EFFECTS OF ILLUMINATION ON THE VITAMIN D AND SECONDARY METABOLITE CONTENTS OF THE OYSTER MUSHROOM, *Pleurotus ostreatus*. (Jacq.)P.Kumm.

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SR/2304/RPR/25/46

UNIVERSITY OF BENIN

BENIN CITY

OCTOBER, 2025.

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (BSC.) IN PLANT BIOLOGY AND BIOTECHNOLOGY.

STUDENT'S THESIS

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CERTIFICATION

This is to certify that this project work was carried out by OSUOJI Chukwuemeka Oluwatimilehin in the department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Nigeria.

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(Project Supervisor)

Signed: ---------- ----------

Prof. B. IKHAJIABGE Date

(Head of Department)

DEDICATION

This work is dedicated to the Almighty God for His endless mercy, guidance, and blessings throughout the course of this study. I also dedicate it to my parents, Retired Captain Kelechi Osuoji and Mrs. Olasumbo Osuoji, for their unwavering love, continuous support, and generous provision, which made the successful completion of this work possible..

ACKNOWLEDGEMENTS

Firstly, I want to express my gratitude to the Almighty God for His grace and mercies, throughout the duration of this project. Without Him, none of this would have been possible. I am deeply grateful to my supervisor, Prof E.O.Akpaja, for his continuous guidance, unwavering support, encouragement and valuable advice throughout this research work. Your patience and insightful advice have been instrumental to the success of this project.

My sincere appreciation also goes to Dr. (Mrs.) Adeogun and Miss Faith for their help, assistance, and valuable insights towards the successful completion of this project.

Special thanks as well to Head of Department, Prof. B. Ikhajiagbe for his leadership and contribution to the academic excellence of the department. I also wish to thank all my lecturers and the entire staff of the Department of Plant biology and biotechnology, University of Benin, for imparting knowledge, guidance, and support throughout my academic journey.

My deepest gratitude goes to my parents, retired Captain Kelechi Osuoji and Mrs. Olasumbo Osuoji, and to my siblings,for their unwavering love, prayers, and support. Your encouragement has been my greatest motivation. Finally, I extend my appreciation to all my friends and colleagues who assisted me in various ways during this project. Thank you all for your kindness and support.

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ABSTRACT

This study examined the effects of illumination on the vitamin D and secondary metabolite contents of *Pleurotus ostreatus* (oyster mushroom) under different exposure durations. Fresh samples of the mushroom were subjected to varying illumination periods using both sunlight and ultraviolet (UV) light for 1, 2, 4, 6, and 8 hours. Ethanol extracts of the samples were subsequently analyzed for vitamin D and secondary metabolite contents. The results revealed that exposure to sunlight for six hours yielded the highest vitamin D content, indicating this as the optimum illumination period for vitamin D synthesis in *P. ostreatus*. Conversely, exposure to ultraviolet light showed inconsistent effects, with prolonged exposure leading to denaturation and a decline in vitamin D concentration. The impact of illumination on secondary metabolite constituents varied according to the observed secondary metabolite, light-sensitive compounds such as flavonoids and phenols decreased with extended exposure with flavonoids showing an optimum value display at 6 hours (0.39 mg/g) but decreased slightly to 0.36 mg/g after 8 hours, while saponins and terpenoids increased, possibly due to the softening of tissue cells that enhanced their production. Cardiac glycosides, being more resistant to light, remained relatively stable across treatments, maintaining (0.35 mg/g), with no statistically significant difference ($p > 0.05$). These findings highlight the significance of the effects of exposure of mushroom samples to sunlight and ultraviolet light at different time periods. The study also provides a basis for further research on optimal illumination at different time points to enhance and maximize vitamin D and phytochemical components of *Pleurotus ostreatus.*

CHAPTER ONE

1.1 INTRODUCTION

Pleurotus ostreatus, widely known as the oyster mushroom, ranks among the most extensively cultivated edible fungi worldwide. Its popularity stems from its rapid growth, high nutritional value, adaptability to diverse growth substrates, and significant potential in biotechnology. Taxonomically, it belongs to the phylum Basidiomycota, class Agaricomycetes, and family Pleurotaceae (Chang & Miles, 2004). As a saprophytic fungus, *P. ostreatus* naturally colonizes lignocellulosic materials, particularly decaying hardwoods such as beech and oak, where it plays a critical ecological role in the decomposition of organic matter. Since its first scientific description in 1775 by Nikolaus Joseph von Jacquin as *Agaricus ostreatus* and subsequent reclassification by Kummer in 1871, this mushroom has emerged not only as a culinary delicacy but also as a model organism in environmental biotechnology, medical research, and functional food development (Sánchez, 2010).

Morphologically, *P. ostreatus* is recognizable by its fan-shaped or oyster-shaped cap, short lateral stipe, and white to gray gills that extend decurrently along the stem. Its remarkable adaptability enables it to thrive on various agricultural residues, including straw, sawdust, coffee grounds, and corn cobs, making it an essential component of sustainable agriculture and circular bioeconomy initiatives (Gregori *et al*., 2007). Beyond its nutritional attributes, the mushroom is rich in bioactive compounds, including β-glucans, lovastatin, polysaccharides, phenolics, and flavonoids, which have demonstrated antioxidant, antimicrobial, cholesterol-lowering, and immunomodulatory effects, highlighting its role as a functional food (Heleno *et al*., 2015; Vetvicka & Novak, 2011). Furthermore, *P. ostreatus* produces a potent array of ligninolytic enzymes, including laccase and manganese peroxidase, that enable the breakdown of complex pollutants, making it a valuable organism for mycoremediation and environmental cleanup efforts (Rodríguez *et al*., 2004; Singh, 2006).

1.2 Nutritional and Pharmacological Importance

Nutritionally, *P. ostreatus* is a rich source of protein, essential amino acids, dietary fiber, vitamins (notably the B-complex), and minerals such as potassium, iron, and selenium (Manzi *et al*., 2001). Its low-fat content, combined with bioactive compounds like polysaccharides, phenolics, and flavonoids, contributes to its antioxidant, antimicrobial, and immunomodulatory properties (Heleno *et al*., 2015; Gunde-Cimerman, 1999). From a pharmacological standpoint, the presence of β-glucans, particularly pleuran, has been linked to immune system enhancement, anti-inflammatory activity, and inhibitory effects against certain cancer cell lines (Vetvicka & Novak, 2011). Additionally, the natural statin lovastatin found in *P. ostreatus* may help reduce cholesterol levels, suggesting its potential as a nutraceutical or functional food ingredient (Alam *et al*., 2009).

1.3 Environmental and Biotechnological Applications

P. ostreatus is extensively studied for environmental applications, especially in mycoremediation. Its ligninolytic enzymes can degrade a wide range of environmental pollutants, including polycyclic aromatic hydrocarbons (PAHs), dyes, pesticides, plastics, and even oil residues. The mushroom's ability to grow on contaminated soils and accumulate or detoxify heavy metals further underscores its utility in bioremediation (Pointing, 2001; Bennett *et al*., 2002). Beyond environmental remediation, *P. ostreatus* is applied in biotechnology for enzyme production, bioethanol generation, and the conversion of spent mushroom substrate (SMS) into animal feed or soil conditioners (Gregori et al., 2007; Royse et al., 2017). Recent research has also explored its role in green synthesis of nanoparticles and the development of biodegradable materials, broadening its industrial potential (Sarsaiya *et al*., 2019). Despite its versatility, challenges remain in optimizing cultivation practices, increasing yields, and enhancing disease resistance, prompting ongoing studies in strain improvement, substrate optimization, and metabolic profiling (Pathmashini *et al*., 2008).

Plate 1: *Pleurotus ostreatus*

1.5. Justification of Study

Light is a key environmental factor that influences the physiological and biochemical characteristics of mushrooms, despite their non-photosynthetic nature. *Pleurotus ostreatus* (oyster mushroom) is recognized for its high nutritional content and abundance of phytochemicals such as phenolics, flavonoids, polysaccharides, terpenoids, and ergosterol-derived vitamin D_2, which contribute to its strong antioxidant and medicinal properties (Cheung, 2010; Mattila *et al*., 1994).

Despite growing interest in functional foods, limited research has examined how different light sources—particularly sunlight and ultraviolet (UV) light—affect the phytochemical composition of *P. ostreatus*. Light exposure has been shown to stimulate the biosynthesis of secondary metabolites, enhancing antioxidant activity and nutritional quality (Zhu *et al*., 2013). For instance, ultraviolet illumination promotes the photochemical conversion of ergosterol to vitamin D_2, thereby improving the mushroom's vitamin profile and health benefits (Jasinghe & Perera, 2005).

Studying the effects of sunlight and UV light on *P. ostreatus* is therefore justified because it can provide a low-cost, natural, and sustainable method to increase the production of valuable phytochemicals. This research will help optimize cultivation and post-harvest

illumination strategies to enhance the nutritional, pharmaceutical, and commercial value of Pleurotus ostreatus. Moreover, it supports the broader goal of developing functional foods that promote human health and well-being (Chang & Wasser, 2012).

1.6 Origin and Taxonomic History

Pleurotus ostreatus is a saprotrophic basidiomycete in the family Pleurotaceae. Its formal taxonomic history began in 1775 when Jacquin described it as *Agaricus ostreatus*, followed by its reclassification under the genus Pleurotus by Kummer in 1871, reflecting its unique morphological and ecological features compared to other Agaricus species (Kummer, 1871; Jacquin, 1775; Chang & Miles, 2004). The genus name Pleurotus derives from Greek, meaning "side ear," referring to the lateral attachment of its cap to substrates, while the species epithet ostreatus describes the oyster-like shape of the fruiting body. The species produces shelf-like clusters, exhibits white to lilac spore prints, and is widely recognized as one of the easiest mushrooms to identify and cultivate.

1.7 Evolutionary and Geographic Origin

Evolutionary studies suggest that *P. ostreatus* likely originated in the temperate forests of the Northern Hemisphere, particularly East Asia, which is considered a center of diversification for many Pleurotus species (Zhang *et al*., 2010). Phylogenetic analyses indicate that the *P. ostreatus* species complex began diversifying around 39 million years ago during the late Eocene, a period characterized by climate shifts and geological events that facilitated fungal dispersal across continents (Vilgalys & Moncalvo, 1999; Zhang *et al*., 2010). Today, it is cosmopolitan, naturally occurring on decaying hardwoods like beech (Fagus), oak (Quercus), and poplar (Populus) across Europe, North America, and Asia, owing to its saprotrophic lifestyle and efficient lignin-degrading enzymatic system (Kües & Liu, 2000; Sánchez, 2010).

1.8. Domestication and Cultivation History

Although naturally occurring in forests, large-scale cultivation of *P. ostreatus* began in Germany during World War I as a response to meat shortages (Chang & Miles, 2004). Its cultivation has since spread globally, especially in Asia, with countries such as China, India, and Thailand emerging as major producers. Presently, it is the second most cultivated mushroom worldwide after Agaricus bisporus due to its rapid growth, adaptability, and ability to utilize diverse agricultural waste as substrates (Royse et al., 2017; Sánchez, 2010). Continuous research aims to optimize cultivation conditions, improve yield, and fully exploit its nutritional and biotechnological potential.

1.9. COMMON/OTHER NAMES OF *Pleurotus ostreatus*

Pleurotus ostreatus is known by several common names and regional or alternative names worldwide. Here are some of the most widely used:

Common or Other Names for *Pleurotus ostreatus*:

1. Oyster mushroom – This is the most widely used English common name, due to the shell-like shape of the cap resembling an oyster.
2. Tree oyster mushroom – Refers to its natural growth on decaying wood or tree trunks.
3. Pearl oyster mushroom – A specific variety with a pale or white cap; the name emphasizes its color.
4. Seta de ostra – Spanish name, meaning "oyster mushroom".
5. Champignon en huître – French for "oyster mushroom".
6. Kalabhatta – A local name in some Indian languages, though names vary regionally.
7. Dhingri – Common Hindi name in India for cultivated oyster mushrooms.
8. Pako – A local name in parts of the Philippines and Southeast Asia.

1.10. Phytochemical Properties of *Pleurotus ostreatus*

Pleurotus ostreatus, commonly known as the oyster mushroom, is a highly nutritious and medicinally valuable fungus. It contains a wide range of bioactive phytochemicals that play important roles in maintaining health and preventing disease. These compounds include

phenolics, flavonoids, polysaccharides, terpenoids, sterols, alkaloids, and saponins, all of which contribute to its antioxidant, antimicrobial, and therapeutic effects (Jayakumar *et al*., 2009; Cheung, 2010).

1.10.1 Phenolic Compounds

Phenolic compounds are among the most significant phytochemicals in *P. ostreatus*. They include gallic acid, ferulic acid, caffeic acid, vanillic acid, and p-coumaric acid, which are known for their powerful antioxidant activities. These compounds protect biological tissues by scavenging free radicals, reducing oxidative stress, and preventing lipid peroxidation (Barros *et al*., 2007).

The total phenolic content in *P. ostreatus* depends on factors such as the growth substrate, temperature, and light exposure. Mushrooms grown under controlled illumination—especially sunlight or UV light—often exhibit higher phenolic content, which enhances their antioxidant and medicinal properties.

1.10.2. Flavonoids

Flavonoids are another important group of bioactive compounds found in *P. ostreatus*, including quercetin, catechin, and rutin. These compounds exhibit antioxidant, anti-inflammatory, and antimicrobial effects by neutralizing reactive oxygen species (ROS) and enhancing cellular defense systems (Heleno *et al*., 2015).

Although mushrooms generally contain lower flavonoid levels compared to plants, the flavonoid concentration in *P. ostreatus* is sufficient to contribute to its health benefits. The content of flavonoids can also increase with proper illumination, which stimulates secondary metabolite synthesis.

1.10.3. Polysaccharides

Polysaccharides are the most abundant bioactive compounds in *P. ostreatus*, particularly β-glucans, heteroglucans, and heteropolysaccharides. These compounds are renowned for their immunomodulatory, antitumor, and cholesterol-lowering effects (Borchers *et al*., 2008).

β-glucans stimulate immune cells such as macrophages and natural killer cells, enhancing the body's defense mechanisms against infections and cancer. In addition, these polysaccharides can regulate blood sugar levels and improve gut health. The yield and structure of polysaccharides are influenced by cultivation conditions, including substrate type and illumination level.

1.10.4 Terpenoids

Pleurotus ostreatus also produces terpenoid compounds, which are responsible for its aroma and contribute to its antimicrobial and antiviral properties. Terpenoids act as natural defense agents against bacteria and fungi and may have anti-inflammatory and anticancer potentials (Paterson, 2006).

Although they are present in lower amounts than polysaccharides or phenolics, terpenoids play a vital role in the overall therapeutic activity of *P. ostreatus* and are increasingly being studied for their pharmacological relevance.

1.10.5 Sterols and Ergosterol-Derived Compounds

One of the key sterols present in *P. ostreatus* is ergosterol, which serves as the precursor of vitamin D_2. When the mushroom is exposed to sunlight or ultraviolet (UV) light, ergosterol undergoes a photochemical transformation into vitamin D_2 (Jasinghe & Perera, 2005). This conversion enhances the mushroom's nutritional quality, making it an excellent non-animal source of vitamin D_2.

Vitamin D_2 plays a crucial role in calcium metabolism, bone health, and immune function. Therefore, light exposure during or after cultivation can significantly improve the vitamin D_2 content of *P. ostreatus*, providing additional health benefits.

1.10.6. Alkaloids

P. ostreatus contains small quantities of alkaloids, which are nitrogenous compounds known for their analgesic, antimicrobial, and anti-inflammatory effects. These bioactive molecules contribute to the mushroom's therapeutic potential by inhibiting bacterial growth and modulating pain and inflammation (Okwu & Josiah, 2006).

Though present in minor amounts, alkaloids add to the synergistic health benefits of the mushroom's complex phytochemical profile.

1.10.7. Saponins

Saponins are another group of phytochemicals detected in *P. ostreatus*. They are known for their cholesterol-lowering, antioxidant, and immune-boosting effects. Saponins can form complexes with cholesterol, thereby reducing its intestinal absorption and lowering blood cholesterol levels. They also enhance immune responses and protect against certain pathogens.

The concentration of saponins in *P. ostreatus* may vary with environmental and cultivation factors, including substrate and illumination intensity.

1.10.7. Antioxidant Properties and Biological Significance

The overall antioxidant activity of *Pleurotus ostreatus* results from the combined effects of phenolics, flavonoids, ergosterol, and ergothioneine—a unique antioxidant amino acid found in mushrooms (Cheung, 2010). These compounds work together to reduce oxidative stress, prevent DNA and cellular damage, and lower the risk of chronic diseases such as cancer, diabetes, and cardiovascular disorders.

Environmental conditions, particularly illumination, can significantly enhance these antioxidant properties by stimulating the synthesis of phenolic compounds and vitamin D_2

In summary, *Pleurotus ostreatus* contains a diverse range of phytochemicals, including phenolics, flavonoids, polysaccharides, terpenoids, sterols, alkaloids, and saponins, all of which contribute to its nutritional and therapeutic value. These compounds provide strong antioxidant, immunomodulatory, antimicrobial, and anticancer effects. Light exposure, especially from sunlight and UV sources, can further enhance the production of these bioactive compounds, improving the overall health-promoting potential of the mushroom.

1.11. Vitamin D in Pleurotus ostreatus

Pleurotus ostreatus (commonly known as the oyster mushroom) is one of the few natural, non-animal sources of vitamin D_2 (ergocalciferol). This vitamin plays a vital role in calcium and phosphorus metabolism, bone development, immune regulation, and the prevention of metabolic bone diseases such as rickets, osteoporosis, and osteomalacia. In humans, vitamin D_3 (cholecalciferol) is synthesized in the skin from 7-dehydrocholesterol upon

exposure to sunlight. Similarly, in mushrooms, vitamin D_2 is produced by photochemical conversion of ergosterol, a major sterol in fungal cell membranes.

When *P. ostreatus* is exposed to sunlight or ultraviolet (UV-B) radiation, ergosterol absorbs the UV energy and is converted into previtamin D_2, which then spontaneously isomerizes into vitamin D_2 through a heat-dependent process (Jasinghe & Perera, 2005). This mechanism closely mirrors how human skin produces vitamin D_3, making *P. ostreatus* an excellent dietary alternative for vegetarians and individuals who avoid animal-derived foods.

The amount of vitamin D_2 produced in *P. ostreatus* depends on several factors, including the intensity, duration, and wavelength of illumination, as well as the surface moisture and morphology of the fruiting body. Jasinghe and Perera (2005) reported that mushrooms exposed to UV-B radiation exhibited up to a 200-fold increase in vitamin D_2 content compared to dark-grown mushrooms. Similarly, Mau *et al*. (1998) and Phillips *et al*. (2012) found that both pre-harvest and post-harvest light exposure significantly enhance vitamin D_2 levels without negatively affecting other nutritional or sensory properties.

Moreover, *P. ostreatus* retains a considerable portion of its vitamin D_2 even after drying and storage, suggesting that UV-treated mushrooms can serve as a stable source of vitamin D_2 in the human diet (Keegan *et al*., 2013). This is especially relevant for populations living in regions with limited sunlight exposure or dietary restrictions that exclude animal products. The bioavailability of mushroom-derived vitamin D_2 in humans has been confirmed to be comparable to that of synthetic or supplement forms (Calvo *et al*., 2013).

Beyond its nutritional importance, vitamin D_2 in *P. ostreatus* also contributes to the mushroom's antioxidant, anti-inflammatory, and immunomodulatory activities. Vitamin D_2 helps regulate gene expression involved in immune defense and calcium homeostasis, thereby supporting cardiovascular health and cellular protection against oxidative stress (Holick, 2007; Keegan *et al*., 2013).

In summary, *Pleurotus ostreatus* serves as a natural, sustainable, and functional food source of vitamin D_2. Controlled exposure to sunlight or ultraviolet light during cultivation or after harvest offers a simple, cost-effective strategy to enhance its vitamin D content, thereby

improving its nutritional and medicinal quality. This makes *P. ostreatus* a valuable addition to diets aimed at preventing vitamin D deficiency and promoting overall health.

1.4 AIM AND OBJECTIVES

Aim:

This study aims to investigate the effect of sunlight and ultraviolet (UV) light illumination on the phytochemical properties of *Pleurotus ostreatus*. It seeks to determine how these light sources influence the synthesis and concentration of key bioactive compounds—such as phenolics, flavonoids, polysaccharides, and vitamin D_2—and their associated antioxidant activities. The goal is to identify the optimal illumination condition that enhances the nutritional and medicinal quality of *P. ostreatus.*

Objectives:

The objectives are as follows:

1. To determine the effect of illumination using sunlight and ultraviolet light exposure on the phytochemical composition of *Pleurotus ostreatus*.
2. To assess the relationship between illumination type and exposure period on the Phytochemical activity in *P. ostreatus*.

To identify the optimal illumination condition for enhancing the vitamin D contents of *Pleurotus ostreatus*.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Sample Collection

Fresh oyster mushrooms (*Pleurotus ostreatus*) were collected from a Mycrofarms at Isihor, in Benin City, Edo State. The samples were cleaned to remove dirt and then placed on clean surfaces. Each sample was properly labeled to avoid mixing before they were taken to the laboratory for analysis.

2.1.2 Sample Preparation

The samples were then chopped into bits and weighed. After weighing, the samples were exposed to different illumination sources (sunlight and ultraviolet light) at time periods of 1 hour, 2 hours, 4 hours, 6 hours, 8 hours.

2.2 Methodology

2.2.1 Methodology: Carr-Price Reaction for Vitamin D

Principle: The Carr-Price reaction is a colorimetric test that measures the reaction between vitamin D (primarily D2 or D3) and antimony trichloride ($SbCl_3$) in chloroform, producing a blue color. The intensity of the blue color is proportional to the vitamin D content.

Procedure

Preparation of Reagents,

Dissolve antimony trichloride in chloroform to make a 0.5–1% w/v solution. This solution is sensitive to moisture, so prepare it immediately before use in a dry environment.

Sample Preparation,

Dissolve the vitamin D sample (pure or extracted) in chloroform to get a suitable concentration (commonly 0.1–1 mg/mL).

Reaction,

In a clean test tube, add 1–2 mL of the sample's chloroform solution.

Add 1–2 drops of the $SbCl_3$ reagent to the solution.

Mix gently by swirling.

Observation,

A blue color appears immediately if vitamin D is present.

The intensity of the blue color is highest at the beginning and may fade over a few minutes.

Quantitative Estimation (Optional)

Measure the absorbance of the blue complex using a spectrophotometer at 620–650 nm.

Compare with a standard curve prepared using known concentrations of vitamin D.

2.2.2 Qualitative Phytochemical Screening

The qualitative phytochemical tests were conducted according to the methods of Sofowora (1993), Trease and Evans (2002), and Harborne (1998).

Various tests were performed to identify the types of phytochemicals present in the mushroom extracts.

2.2.1 Test for Flavonoids (Shindo's Test)

2 mL of the extract was mixed with 2 mL of 5% sodium hydroxide (NaOH). After mixing, 2 mL of dilute hydrochloric acid (HCl) was added. The appearance of a yellow color confirmed the presence of flavonoids.

2.2.2 Test for Phenols (Ferric Chloride Test)

2 mL of the extract was treated with 5% ferric chloride solution. The appearance of a deep blue or black color indicated the presence of phenols.

2.2.3 Test for Saponins (Foam Test)

2 mL of the extract was mixed with 6 mL of water in a test tube and shaken vigorously. The stable foam that persisted for several minutes confirmed the presence of saponins.

2.2.4 Test for Steroids (Libermann–Burchard Test)

2 mL of the extract was mixed with 5 drops of chloroform, 4 drops of acetic anhydride, and 1 drop of concentrated sulfuric acid. A color change from purple to blue or green indicated the presence of steroids.

2.2.5 Test for Tannins

Three drops of 0.1% ferric chloride were added to the extract. A brownish-green or blue-green color showed the presence of tannins.

2.2.6 Test for Cardiac Glycosides (Keller–Killiani Test)

1 mL of the extract was mixed with 2 mL of glacial acetic acid and 1 drop of ferric chloride, then 1 mL of concentrated sulfuric acid was added. A brown ring at the boundary indicated the presence of cardiac glycosides.

2.3 Quantitative Analysis of Phytochemicals

2.3.1 Preparation of Extracts

50 mg of the mushroom extract was weighed and dissolved in 50 mL of methanol and distilled water to make a stock solution. This was used for further quantitative analysis.

2.3.2 Determination of Total Flavonoid Content (TFC)

The total flavonoid content was determined using the method of Ayoola et al. (2008).

2 mL of 2% aluminum chloride ($AlCl_3$) in ethanol was added to 2 mL of the extract. The absorbance of the mixture was measured at 415 nm using a UV–Visible spectrophotometer. The flavonoid concentration was calculated using a quercetin standard curve and expressed as mg quercetin equivalent per gram (mg QE/g) of extract.

Data Analysis

The data obtained from our experiments were analyzed using descriptive and inferential statistics. We computed mean values and standard deviations to effectively summarize the findings. To evaluate the effects of illumination time and treatment type on the vitamin D and secondary metabolite contents of *Pleurotus ostreatus*, we conducted a two-way Analysis of Variance (ANOVA).

CHAPTER THREE

RESULTS AND DATA ANALYSIS

This chapter reports the results of an experimental investigation into the effects of illumination on vitamin D and phytochemical composition in *Pleurotus ostreatus* (oyster mushroom). The objective was to determine the influence of different light sources, specifically sunlight and ultraviolet light, and varying exposure durations on vitamin D production and the concentrations of key secondary metabolites, including flavonoids, terpenoids, steroids, cardiac glycosides, phenols, and saponins.

Vitamin D concentration in *Pleurotus ostreatus* increased with extended light exposure. Control samples, which were not exposed to light, exhibited the lowest vitamin D content, whereas samples exposed for several hours demonstrated higher concentrations.

Plate 2: Oyster mushroom samples subjected to sunlight exposure

Table 3.1: *Vitamin D content of (µg/g) Pleurotus ostreatus under different light sources and exposure durations*

Light Source	4 Hours (µg/g ± SEM)	6 Hours (µg/g ± SEM)	8 Hours (µg/g ± SEM)
Sunlight	14.33 ± 0.18	15.13 ± 0.17	13.33 ± 0.13
Ultraviolet Light	13.73 ± 0.13	12.67 ± 0.48	12.80 ± 0.61

**Mean of three replicates ± standard error*

Both sunlight and ultraviolet light increased the vitamin D content of *Pleurotus ostreatus*, with sunlight resulting in higher concentrations at all exposure durations. The increase in vitamin D from 4 to 6 hours of exposure was statistically significant ($p < 0.05$), demonstrating that vitamin D synthesis in the mushroom is dependent on exposure time.

After 8 hours of exposure, vitamin D levels declined in both sunlight- and ultraviolet-treated samples. This pattern indicates that prolonged illumination likely causes photodegradation of vitamin D as a result of excessive radiation.

Two-way ANOVA confirmed that both light source and exposure duration significantly affected vitamin D concentration ($p < 0.05$). The interaction between light source and exposure time was also significant, indicating that the rate of vitamin D formation depended on the type of illumination. Sunlight exposure for approximately 6 hours produced the highest and most stable vitamin D yield in oyster mushrooms.

This section presents the results of phytochemical screening and quantification for *Pleurotus ostreatus* samples exposed to sunlight for 6 and 8 hours. The metabolites analyzed were flavonoids, terpenoids, steroids, cardiac glycosides, phenols, and saponins.

Plate 3: Ethanolic extract of oyster mushroom samples

Table 3.2: Phytochemical composition (mg/g ± SEM) of Pleurotus ostreatus exposed to sunlight for different durations

Phytochemical	6 Hours	8 Hours
Flavonoids	0.39 ± 0.00	0.36 ± 0.00
Terpenoids	0.21 ± 0.00	0.22 ± 0.00
Steroids	0.07 ± 0.00	0.20 ± 0.00
Cardiac Glycosides	0.35 ± 0.00	0.35 ± 0.00
Phenols	0.19 ± 0.00	0.05 ± 0.00
Saponins	0.02 ± 0.00	0.03 ± 0.00

**Mean of three replicates ± standard error*

Phytochemical data indicated distinct responses to sunlight exposure. Flavonoid concentration peaked at 6 hours (0.39 mg/g) and decreased to 0.36 mg/g after 8 hours. This reduction was statistically significant ($p < 0.05$), indicating that prolonged exposure may induce light-mediated degradation of flavonoids. exposure.

Terpenoid and saponin concentrations increased slightly after 8 hours of sunlight exposure. This statistically significant difference ($p < 0.05$) suggests that moderate light intensity enhances their synthesis, potentially as a protective response to photo-oxidative stress.

Steroid concentration increased substantially from 0.07 mg/g to 0.20 mg/g with longer exposure, a statistically significant rise ($p < 0.01$), indicating that extended drying and illumination promote steroid biosynthesis.

Phenolic compounds, Phenolic compound concentrations declined sharply from 0.19 mg/g to 0.05 mg/g, a highly significant decrease ($p < 0.01$). This trend supports the hypothesis that phenols are sensitive to light and degrade during extended illumination.remained relatively stable across both time points (0.35 mg/g), with no statistically significant difference ($p > 0.05$).

ANOVA results indicated significant main effects of both phytochemical type and exposure duration ($p < 0.05$), as well as a strong interaction between these factors. This finding demonstrates that the effect of light exposure varied depending on the specific metabolite.

3.4 Summary of Findings

The study demonstrated that illumination is a critical factor influencing both vitamin D synthesis and phytochemical composition in *Pleurotus ostreatus*.

1. Vitamin D concentration increased significantly with exposure time up to 6 hours, then declined at 8 hours, likely due to photodegradation.
2. Phytochemical compounds exhibited varied responses to prolonged exposure. Steroid and terpenoid concentrations increased, whereas flavonoid and phenolic compound concentrations decreased.
3. Two-way ANOVA confirmed significant differences among exposure durations and metabolite types ($p < 0.05$). The significant interaction between time and compound type indicates that the influence of light exposure is specific to each compound.

These findings suggest that moderate sunlight or ultraviolet exposure (about 6 hours) is optimal for maintaining balanced phytochemical content and maximizing vitamin D enrichment in oyster mushrooms.

CHAPTER 4

Discussion

The phytochemical analysis of *Pleurotus ostreatus* ethanol extract after 6 and 8 hours of drying showed clear differences in the levels of flavonoids, terpenoids, steroids, cardiac glycosides, phenols, and saponins. These variations indicate that drying time plays an important role in determining the phytochemical composition of the mushroom. Prolonged drying can either increase or decrease certain compounds, depending on the combined effects of heat and light on their chemical stability.

Flavonoids recorded the highest concentration among the analyzed compounds, with 0.3889 ± 0.0000 mg/g at 6 hours and 0.3568 ± 0.0002 mg/g at 8 hours. The slight reduction after 8 hours suggests that flavonoids are somewhat sensitive to prolonged heat and light exposure, which may lead to oxidation or molecular breakdown (Khatun *et al*., 2015). Flavonoids are known antioxidant agents that help neutralize free radicals and protect living cells from oxidative damage (Oke *et al*., 2013). Therefore, the observed decline after longer drying may indicate a slight decrease in antioxidant capacity.

Terpenoids showed a small increase, from 0.2115 ± 0.0004 mg/g at 6 hours to 0.2176 ± 0.002 mg/g at 8 hours. This increase suggests that terpenoids are relatively stable and that extended drying may enhance their extraction as the mushroom tissues become softer and release more compounds (Oboh & Shode, 2002). Terpenoids contribute to the antimicrobial and anti-inflammatory properties of mushrooms and play a role in their medicinal potential (Adebayo *et al*., 2014).

Steroids increased significantly from 0.0680 ± 0.0006 mg/g to 0.2010 ± 0.0007 mg/g after 8 hours of drying. This major rise indicates that longer drying favors the formation or easier extraction of steroid compounds. It is possible that precursor molecules are converted into detectable steroid forms during prolonged drying (Adejumo & Awosanya, 2005). Previous studies have shown similar results, with moderate heating enhancing sterol content by softening the cell wall and improving release (Effiong *et al*., 2024).

Cardiac glycosides (listed as “cardio glucosidase”) remained stable, with 0.3509 ± 0.001 mg/g after 6 hours and 0.3465 ± 0.0006 mg/g after 8 hours. The small difference indicates that these

compounds are resistant to breakdown under the drying conditions used (Akinmoladun *et al*., 2007). This stability means that their beneficial roles, such as heart-strengthening effects, are preserved throughout the process.

Phenols decreased sharply from 0.1956 ± 0.001 mg/g at 6 hours to 0.0527 ± 0.003 mg/g at 8 hours. This strong decline shows that phenolic compounds are highly sensitive to heat and oxidation. Phenols are among the main antioxidant agents in mushrooms, and their reduction may lower the antioxidant strength of the extract (Mattila *et al*., 2000; Kakon *et al*., 2012). Similar reductions have been reported in earlier studies, in which prolonged drying or high light intensity led to oxidative degradation of phenolic compounds (Khatun *et al*., 2015).

Saponins increased slightly from 0.0267 ± 0.002 mg/g at 6 hours to 0.0345 ± 0.003 mg/g at 8 hours. This slight rise suggests that saponins are relatively heat-stable and become more extractable over longer drying periods (Adebayo *et al*., 2014). Saponins are known for their antifungal, antibacterial, and cholesterol-lowering effects, which improve the medicinal value of the mushroom.

In summary, drying time significantly influenced the phytochemical content of *Pleurotus ostreatus*.

1. Flavonoids and phenols decreased after 8 hours, indicating some loss of antioxidant compounds.
2. Terpenoids, steroids, and saponins increased, showing greater stability or release with longer exposure.
3. Cardiac glycosides remained unchanged, suggesting resistance to degradation.

These findings suggest that a 6-hour drying period helps preserve antioxidant compounds, such as flavonoids and phenols, while an 8-hour drying period enhances compounds such as steroids and terpenoids. This agrees with previous studies, which reported that both the duration and method of drying significantly influence the phytochemical yield and nutritional quality of mushrooms (Akyuz *et al*., 2010; Dulay *et al*., 2015; Effiong *et al*., 2024).

At the control stage, both sunlight and ultraviolet samples had similar values (around 9 units), showing no difference before light exposure. After 1 hour, both treatments showed a sharp increase

to about 15–16 units, suggesting that short-term light exposure greatly enhanced the compound's concentration or activity of vitamin D (Adejumo & Awosanya, 2005).

After 2 hours, the sunlight-exposed samples maintained high values (~15 units), while UV-treated samples dropped slightly (~11–12 units). This shows that prolonged ultraviolet exposure may begin to break down vitamin D compounds (Adeyeye & Adesina, 2024). By 4 hours, sunlight samples remained high (~13–14 units), while ultraviolet light caused a noticeable decrease (~5 units). This could be due to photodegradation of sensitive molecules such as phenolics, vitamins, or enzymes (Mattila et al., 2000; Kakon *et al*., 2012).

At 6 hours, both treatments improved again. Sunlight remained steady at around 15 units, while UV light samples rose slightly to 12 units, possibly due to adaptive or stabilizing processes that restored some compounds (Effiong *et al*., 2024).

After 8 hours, sunlight samples slightly decreased (~10 units), whereas ultraviolet samples increased again (~15 units). This rebound might result from stress-induced production of secondary metabolites(Dulay *et al*., 2015).

Overall, sunlight exposure showed a steady, consistent effect, maintaining high vitamin D levels for most of the period. In contrast, ultraviolet exposure caused fluctuating results, with an initial increase, a mid-duration drop, and a later rebound. This pattern suggests that ultraviolet light can both stimulate and degrade vitamin D compounds, depending on the duration of exposure (Jasinghe & Perera, 2006; Teichmann *et al*., 2007).

These results indicate that controlled sunlight exposure supports stable development of the vitamin D compound, whereas ultraviolet light requires precise timing to avoid degradation. In mushrooms like *Pleurotus ostreatus*, moderate exposure improves phytochemical accumulation and vitamin D formation, but too much ultra violet light can cause oxidative stress and compound breakdown (Akyuz *et al*., 2010; Kalac, 2016).

Conclusion

The results of this study clearly demonstrate that light exposure plays a vital role in determining the phytochemical composition and nutritional quality of *Pleurotus ostreatus*. The variations observed in flavonoids, terpenoids, steroids, cardiac glycosides, phenols, and saponins after 6 and 8 hours of illumination This study highlights the critical influence of light exposure on the phytochemical composition and nutritional quality of Pleurotus ostreatus. It found that a shorter illumination period of 6 hours is more effective in preserving sensitive compounds like flavonoids and phenols, which are crucial for their antioxidant properties. In contrast, an 8-hour exposure leads to higher levels of more stable compounds such as terpenoids, steroids, and saponins, which have various health benefits.

Cardiac glycosides remained stable regardless of exposure time, indicating their resilience. Sunlight exposure produced consistent effects on compound synthesis, while ultraviolet light exposure showed varying effects over time, suggesting both stimulation and potential damage. Overall, about 6 hours of natural sunlight is recommended for optimizing the mushroom's bioactive components, balancing nutrient preservation with compound development. These findings align with previous research on the significance of processing conditions on the yield and biological activity of edible mushrooms.

Recommendations

1. Optimal Illumination Duration:Based on the findings, an exposure time of 6 hours under sunlight is recommended to retain a high concentration of antioxidant compounds while minimizing nutrient loss.
2. Avoid Excessive Exposure:Extended exposure to sunlight beyond 8 hours should be avoided for compounds sensitive to oxidation and heat degradation, such as phenols and flavonoids.
3. Further Studies:Future research should explore the combined effects of illumination, humidity, and light wavelength on the stability of specific phytochemicals and vitamins in *Pleurotus ostreatus*.It would also be useful to examine how these compounds influence antioxidant, antimicrobial, and nutritional activity in different mushroom species.

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APPENDIX

This appendix presents detailed statistical results from analyses of the vitamin D and phytochemical contents of *Pleurotus ostreatus* (oyster mushroom) under sunlight and ultraviolet illumination. Various exposure durations were tested to evaluate their effects on vitamin D synthesis and phytochemical variation. Statistical analyses, including one-way ANOVA, two-way ANOVA, and the Brown–Forsythe test, were performed to determine the significance level and the interaction between the treatment factors.

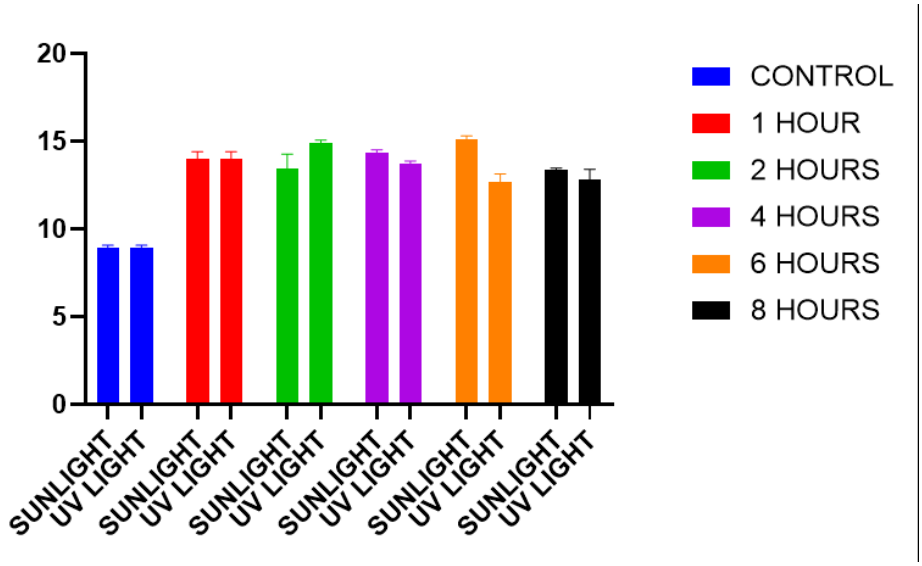

Figure A3.1 Vitamin D content of *Pleurotus ostreatus* under different light sources and exposure durations

The bar chart titled "" shows the effects of different exposure durations (1, 2, 4, 6, and 8 hours) under sunlight and ultraviolet light on the measured compound or activity of vitamin D in *Pleurotus ostreatus*. The results reveal clear differences in how both light sources influenced the samples over time.

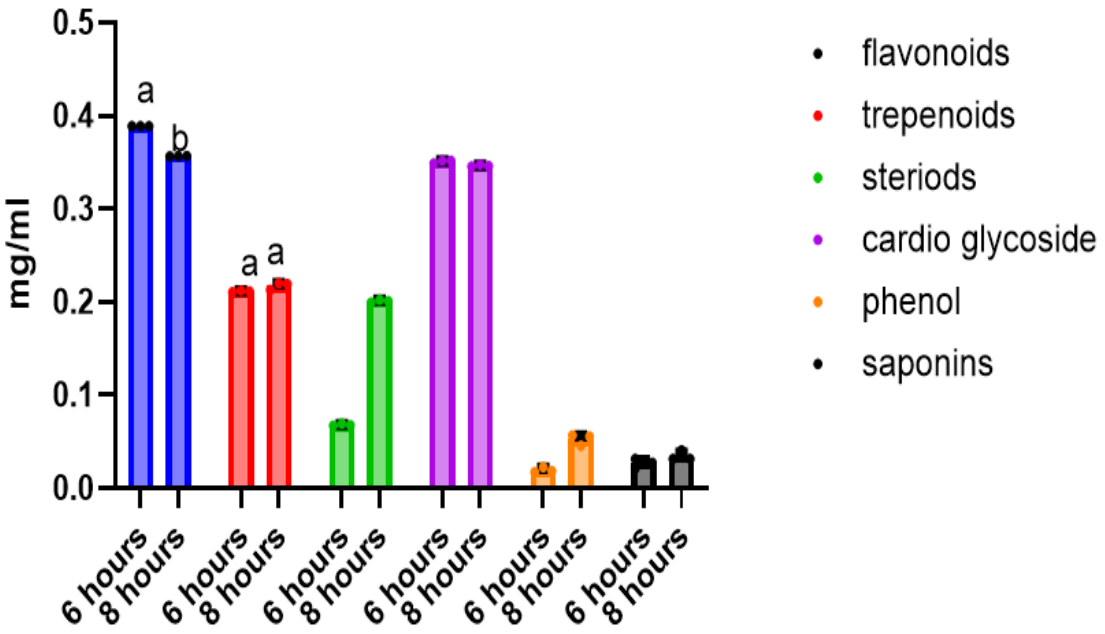

Figure A3.2 Phytochemical composition (mg/g ± SEM) of Pleurotus ostreatus exposed to sunlight for different durations