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Characterisation and phytochemical profile of sunlight exposed freeze-dried oyster mushroom (*Pleurotus ostreatus*) powder

*Nongmaithem, B.D. and Chinnappan, A.K.

Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu - 641043, India

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Abstract

Mushrooms are vitamin B and D rich fungi which belong to the division Basidiomycota. Oyster mushroom (*Pleurotus ostreatus)* has distinct flavour, contains high nutrients and phytochemicals. Phytochemicals are naturally existing compounds which play with nutrients to protect against diseases. The aim of the study is to characterise and compare the phytochemical profile of sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder and non-sunlight exposed freeze-dried oyster mushroom (NSEFDOM) powder. The phytochemical constituents of both SEFDOM and NSEFDOM powder were extracted using aqueous, methanol and ethanol. Eighteen metabolites were screened for all the three solvents for both the samples. The phytochemical screening showed that the presence of alkaloids, flavonoids, sterols, proteins, carbohydrates, saponins for both samples were detected while other remaining metabolites were not detected. The results showed that the metabolites such as alkaloids, sterols, anthraquinones and carbohydrates were appreciable in the NSEFDOM powder group when compared to SEFDOM powder samples. Protein content was found to be high in SEFDOM powder group with ethanol extraction. The overall phytochemical extraction efficiency is high in methanol solvent. This study found that the amount of phytochemical content in non-sunlight exposed freeze-dried oyster mushroom powder was higher than sunlight exposed oyster mushroom powder.

1. Introduction

Mushrooms are fleshy edible fungi rich in vitamin B and D, belonging to the division Basidiomycota. The benefits of vitamin D in edible mushrooms have been known when the provitamin D_2 was extracted from wild mushrooms (Keegan *et al*., 2013). Oyster mushrooms (*Pleurotus ostreatus)* also popularly known as Dhingri in India and it can be cultivated throughout the year (Biswas and Kuiry, 2013). The major oyster mushroom production states in India are Orissa, Karnataka, Maharashtra, Andhra Pradesh, Madhya Pradesh, West Bengal and most of the North Eastern hill states. According to the National Horticulture Board (2021-2022), Bihar produced more than 28,000 tonnes of all varieties of mushroom and is the largest mushroom producer (10.82% of total mushroom) in India. According to the Oyster Mushroom Global Market Report 2023, the marketing size of oyster mushrooms increased to 6.1% from 2022 to 2023 and China is the leading country for oyster mushroom production in the World (The Business Research Company, 2023).

Mushrooms are rich sources of B vitamins such as thiamine, riboflavin, niacin, biotin, vitamin C and vitamin D as ergosterol. Mushrooms are the only vegan source which can convert Vitamin D_2 from its precursor ergosterol by exposure to sunlight or Ultraviolet rays, due to isomerism. They contain high protein, low calorie, low fat, and a high proportion of polyunsaturated fatty acid (PUFA) around 72-85% of total fatty acid. They contain all essential amino acids which are required by healthy adults and abundant amounts of antioxidants. Mushrooms also contain many mineral salts. The most unique character of mushrooms is that they contain vitamin B_{12} which is not present in vegetable sources. Mushrooms are the one food which can give various nutrients so they are a useful way of getting multiple nutrients at same time for vegetarians. Mushrooms have the properties of antifungal, antitumor, antiviral, anti-inflammatory, antibacterial and contain distinct flavor and high antioxidative properties. The antioxidants can prevent free radicals and also prevent some diseases such as atherosclerosis, diabetes and cancer (Singh, 2017). Oyster mushrooms are commonly grown on decaying

*Corresponding author.
Email: *beitanong@gmail.com*

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woods or dying trunks of coniferous woods. Currently, in India oyster mushrooms are popularly grown indoors using sterilized paddy straw or wheat straw in polythene bags and made available throughout the year. The fruiting body of oyster mushrooms are consumed as soup, soup powder, gravy, pickle, fried items and also consumed raw as salad with other vegetables. The main view of the study is that regular consumption of mushrooms can get an abundant amount of Vitamin D and plenty of phytochemicals which can prevent deficiency diseases. The main objective of the study is to characterise and compare the phytochemical profile of sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder and non-sunlight exposed freeze-dried oyster mushroom (NSEFDOM) powder. This study also focuses on the antioxidant activities using DPPH free radical scavenging assays and to identify functional groups by FTIR spectroscopy for both the samples.

2. Materials and methods

2.1 Sample selection

Fresh oyster mushrooms were purchased from the Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for analysis.

2.2 Pre-preparation of oyster mushroom

Oyster mushrooms were collected and cleaned; removing stalks 2 cm from the leafy part of the mushroom and dividing them into two samples:

Sample 1 - Oyster mushrooms were sliced into 1×1 sq.cm. Sliced mushrooms were exposed gills up towards the sun for 30 mins (1–1:30 pm). Mushrooms were exposed to the sun to increase the content of Vitamin D_2. After exposure to sunlight, they were lyophilized.

Sample 2 - Similar to sample 1, oyster mushrooms were sliced and kept for lyophilisation without exposure to sun. Both the samples were lyophilized under the temperature of -45°C and vacuum pressure about 0.010-0.012 for 2 days. Freeze drying method is a convenient method in terms of retaining colour, texture and appearance (Argyropoulos *et al*., 2011).

Sunlight exposed freeze-dried oyster mushrooms (sample 1) and non-sunlight exposed freeze-dried oyster mushrooms (sample 2) were powdered after lyophilisation and screened with 1 mm pore size mesh sieve. Stored the powder in an airtight container and kept in the refrigerator (-4°C) for further analysis.

2.3 Preparation of oyster mushroom extracts

A total of six conical flasks were labelled as A, B, C, D, E and F. The 1 g of mushroom powder from sample 1 was added into each conical flask (A, B and C). From sample 2, added 1 g of mushroom powder in each conical flask (B, C and D). Water, Methanol and Ethanol were used as solvent for extraction. For the extraction of polyphenolic compounds, alkaloids, flavonoids, terpenoids and saponins were quantified more in ethanol extraction (Koffi *et al*., 2010; Ogidi *et al*., 2016). Poured 50 mL of relevant solvent to each conical flask.

'A' contains SEFDOM powder with Aqueous, 'B' contains SEFDOM powder with Methanol and 'C' contains SEFDOM powder with Ethanol. 'D' contains NSEFDOM powder with Aqueous, 'E' contains NSEFDOM powder with Methanol and 'F' contains NSEFDOM powder with Ethanol.

Extraction was performed by using the "Shaking Incubation method". Shaker incubator is one of conventional extraction methods. At a specific time, temperature and speed of the shaker, the powder sample is mixed with solvent and extraction is done inside the shaker incubator (Mirheli *et al.,* 2018). The mixtures were shaken for 30 mins in the Shaker Incubator at a temperature of 30°C at speed 7 and kept overnight in the refrigerator. Filtrated the extracts through 11.5 µm whatman filter paper. The extract was screened for phytochemical constituents, antioxidant activity and FTIR spectra.

2.4 Phytochemical screening of oyster mushroom powder extracts

The six extracts were subjected for phytochemical screening to identify the presence of metabolites like alkaloids, flavonoids, sterols, terpenoids, anthraquinones, anthocyanin, proteins, phenolic compounds, quinones, carbohydrates, tannins, saponins, cardiac glycosides, glycosides, lignins, coumarins and volatile oils.

2.5 Antioxidant activity using DPPH free radical scavenging assay

DPPH free radical scavenging assay is an antioxidant assay based on electron-transfer which produces a violet solution in ethanol (Garcia *et al.,* 2012). All the six samples were analysed antioxidant activity by using DPPH free radical scavenging assay which was based on ascorbic acid (AA) as standard solution. This analysis showed the percentage inhibition against the different concentration of the samples and compared samples with which solvent extract was more in antioxidant activity.

2.6 Fourier transform infrared spectroscopy analysis

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Fourier infrared absorption spectroscopy was carried to detect the presence of functional groups. All the six samples were used for FTIR (SHIMADZU) analysis, the range between 400-4000 cm^{-1}. FTIR is a rapid and most powerful tool to detect functional groups content in substances (Mane and Khilare, 2021). Phytochemical screening, antioxidant activity and FTIR analysis were performed in Bharat Ratna Prof. C.N.R Rao Research Centre, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

2.7 Statistical analysis

The percentage inhibition of sunlight exposed freeze -dried oyster mushroom (SEFDOM) powder extracts and non-sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder extracts were calculated and comparison of correlation coefficient of p-values were performed by Sigma plot version 14.5 by Pearson Correlation.

3. Results and discussion

3.1 Phytochemical screening

Sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder and non-sunlight exposed freeze-dried oyster mushroom (NSEFDOM) powder with different solvents were screened for phytochemicals. The result is given in Table 1, which shows the presence and absence of phytochemical constituents of the solvent extracts of both the groups that are sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder and non-sunlight exposed freeze-dried oyster mushroom (NSEFDOM) powder. The result shows that a highly appreciable amount of alkaloids is found in sample 2 with aqueous extract while in sample 1 with methanol

Table 1. Phytochemical screening for sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder and non-sunlight exposed freeze-dried oyster mushroom (NSEFDOM) powder with different solvents.

S/N.	Phytochemical constituents	Test Performed	Sunlight exposed freeze-dried oyster mushroom powder			Non-Sunlight exposed freeze-dried oyster mushroom powder		
			Aqueous extract (A)	Methanol extract (B)	Ethanol extract (C)	Aqueous extract (D)	Methanol extract (E)	Ethanol extract (F)
1	Alkaloids	+Mayer's reagent	-	+	+	+	+	+
		+ Dragendorff's reagent	+	+	-	+	-	+
2	Flavonoids	Alkaline test	-	-	-	-	-	-
		+ H_2SO_4	+	+	+	+	+	+
		+ lead acetate	+	+	-	+	+	-
		Shinoda test	-	-	-	-	-	-
3	Sterols	Liebermann Test	+	+	-	+	+	+
4	Terpenoids	Libermann test	-	-	-	-	-	-
5	Anthraquinone	Borntrager's test	-	-	-	-	+	-
6	Anthocyanin	HCl Test	-	-	-	-	-	-
7	Protein	+ 2% Ninhydrin reagent	+	+	+	+	+	+
		+ 2% $CuSO_4$ + 95% ethanol	-	-	-	-	-	-
		+KOH pellet	+	+	+	+	+	+
		+conc. HNO_3						
8	Phenolic compounds	+ 5% neutral $FeCl_3$	-	-	-	-	-	-
9	Quinones	Conc. HCl	-	-	-	-	-	-
10	Carbohydrates	Molisch's test	+	+	+	+	+	+
		Fehling's test	-	+	+	+	+	+
11	Tannin	Braymer's test	-	-	-	-	-	-
12	Saponins	Shaken with water	-	+	+	+	+	+
13	Phytates	+ ammonium thiocyanate + $FeCl_3$	-	-	-	-	-	-
14	Cardiac glycosides	+ Baljet reagent	-	-	-	-	-	-
		Bromine water test	-	-	-	-	-	-
		Keller -killani test	-	-	-	-	+	+
15	Glycosides	Borntrager's test	-	-	-	-	-	-
16	Lignin	+ Gallic acid	-	-	-	-	-	-
17	Coumarins	Fluorescence test	-	-	-	-	-	-
18	Volatile oils	Fluorescence test	-	-	-	-	-	-

(+) = Appreciable amount, (-) = Completely absent

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extract. Highly appreciable amount of flavonoids in both the groups with aqueous and methanol extracts. Appreciable amount of sterols in both the groups but absence in ethanol extract of sample 1. Appreciable amounts of protein and carbohydrates are found in all the samples. Saponins are present in appreciable amounts in both the groups but absent in aqueous extract of sample 1. For overall, a highly appreciable amount of phytochemical is found in sample 2 that is non-sunlight exposed freeze-dried oyster mushroom (NSEFDOM) powder when compared to sample 1 and sunlight exposed freeze-dried oyster mushroom (NSEFDOM) powder. Though the amount of vitamin D_2 is higher when mushrooms are exposed to sunlight because the isomerism of pro-vitamin D_2 to pre-vitamin D_2 (Keegan *et al.,* 2013).

3.2 Antioxidant activity using DPPH

Antioxidant activity of aqueous extract, methanol extract and ethanol extract of both the samples- sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder and non-sunlight exposed freeze-dried oyster mushroom (NSEFDOM) powder were analysed by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay with standard ascorbic acid. The values of free radical scavenging activity of all samples are presented in Table 2 and the percentage inhibition of all samples in different concentrations are represented in Figure 1.

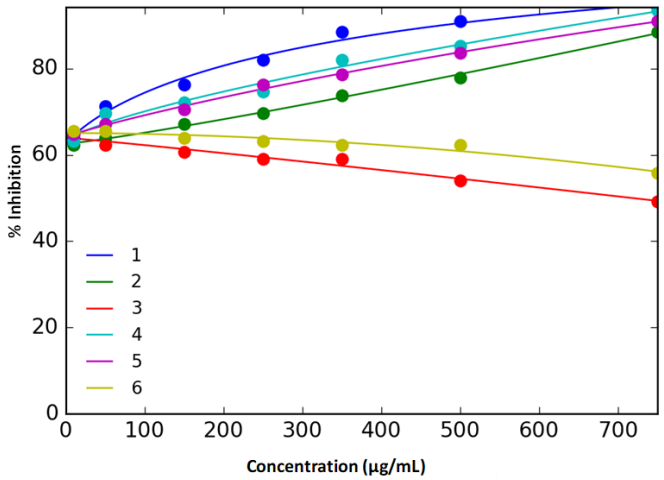

Figure 1. Percentage (%) inhibition curve of sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder and non-sunlight exposed freeze-dried oyster mushroom (NSEFDOM) powder.

Table 2 shows that the percentage (%) inhibition of sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder of aqueous extract (A), methanol extract (B) and ethanol extract (C) and Non-sunlight exposed freeze-dried oyster mushroom (NSEFDOM) powder of aqueous extract (D), methanol extract (E) and ethanol extract (F) against different concentration of samples. Sample 'A' and 'B' show highest inhibition at 750 µl and lowest inhibition at 10 µl of concentration and sample 'C' shows highest inhibition at 10 µl and lowest inhibition at 750 µl of concentration. Whereas, sample 'D' and 'E' also show that the highest inhibition is at 750 µl and lowest inhibition at 10 µl of concentration but sample 'F' shows the highest inhibition at 10 µl and 50 µl of concentration and lowest inhibition at 750 µl of concentration. From the above interpretation there is no difference in terms of percentage (%) inhibition activity of sample 1 and sample 2.

The percentage inhibition of both the groups - sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder extracts and non-sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder extracts were calculated and compared by Pearson Correlation and multiple linear regression. There is positive correlation between sample 1 and sample 2 (p-value <0.005) which means both the groups are positively significant. But there is not significantly different in both samples.

3.3 Fourier transform infrared spectroscopy spectra for functional groups

The presence of existing functional groups of the solvent extracted samples was determined by using FTIR spectra. In FTIR spectra, the x-axis denotes the infrared spectrum, which plots the intensity of infrared spectra. The peaks are known as absorbance bands. The y-axis denotes the amount of infrared light transmitted/ absorbed by the sample which is analysed (Jennifer, 2018). The functional groups and quantified frequencies were fallen between mid IR spectra (ie.,400-4000 cm^{-1}), frequency numbers lie: secondary metabolites (< 600 cm^{-1}) aromatic compounds (<1500 cm^{-1}) C=C, C=O and C=N groups (1500-2000 cm^{-1}), C≡C and C≡N groups (2000-2500 cm^{-1}), alcoholic/phenolic like O-H, N-H, C-H groups (2500-4000 cm^{-1}) (Asep and Rosi, 2019). The frequency numbers and peak are denoted in the following figures (2A, 2B, 2C, 2D, 2E and 2F). A series of bands in FTIR spectra of all the samples were observed.

Figure 2A shows major peak at 3348.42 cm-1 lies in the region of phenolic/ alcoholic group, at 1635.64 cm-1 lies in the region of alkene group and another peak at 686.66 cm-1 lie in the region of aromatic compound group and some fingerprints lie in the region < 600 cm-1 frequency number which shows that presence of high amount of secondary metabolites in sample 'A' .

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Table 2. DPPH free radical scavenging activity of sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder and non-sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder.

S/N.	Concentration (µL)	Sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder (% inhibition)			Non-sunlight exposed freeze-dried oyster mushroom (NSEFDOM) powder (% inhibition)		
		Aqueous extract (A)	Methanol extract (B)	Ethanol extract (C)	Aqueous extract (D)	Methanol extract (E)	Ethanol extract (F)
1	10	63.93	62.30	64.75	63.11	64.75	65.57
2	50	71.31	63.93	62.30	69.67	67.21	65.57
3	150	76.26	67.21	60.66	72.13	70.49	63.93
4	250	81.97	69.67	59.02	74.59	76.23	63.11
5	350	88.52	73.77	59.02	81.97	78.69	62.30
6	500	90.98	77.87	54.10	85.25	83.61	62.30
7	750	94.26	88.52	49.18	93.44	90.98	55.74

Figure 2B shows major peak at 3356.14 and another two peaks at 2947.23 and 2831.50 (cm-1) lies in the region of phenolic/ alcoholic group, at 1666.650 cm-1 lies in the region of alkene group and another peak at 686.66 cm-1 lie in the region of aromatic compound group and showing multiple fingerprints in region < 600 cm-1 which means that abundant amount of secondary metabolites are present in sample 'B'.

Figure 2C shows major peak at 3356.14 cm-1 lies in the region of phenolic/ alcoholic group, at 1666.50 cm-1 lies in the region of alkene group and another peak at 671.23 cm-1 lie in the region of aromatic compound group multiple finger print are shown which means present of secondary metabolites in sample 'C'.

Figure 2D shows major peak at 3317.56 cm-1 lies in the region of phenolic/ alcoholic group, at 1635.64 cm-1 lies in the region of alkene group and another peak at 686.66 cm-1 lie in the region of aromatic compound group and few number of fingerprints shows less present of secondary metabolites in sample 'D' .

Figure 2E shows major peak at 3356.14 cm-1 lies in the region of phenolic/ alcoholic group, at 1635.64 cm-1 lies in the region of alkene group and another peak at 686.66 cm-1 lie in the region of aromatic compound group and multiple fingerprints lie between 400-600 cm-1 frequency number, more secondary metabolites are present in sample 'E' when compare to other samples.

Figure 2F shows major peak at 3348.42 cm-1 lies in the region of phenolic/alcoholic group, at 1651.07 cm-1 lies in the region of alkene group and another peak at 686.66 cm-1 lie in the region of aromatic compound group and some fingerprints shows in the region between 400-600 cm-1. So, sample 'F' also presents some secondary metabolites.

From the above interpretation, there is not much difference in presence of phenolic/alcoholic group, alkene group and aromatic group in both samples 1 and 2, whereas, the result was found to be higher of secondary metabolite in sample 'E' ie., non-sunlight exposed freeze-dried oyster mushrooms (NSEFDOM) powder with methanol extracts. Hence, the result shows that sample 2 contains more secondary metabolites when compared to sample 1.

4. Conclusion

Oyster mushrooms have a distinct flavour, contain high nutrients and phytochemicals. The present study concluded that the presence of phytochemicals in NSEFDOM powder extracts were higher when compared to SEFDOM powder extracts. But in the view of antioxidant activity, there is not much difference in percentage inhibition for both the groups when the correlation coefficient of p-values is <0.005. From the FTIR spectra, this study also concluded that both the groups contain appreciable amounts of alcoholic/ phenolic compounds, alkenes and aromatic compounds. But the presence of secondary metabolites is higher in sample1 when compared to sample 2. Hence this study concluded that non-sunlight exposed freeze-dried oyster mushroom (NSEFDOM) powder contained more phytochemicals than sunlight exposed freeze-dried oyster mushroom (SEFDOM) powder when there is no difference in antioxidant and functional groups. Sunlight exposure of mushrooms might increase ergosterol levels but phytochemical constituents may be lost in the process. Therefore, consumption of oyster mushrooms without exposure to sunlight is better for the phytochemicals.

Conflict of interest

The authors declare no conflict of interest.

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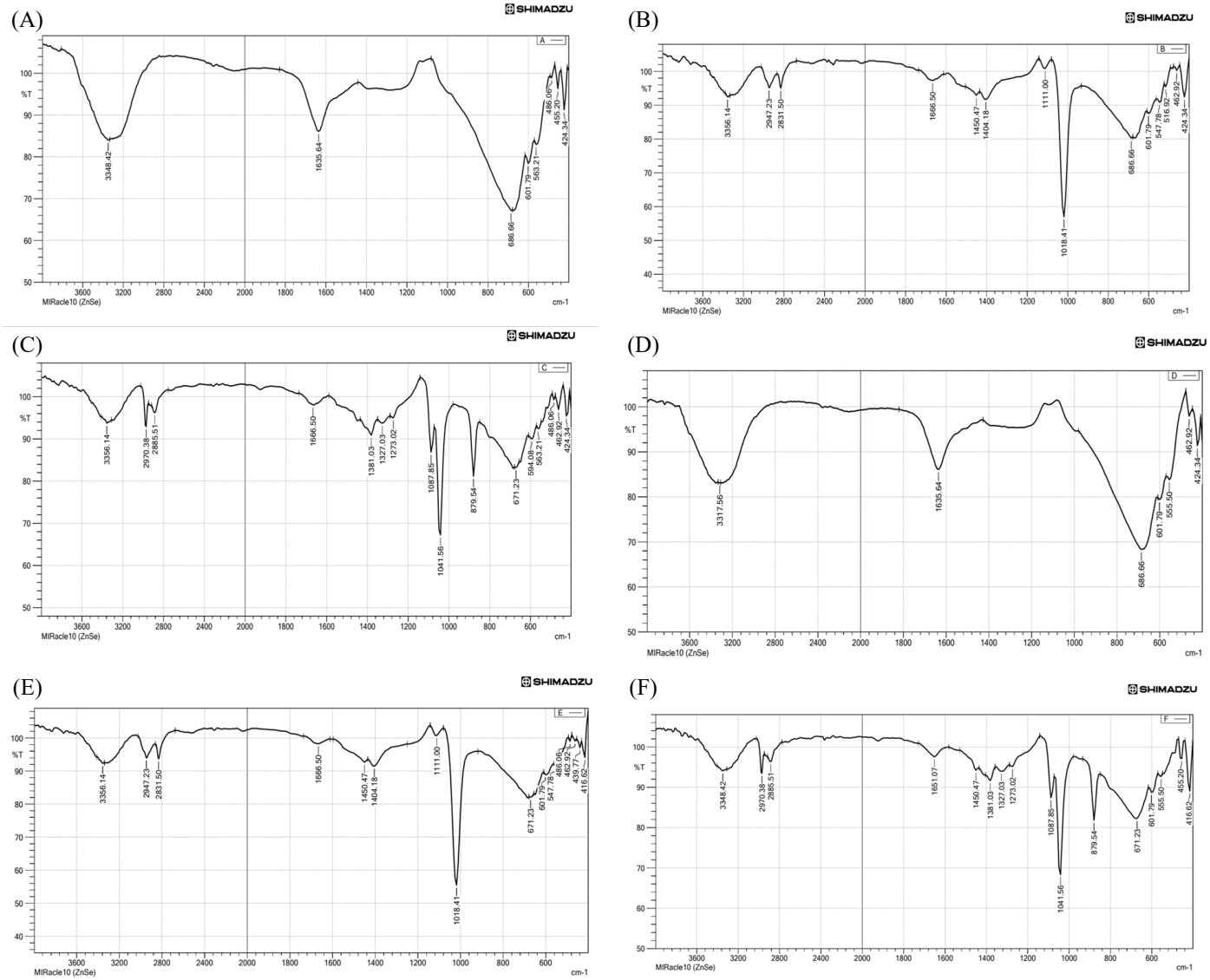

Figure 2. FTIR spectra of aqueous extract of sunlight exposed freeze-dried oyster mushroom powder. A: Sample A, B: Sample B, C: Sample C, D: Sample D, E: Sample E, and F: Sample F.

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