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Antimicrobial activity and identification of secondary metabolites in summer oyster mushroom (*Pleurotus ostreatus*) extracts

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Abstract. *Devi NB, Kalpana CA. 2025. Antimicrobial activity and identification of secondary metabolites in summer oyster mushroom (*Pleurotus ostreatus*) extracts. Asian J Agric 9: 31-39.* Consumption of oyster mushrooms is increasing due to their unique flavor, meaty taste, and extensive medicinal potential. The main aim of this study is to analyze and compare antimicrobial assays and identify secondary metabolites present after different drying techniques of oyster mushrooms. Oyster mushrooms were dried three different ways: Sun-Dried (SD), Cabinet-Dried (CD), and Freeze-Dried (FD). The dried samples were powdered and macerated using ethanol as a solvent extract and ultrasound treated. An antimicrobial assay was performed for the extracts using two Gram-positive and two Gram-negative bacteria. Gentamycin was used as a positive control and ethanol as a negative control. Further phytochemical screening was performed on the extracts to identify secondary metabolites, and other organic and inorganic compounds were identified by Fourier Transform Infrared Spectroscopy (FTIR) analysis. The study revealed that the mean zone of inhibition by SD, CD and FD oyster mushroom extracts against *Staphylococcus aureus* were 15.6 mm, 17.2 mm and 13 mm respectively while against *Bacillus cereus* were 0 mm, 0 mm and 14 mm respectively. And the mean zone of inhibition by SD, CD and FD oyster mushroom extracts against *Escherichia* were 12.6 mm, 7.8 mm and 15.4 mm respectively whereas against *Pseudomonas aeruginosa* were 1 mm, 0 mm and 11.4 mm respectively. The study also found that all three samples contained good amounts of alkaloids and carbohydrates, flavonoids, sterols, anthraquinones, and cardiac glycosides. All the samples showed bands in the IR regions of 400-4000 cm^{-1}. The study concluded that the FD oyster mushroom preserved more secondary metabolites that had potential role in antimicrobial activity. The present work extends the finding of previous work by adopting ultrasonication as an extraction process, to compare the antimicrobial activity and identification of secondary metabolites of different drying method for summer-grown oyster mushroom cultivated in Coimbatore District of Tamil Nadu, India.

Keywords: *Dhingri*, FTIR, lyophilization, phytochemical, ultrasonication

Abbreviations: CD: Cabinet-Dried, FD: Freeze-Dried, FTIR: Fourier Transform Infrared Spectroscopy, SD: Sun-Dried, UAE: Ultrasonication-Assisted Extraction

INTRODUCTION

Oyster mushrooms (*Pleurotus ostreatus)*, also well known as *dhingri* in India, belong to the family Basidiomycetes and are the second most produced edible fungus due to their rich nutritional properties and easy cultivation (El-Razek et al. 2020). The best growing seasons for mushrooms are March/April (summer mushrooms) in hilly areas and September/October (winter mushrooms) in lower regions. The *P. ostreatus* is one of the most commercially cultivated *Pleurotu*s species. Growing *Pleurotu*s species is easy and inexpensive; it can be grown yearly in under-maintained climate conditions. In India, oyster mushrooms are mostly cultivated and produced in eastern regions like Odisha and West Bengal, most of the northeastern states, southern regions, such as Karnataka and Andhra Pradesh, and western regions, such as Maharashtra and Madhya Pradesh in Central India (National Horticulture Board of India) grey oyster mushrooms and white oyster mushrooms are suited to cultivation in Tamil Nadu (TNAU Agritech Portal).

Mushrooms have a long history of consumption and cultivation around the world because of their unique umami flavor and meaty taste, as well as their medicinal properties (Zhou et al. 2024). The *P. ostreatus* is a rich source of major nutrients, including protein, carbohydrates, dietary fiber, minerals, and vitamins, especially Vitamin D (Corrêa et al. 2016) and it is low in fat, calories, and cholesterol (Fakoya et al. 2020). The Vitamin D content is naturally undetectable in indoor-cultivated fresh mushrooms unless irradiated with natural or artificial ultraviolet rays (Hu et al. 2020). Mushrooms are the only vegan source of Vitamin D2 since the conversion of ergosterol to ergocalciferol occurs by natural or artificial ultraviolet rays, which produce adequate Vitamin D in mushrooms (Cardwell et al. 2018). One study on the application of UV-B irradiation and air-drying club together with oyster mushroom powder for the production of Vitamin D (ergocalciferol) revealed that it could be the best strategy to deliver ergocalciferol in foods (Pedrali et al. 2020). According to the study findings from NHANES III, a lower risk of total mortality rate is associated with the consumption of mushrooms in US adults (Ba et al. 2021).

Oyster mushrooms are also rich sources of bioactive compounds that have antioxidant, antimicrobial, antitumor, and anti-inflammatory effects. A previous study showed

that aqueous extracts of the fruiting body of FD oyster mushrooms had the strongest inhibition of the growth of most fungi, and an alcohol-based extract had less inhibition of antimicrobial activity against most tested microorganisms (Younis et al. 2015). Another study by El-Razek et al. (2020) showed that aqueous and ethanol extracts of oyster mushrooms had significant antibacterial properties against *Salmonella typhimurium*, *Escherichia coli*, and *Staphylococcus aureus*.

Oyster mushrooms contain both primary and secondary metabolites. Primary metabolites are essential for the growth and development of an organism. In contrast secondary metabolites are chemical compounds essential for biological activity that are produced by the organisms for protection against environmental shocks or predatory threats (Herawati et al. 2021). Secondary metabolites, also known as phytochemicals, include alkaloids, flavonoids, sterols, terpenoids, anthraquinones, and glycosides and are more concentrated in the fruiting parts of mushrooms (Al Qutaibi and Kagne 2024). In a previous study by the same authors (Nongmaithem and Chinnapan 2023) on the effect of the screening of phytochemical constituents, some oyster mushrooms were FD, and others were exposed to sunlight for 30 minutes followed by FD, then extracted into a powder in different solvents (aqueous, methanol, and ethanol). The study observed that more secondary metabolites were found in FD ethanol extracts, while protein was found in higher quantities in the second sample. Still, there was no difference in the secondary metabolites present in the methanol extracts in the samples.

Since mushrooms contain 80-90% moisture, they are highly perishable (Schill et al. 2021); therefore, it is necessary to reduce their water content by adopting drying methods that extend their shelf life (Van Hung et al. 2020). Sun Drying (SD), Cabinet Drying (CD) and Freeze Drying (FD) were adopted in the present study. SD is low cost and traditional method of drying. SD preserved organoleptic characteristics and physio-chemical properties of oyster mushroom (Maray et al. 2018). Cabinet Drying (CD) is a simple and easy method, has been promoted as appropriate technologies at lower temperature because of low investment. Freeze Drying (FD) is advanced, costly and excellent method of drying which retains the color, texture and nutrients of foods (Shams et al. 2022). No previous studies have highlighted specifically summer harvested oyster mushrooms. The present work extends the finding of previous work by adopting ultrasonication as extraction process and the comparison of different drying methods of *P. ostreatus* in terms of the identification of secondary metabolites, antibacterial activity from Coimbatore District, Tamil Nadu, India.

The main goal of this study was to evaluate the antibacterial activity against two Gram-positive and two Gram-negative bacteria, identify bioactive compounds, and screen primary and secondary metabolites (phytochemicals) from oyster mushroom powder extracts produced via different drying methods.

MATERIALS AND METHODS

Sample procurement and identification

Fresh oyster mushrooms (*P. ostreatus*) were purchased from a farm in Coimbatore, Tamil Nadu, India. They were grown in a mushroom hut (maintained temperature of 18-26°C due to the hot climate in Tamil Nadu) and harvested between April and May (summer mushroom). Sample identification was carried out at the Centre of Advanced Studies in Botany, University of Madras, Tamil Nadu.

Sample preparation

Oyster mushrooms were cleaned and washed thoroughly, and their fruiting parts were separated from their stalks. They were cut into pieces (1×1 cm^2) and divided into three portions, then dried by the following methods.

Sun drying

Sliced oyster mushrooms were Sun-Dried (SD) at 30-35°C, gills up toward the sunlight, for 2-3 days from 11 am to 3 pm when UV was extreme (10±1) in Coimbatore District, Tamil Nadu. SD is the traditional and most convenient method of drying for the removal of moisture content from the mushrooms.

Cabinet drying

Thinly sliced oyster mushrooms were arranged on a tray in a single layer. A cabinet dryer was preheated for 15 minutes to obtain a constant temperature of 50-55°C, sample was kept in the dryer until the moisture was lost (around 10 hours).

Freeze drying

Sliced mushrooms were kept in a lyophilizer at 45±5°C with vacuum pressure of about 0.062 mbar for 36 h until their moisture was lost. Freeze-Dried (FD) is among the best methods for removing moisture without changing the color or texture of food products and with minimal volume change (Bhatta et al. 2020).

Drying extends the shelf life of oyster mushrooms. The dried oyster mushrooms were powdered finely in a grinding machine, sieved through a 0.5 mm pore-size sieve, and kept in an airtight container <2°C for further analysis. The samples were labeled Sun-Dried (SD), Cabinet-Dried (CD), and Freeze-Dried (FD) oyster mushroom powder.

Extract preparation from powdered oyster mushrooms

Ultrasonication-Assisted Extraction (UAE) was adopted for solvent extraction. UAE is a non-conventional method of solvent extraction from mushroom powder using an ultrasound device. The UAE method of extraction is reported to yield high ergosterol and metabolites from mushrooms in less time than the Soxhlet extraction method (Papoutsis et al. 2020). The protocol followed for the extraction of phytochemicals and metabolites was that of Patil et al. (2018) with some modifications to the solvent used and time.

Five grams of SD, CD, and FD oyster mushroom powder were added separately to three 250 mL glass

beakers and mixed with 100 mL of ethanol (HPLC grade). Ethanol is Generally Recognized as Safe (GRAS) which was adopted as a solvent extract other than methanol and water because of non-toxic and easy to evaporate. Nutraceutical and food supplements of mushroom extracts, either aqueous or alcohol-based could be dried or solution, and are available as powder or tablets form (Radzki et al. 2023). All three samples were covered with aluminum foil to prevent evaporation during extraction and were ultrasonicated (Labman digital ultrasonicator cleaner, 100W power rating, 40±3 Khz ultrasonication frequency) for 30 minutes at 50±5°C. After ultrasonication, the extracts were centrifuged at 3500 rpm for 15 minutes. The supernatant was separated and filtered through 11µm Whatman filter paper. The extracts were stored at -4°C until further analysis.

Antimicrobial assay

An antibacterial assay was performed following the Kirby-Bauer method in accordance with the standards set by the Advanced Research Laboratory, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, India. In brief, 4-5 mL of broth medium was incubated at 35°C for 2-6 hours to maximize turbidity. The culture was diluted in sterile saline to 1-2 x 10^8 CFU/mL for *S. aureus* (ATCC 6538), *B. cereus* (ATCC10987), *E. coli* (ATCC 25922*)*, and *Pseudomonas aeruginosa* (ATCC27853). The medium was punctured to make a 6 mm diameter well and the extracts were filled in different concentrations, such as 10 µL, 20 µL, 30 µL, 40 µL, and 50 µL. The Petri plates were inverted for complete diffusion, and the inhibition zones were examined by measuring the diameter (in mm) formed around the well after 24 hours' of incubation at 37°C. The zones were measured using a standard (Hi-Media) scale (Witasari et al. 2022). *Gentamycin*, well established antibiotic was randomly selected (easily available) as a positive control and ethanol as a negative control because ethanol was used as a solvent for extraction of bioactive compounds from oyster mushrooms. The two Gram-positive bacteria such as *S. aureus* and *B. cereus*, while two Gram-negative bacteria namely *E. coli*, and *P. aeruginosa* were studied against positive control "Gentamycin*"* since there was less reported of the specific bacteria in different drying of oyster mushroom extracts.

Phytochemical screening

Identification of the phytochemical constituents in naturally available food facilitates the finding of potent pharmacological and therapeutic activity. Conventional ways of performing phytochemical screening tests are more economical, easily affordable, and still good choices for qualitative phytochemical constituents. Phytochemical constituents were screened as described in Shaikh and Patil (2020) as follows:

Identification of alkaloids in mushroom extracts by Mayer's test and Dragendorff's test. A color change of creamy color precipitate and reddish brown precipitate respectively denoted the presence of alkaloids.

Identification of flavonoids by adding concentrated H_2SO_4 test. A change in color to reddish orange color indicated the presence of flavonoids in the extract. Identification of sterols in the mushroom extracts by Libermann's test, the presence of a reddish brown ring indicated that the extract contained sterols. Anthraquinones was identified by Borntrager's test, reddish orange color indicated the presence of anthraquinones. Identification of protein by 2% Ninhydrin reagent, showing a color of purple indicated the presence of protein in the extracts. Identification of carbohydrates by Molisch's test and Fehling's test), showing violet ring and red precipitate indicated the presence of carbohydrates. Identification of cardiac glycosides by Keller-Killani test, forming of brown ring indicated the presence of cardiac glycosides in the mushroom extracts.

Identification of functional groups by FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) of the extracts was performed to identify the presence of functional groups. FTIR analysis is the most powerful and fastest tool to detect the functional groups present in a substance (Mane and Khilare 2021). FTIR (SHIMADZU) was performed for the liquid extracts in the region between 400-4000 cm^{-1}.

Statistical analysis

Zone of inhibition against *S. aureus*, *B. cereus*, *E. coli*, and *P. aeruginosa* in different concentrations of SD, CD, and FD oyster mushroom extracts were calculated and compared within the group by one sample t-test at 95% confidence interval of the difference. A significant 2-tailed was analysed and *p*-value of <0.01 and <0.05 are at 99% and 95% significant respectively. It was performed in IBM SPSS Statistics version 21.

RESULTS AND DISCUSSION

Antimicrobial susceptibility of oyster mushroom extracts dried with different methods

SD oyster mushroom powder extract, CD oyster mushroom extract, and FD oyster mushroom powder extract were subjected to an antimicrobial activity test (Kirby-Bauer method). Many studies have reported that *Pleurotus* spp. has medicinal properties, including antimicrobial, antioxidant, anti-viral, anti-diabetic, and anti-inflammatory properties (Masri et al. 2017). A study on antimicrobial activities in methanol, petroleum ether, and acetone extracts of agro-waste-produced oyster mushrooms against five Gram-positive and five Gram-negative bacteria revealed that methanol extract had a broader spectrum of inhibition against the bacteria, followed by acetone extract and petroleum ether (El-Razek et al. 2020). In the current study, ethanol extracts of oyster mushrooms dried with different methods were analyzed for antimicrobial assays.

The pictorial zone of inhibition against the Gram-positive and Gram-negative bacteria by different concentrations of extracts of oyster mushroom dried by

different methods (10µL, 20µL, 30µL, 40µL, and 50µL) (Figure 1). The zone of inhibition against Gram-positive and Gram-negative bacteria by the positive control (Gentamycin) and negative control (ethanol) (Figure 1.A). The pictorial zone of inhibition against the two Gram-positive bacteria (*S. aureus* and *B. cereus*) and two Gram-negative (*E. coli* and *P. aeruginosa*) by the SD oyster mushroom powder extract (Figure 1.B). The zone of inhibition against the two Gram-positive bacteria (*S. aureus* and *B. cereus*) and two Gram-negative (*E. coli* and *P. aeruginosa*) bacteria by the CD oyster mushroom powder extract (Figure 1.C). The pictorial zone of inhibition against the two Gram-positive bacteria (*S. aureus* and *B. cereus*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) by the FD oyster mushroom powder extract (Figure 1.D).

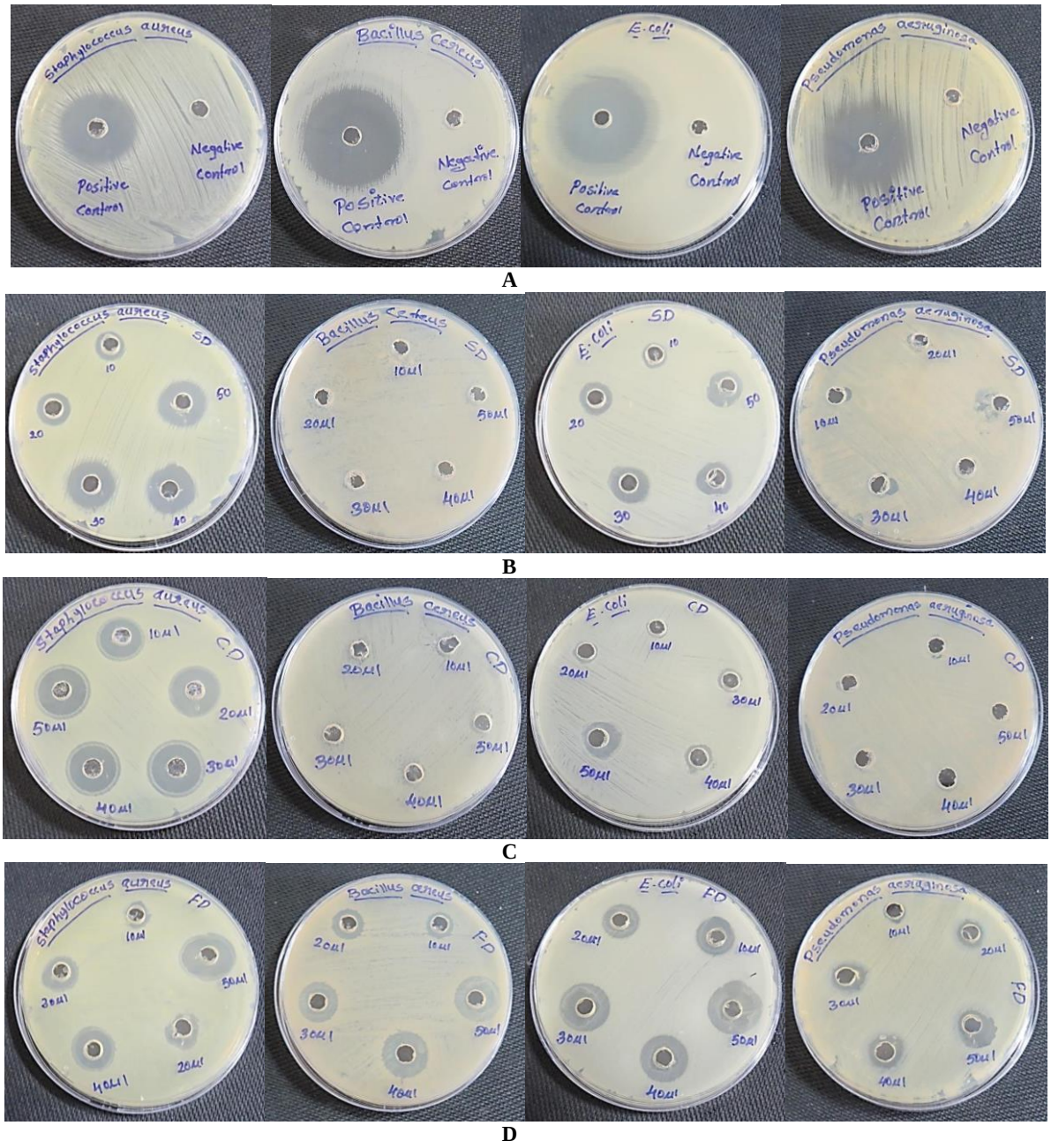

Figure 1. Zone of inhibition against Gram-positive and Gram-negative bacteria by: A. Positive control (Gentamycin) and negative control (ethanol), B. SD oyster mushroom powder extract, C. CD oyster mushroom powder extract, D. FD oyster mushroom powder extract

Table 1. Diameter of inhibition against two Gram-positive bacteria (*S. aureus* and *B. cereus*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) in different concentrations of SD, CD, and FD oyster mushroom extracts

Concentration (µL)	Zone of Inhibition (mm)											
	Gram-positive						Gram-negative					
	S. aureus (mm)			*B. cereus* (mm)			*E. coli* (mm)			*P. aeruginosa* (mm)		
	SD	CD	FD	SD	CD	FD	SD	CD	FD	SD	CD	FD
10	11	15	9	0	0	9	9	0	14	1	0	8
20	12	18	10	0	0	12	11	8	13	0	0	9
30	18	18	13	0	0	14	15	8	16	4	0	12
40	18	17	14	0	0	17	14	9	16	0	0	13
50	19	18	19	0	0	18	14	14	18	0	0	15
Mean zone of inhibition (mm)	15.6	17.2	13	0	0	14	12.6	7.8	15.4	1	0	11.4
p-value	0.001**	0.00**	0.002**	NS	NS	0.001**	0.000**	0.025*	0.000**	0.266	NS	0.001**
Standard (*Gentamycin*)	28 mm			35 mm			31 mm			29 mm		
Negative control (ethanol)	0			0			0			0		

Note: SD: Sun-Dried oyster mushroom extract, CD: Cabinet-Dried oyster mushroom extract, FD: Freeze-Dried oyster mushroom extract. ** significant level at 99.9%, * significant level at 95%, NS: Not Significant

The zone of inhibition by SD, CD, and FD oyster mushroom extracts in different concentrations (10 µL, 20 µL, 30 µL, 40 µL, and 50 µL) against two Gram-positive bacteria (*S. aureus* and *B. cereus*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) compared with positive control (standard Gentamycin). The higher the concentration, the greater the zone of inhibition observed, and the zone of inhibition is denoted in mm. The zone of inhibition by standard Gentamycin was 28 mm and 35 mm for the two Gram-positive bacteria (*S. aureus* and *B. cereus*), respectively, and 31 mm and 29 mm for the two Gram-negative bacteria (*E. coli* and *P. aeruginosa*), respectively. The negative control, ethanol, did not show a zone of inhibition of either the Gram-positive or Gram-negative bacteria. The mean zone of inhibition by SD, CD and FD oyster mushroom extracts against *S. aureus* were 15.6 mm, 17.2 mm and 13 mm respectively while against *B. cereus* were 0 mm, 0 mm and 14 mm respectively. The average zone of inhibition by SD, CD and FD oyster mushroom extracts against *E. coli* were 12.6 mm, 7.8 mm and 15.4 mm respectively whereas against *P. aeruginosa* were 1 mm, 0 mm and 11.4 mm respectively (Table 1).

The CD oyster mushroom extract had a greater ability to inhibit the growth of the Gram-positive bacteria *S. aureus* (p=0.000) followed by SD (p=0.001) and FD (p=0.002) oyster mushroom extracts, there was no significantly difference of drying methods against *S. aureus* while the FD oyster mushroom extract had more ability to inhibit the Gram-positive bacteria *B. cereus* (p=0.001)so, there was significantly affect by drying method to inhibit this bacteria, whereas the other two extracts had no zone of inhibition. The FD oyster mushroom extract had a higher inhibition zone, followed by the SD and CD oyster mushroom extracts for the Gram-negative bacteria *E. coli*, a non-significant difference was observed within the samples. The FD oyster mushroom extract had more ability to inhibit *P. aeruginosa* (p=0.001) than the SD or CD oyster mushroom extracts, as either no inhibition zone or much less inhibition was found for both. So, a significantly difference was observed. A study has shown the significant impact of aqueous and ethanol extracts of freeze-dried oyster mushrooms in antibacterial properties against *S. typhimurium*, *E. coli*, and *S. aureus* (El-Razek et al. 2020). In the present study, thermal degradation of bioactive compounds was observed in SD and CD oyster mushroom extracts compared to FD. SD and CD oyster mushroom extracts did not show antimicrobial properties to inhibit against *B. cereus* and *P. aeruginosa.* In contrast FD oyster mushroom extract showed antimicrobial properties to inhibit both Gram-positive and Gram-negative, showed statistically significant. Therefore, the study demonstrated that different drying methods might affect the bioactive compounds that are responsible for antimicrobial properties since FD oyster mushroom extracts preserved bioactive compounds followed by SD and CD (Table 1).

Phytochemical screening

Phytochemical screening was performed to observe the secondary metabolites present in extracts of oyster mushrooms dried using different methods. Studies have shown that macro-fungi like oyster mushrooms have been used in the pharmaceutical industry due to the presence of secondary metabolites (Al Qutaibi and Kagne 2024). One study by Rahimah et al. (2019) found that 70% ethanol of oyster mushroom extract contained a total phenolic compound (6.67 µg/1 mg) equivalent to 1 mg gallic acid. This same study also confirmed that aqueous was a better solvent than ethanol or acetone. Another study (Senthilkumar et al. 2015) demonstrated that major phytochemical constituents from edible wild *P. ostreatus* collected from the Dharmapuri District, Tamil Nadu, were found to be higher in ethanol extracts than other solvents, such as ethyl acetate, chloroform, and hexane. Ethanol is the most common solvent used for extraction other than water and methanol due to its dissolvability of polar and non-polar solvents; special techniques to be adopted for drying by using water for extraction, and the use of methanol for extraction are limited due to its toxic effect (Rahimah et al. 2019). Ethanol is also recognized as Generally Recognized as Safe GRAS by the US Food and

Drug Administration (FDA 2024). In the present study, ethanol was used as a solvent extraction to screen for 16 phytochemical constituents, and their presence in the extracts of oyster mushroom powders dried by different methods was compared.

Out of 16 phytochemical constituents screened, alkaloids and carbohydrates were present in high amounts in all three samples, shown in yellow as oyster mushroom extract was high in alkaloids, which has the potential role of antibacterial properties. Flavonoids, sterols, anthraquinones, and cardiac glycosides were found appreciably in all three extracts (shown in light green). Anthocyanin was found in the FD oyster mushroom extract in appreciable amounts but not detected in the SD or CD oyster mushroom extracts. Protein was found in appreciable quantities in the SD and CD oyster mushroom extract and high amounts in the FD oyster mushroom extract. Thus, FD retained more anthocyanin and protein content in the mushrooms than did other methods of drying. Terpenoids, phenolic compounds, quinones, tannins, saponins, lignin, coumarins, and volatile oils were not detected (shown in green) in any sample. Such metabolites might be lost during drying or processing. The potential effect of secondary metabolite content in fungi is closely related to its growing conditions, the extraction process, and the solvent used for solvent extraction (Herawati et al. 2021) (Table 2).

Identification of functional groups by FTIR analysis

Mushrooms naturally contain high amounts of polysaccharides, high protein, and low fat. The nutritional quality of mushrooms is closely related to their growing conditions. *Pleurotus* spp. cultivated on paddy straw was found to be abundant in protein and carbohydrates, high in fiber, and low in fat, so it is an excellent choice for a high-protein, low-calorie diet (Aswathy et al. 2024). The functional groups of the SD, CD, and FD oyster mushroom extracts were identified by FTIR analysis in the region of 400-4000 cm^{-1} (Figure 2). According to a previous study (Wickramasinghe et al. 2023): The important broadband lies in the region of 3300 cm^{-1} because the O–H and C–H group stretching predicts the availability of moisture. The band around 2900-2880 cm^{-1} represents the stretching of CH_2 and CH_3 (fatty acids in the cell walls). The band at 1740 cm^{-1} might be the result of the carbonyl stretching of alkyl esters, and the band between 1650-1560 cm^{-1} was determined to be amides of protein. The band between 1500-750 cm^{-1} might be polysaccharide C–O stretching, which is linked with protein, lipids, and polysaccharides. The region around 850-750 cm^{-1} has been identified as a polysaccharide anomeric configuration, the β- glycosides might be linked to the band at 890 cm^{-1}, and α- glycosides might be linked to the band between 860-810 cm^{-1}.

The major peaks for all three samples. The consolidated graph was prepared in Origin software 2024b (learning version). All three samples (SD, CD, and FD) show three specific bands in the region 400-4000 cm^{-1}. The peaks were found to be similar, but there were slight quantitative differences within the bands. SD, CD, and FD were found at the bands 3341.31 cm^{-1}, 3319.76 cm^{-1}, and 3338.62 cm^{-1}, respectively, which depict the availability of moisture. The bands at 2888.62 cm^{-1}, 2891.31 cm^{-1}, and 2888.23 cm^{-1} for SD, CD, and FD, respectively, show the stretching of CH_2 and CH_3, which showed the presence of sterols in phytochemical screening (Table 2). The bands at 1641.01 cm^{-1} (SD) and 1632.93 cm^{-1} (FD) represent the presence of proteins (Table 2), while there was no specific band for the CD extract. Thermal treatment might denatured proteins present in CD oyster mushroom extract. The bands at 1382.33 cm^{-1}, 1385.02 cm^{-1}, and 1387.22 cm^{-1} for the SD, CD, and FD extracts, respectively, show that the presence of the C–O group belongs only to polysaccharides (Akcay and Yalcin 2021) which denoted highly presence of carbohydrates in all the three mushroom extracts (Table 2). The sharp peaks at 1050.89, 1050.89, and 1053.59, respectively, might represent the presence of alkaloids, protein, lipids, and polysaccharide vibration (Table 2). The bands in the regions 878.44 cm^{-1}, 875.74 cm^{-1}, and 886.52 cm^{-1} respectively represented the presence of β- glycosides which showed the presence of flavonoids, cardiac glycosides in the SD, CD, and FD oyster mushroom extracts (Figure 2). There was a significant difference in protein content and no other bioactive compounds present in the three samples.

Table 2. Phytochemical constituents observed in sun-dried, cabinet-dried, and freeze-dried oyster mushroom extracts

Phytochemical constituents	Sun-dried oyster mushroom	Cabinet-dried oyster mushroom	Freeze-dried oyster mushroom
Alkaloids			
Anthocyanin			
Anthraquinone			
Carbohydrates			
Cardiac glycosides			
Coumarins			
Flavonoids			
Lignin			
Phenolic compounds			
Protein			
Quinones			
Saponins			
Sterols			
Tannin			
Terpenoids			
Volatile oils			

Note: ■: Absent, ■: Appreciable amount, ■: High amount

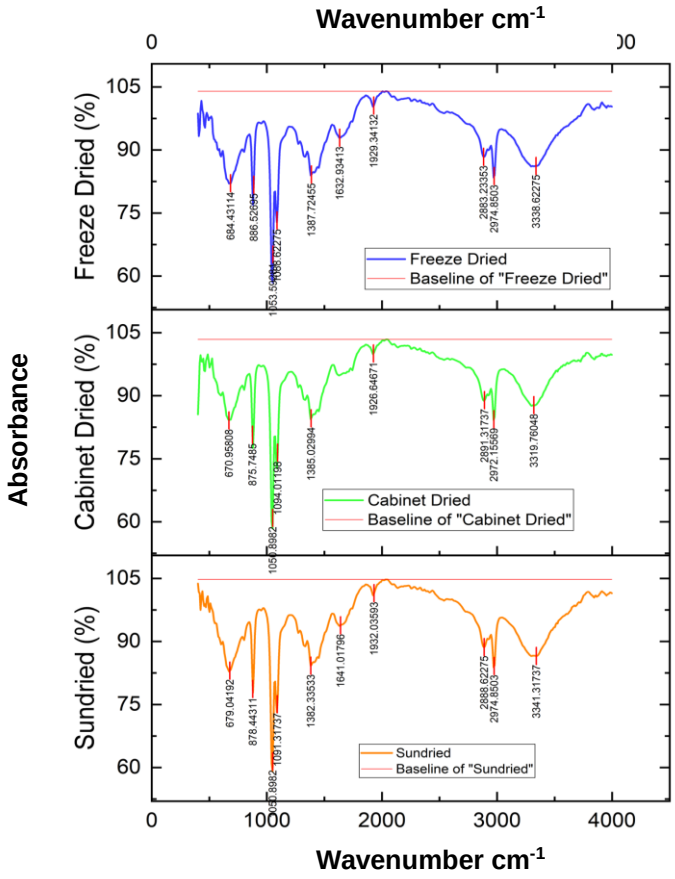

Figure 2. FTIR spectra of sun-dried, cabinet-dried, and freeze-dried oyster mushroom extracts region between 400-4000 cm.[1]

Discussion

Oyster mushrooms are well known for having nutraceuticals and pharmaceutical properties in mankind. They are highly perishable and have a short shelf life of 3-4 days at room temperature because of low ethylene production and high rate of respiration. Spoilage, browning, and off-flavor might happen if the mushrooms are not stored properly. Drying is the best way to extend the shelf life of mushrooms by removing moisture from them. There are different drying methods to reduce moisture content–traditional drying (sun drying) and advanced drying (cabinet drying and freeze drying) were adopted in the present experiment to evaluate whether the different drying methods impact the antimicrobial activity, content of primary and secondary metabolites as well as bioactive compounds. However, the practice of Sun Drying (SD) is affected because of environmental and weather conditions, and the products may be contaminated by pests, insects, dirt, etc. But it is still being adopted because of its convenience and low cost among other drying methods. Cabinet Drying (CD) is drying in a hot dryer with a high temperature within a few hours; it does not depend on weather conditions while Freeze Drying (FD) is an advanced technology that dries at low temperature, it retains nutrients more than other drying methods. Dried and powdered mushrooms can be stored for a year in well air-sealed conditions; this can be incorporated with food products and may be an alternate source of meat for the vegan population (Van Hung et al. 2020).

Ethanol is GRAS which is commonly used as a solvent extract because of non-toxic and easy to evaporate. UAE is a non-conventional method of solvent extraction which was adopted in this experiment because of the less time consumption as well as yielding more by this extraction process (Papoutsis et al. 2020). Characterization of antimicrobial activity, identification of secondary metabolites and other bioactive compounds of different drying of oyster mushrooms for various reasons. Primarily, ethanol extract among the three extracts (SD, CD and FD mushroom extracts) had maximum inhibition against Gram-positive and Gram-negative bacteria. Our findings revealed (shown in Table 1) that FD mushroom extract had maximum inhibition ability against both the Gram-positive bacteria and Gram-negative bacteria followed by SD and CD oyster mushroom extracts. Secondarily, to observe secondary metabolites from the three different extracts. Our finding observed that (Table 2) FD retained protein more than SD and CD; drying techniques did not affect the presence of secondary metabolites. Finally, to identify and compare whether drying techniques influenced functional groups present in all three extracts. The findings showed that (Figure 2) there was no effect in functional groups by drying.

The present study shows that SD, CD, and FD oyster mushroom extracts had average zones of inhibition against *S. aureus* at 15.6 mm, 17.2 mm, and 13 mm respectively, and the average zone of inhibition against *B. cereus* at 0 mm, 0 mm, and 14 mm respectively. SD, CD, and FD oyster mushroom extracts had an average zone of inhibition against *E. coli* at 12.6 mm, 7.8 mm, and 15.4 mm respectively whereas against *P. aeruginosa* at 1 mm, 0 mm, and 11.4 mm respectively. It revealed that the SD and CD oyster mushroom extracts have the potential role of inhibiting bacterial growth against one Gram-positive and one Gram-negative bacteria. In contrast, FD oyster mushroom extract has the highest ability to inhibit both Gram-positive and Gram-negative bacteria. Gram-positive bacteria (*B. cereus*) strain and Gram-negative bacteria (*P. aeruginosa*) were more resistant to SD and CD extracts. Ahmed et al. (2022) compared the antibacterial activity of three solvent extracts (methanol, petroleum ether, and acetone) of oven-dried *P. ostreatus* against Gram-positive and Gram-negative bacteria. Methanol extract showed the average zone of inhibition at 18 mm, 13 mm, 9 mm, and 16 mm against *S. aureus, B. cereus, E. coli,* and *P. aeruginosa* respectively. Petroleum ether extract showed the average zone of inhibition at 18 mm against *S. aureus* whereas it showed negative results for the other three bacteria. Acetone extract showed the average zone of inhibition at 20 mm, 20 mm, and 15 mm against *S. aureus, B. cereus,* and *E. coli* respectively, and a negative result for *P. aeruginosa*. The methanol extract had more antibacterial activity followed by acetone extract and petroleum ether extract was least and Gram-negative bacteria strains were more resistant than Gram-positive bacteria strains.

The phytochemical screening of SD, CD, and FD oyster mushroom ethanol extracts revealed the presence of high amounts of alkaloids, and carbohydrates in all three extracts. It showed a high amount of protein content in FD oyster mushroom extract whereas there was an appreciable amount of protein in SD and FD extracts. All three extracts presented appreciable amounts of flavonoids, sterols, anthraquinones, and cardiac glycosides. Anthocyanin was present in FD oyster mushroom extract whereas it was absent in SD and CD oyster mushroom extracts. A study was reported by Effiong et al. (2024) on the phytochemical constituent of five different solvent extracts (ethanol, methanol, aqueous, chloroform, and n-Hexane) of hot oven-dried *P. ostreatus.* All the extracts were qualitatively present of alkaloids, flavonoids, carbohydrates, tannins, anthocyanins and betacyanins, saponins, phenols, coumarins, quinones, terpenoids, and cardiac glycosides. In contrast, phlorotannins and betacyanins were absent in chloroform and n-hexane extracts. Either glycosides or acids were completely absent in all five extracts. Fakoya et al. (2020) demonstrated the presence of alkaloids, saponins, phenols, tannins, protein, carbohydrates and in ethanol and aqueous extract sun-dried *P. ostreatus* extract while triterpenoids and glycosides were found only in ethanol extract.

From the FTIR analysis, the present study demonstrated that all the major peaks of the three extracts (SD, CD and FD oyster mushroom) fell between the regions 400-4000 cm^{-1}. The stretching bands fell in the specific regions which have significantly related to the presence of sterols, alkaloids, proteins, lipids, carbohydrates, polysaccharide vibration, β- glycosides, flavonoids, and cardiac glycosides in all three samples. The bands at 1641.01 cm^{-1} (SD) and 1632.93 cm^{-1} (FD) represent the presence of proteins while there was no specific band for the CD extract, it might be denatured of protein during drying (Figure 2). FTIR analysis of four different oven-dried mushrooms (*Agaricus bisporous, P. ostreatus, Calocybe* species, and *Ganoderma lucidum*) powder showed specific regions with little difference in quantitative. All the bands are shown in IR-spectra correlated with the oyster mushroom components of protein, fat, carbohydrates, moisture, and polysaccharides including glucans (Wickramasinghe et al. 2023).

In conclusion, oyster mushrooms (*P. ostreatus*) have multiple benefits, as shown through the presence of antimicrobial properties, and they are rich in primary and secondary metabolites like alkaloids, carbohydrates, flavonoids, sterols, anthraquinones, and cardiac glycosides which have a potential role to play in inhibiting Gram-positive and Gram-negative bacteria, also found as the presence of bioactive compounds in the extracts from FTIR analysis. Research on different varieties of oyster mushroom using different techniques of extraction with other solvents can be implemented for future aspects to forge on pharmaceutical and food industries. This study also recommends fortifying traditional food products with oyster mushroom powder to improve their nutritional quality, and it will be therefore necessary to analyze its bioavailability.

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