *7*(1), 44.

Li, J., Li, L. Q., Long, H. P., Liu, J., Jiang, Y. P., Xue, Y., & Liu, J. K. (2021). Xylarinaps A–E, five pairs of naphthalenone derivatives with neuroprotective activities from *Xylaria nigripes*. *Phytochemistry*, 186, 112729.

Peng, W. F., Wang, X., Hong, Z., Zhu, G. X., Li, B. M., Li, Z., & Zhan, S. K. (2015). The anti-depression effect of *Xylaria nigripes* in patients with epilepsy: A multicenter randomized double-blind study. *Seizure*, 29, 26-33.

Wangsawat, N., Ju, Y. M., Phosri, C., Whalley, A. J., & Suwannasai, N. (2021). Twelve new taxa of *Xylaria* associated with termite nests and soil from northeast Thailand. *Biology*, *10*(7), 575.

Yu, H., Han, G., Sun, M., & Ma, T. (2023). Potential targets and pharmacological effects of Wuling Capsule on Alzheimer's disease: A network pharmacology-based analysis. *Future Integrative Medicine*, *2*(1), 10-22.

Zhu, A. H., Song, Z. K., Wang, J. F., Guan, H. W., Qu, Z., & Ma, H. X. (2024). Multi-gene phylogenetic and taxonomic contributions to *Xylaria* (Ascomycota) associated with fallen fruits from China. *MycoKeys*, 106, 23.