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Antibacterial Activity and Quality Assessment of Ergocalciferol in Lyophilized and UV Irradiated Oyster Mushroom (*Pleurotus Ostreatus)* Powder

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Abstract

Mushrooms are highly nutritious fungi and also considered as nutraceuticals. Oyster mushroom (*Pleurotus ostreatus)* is one of the edible mushrooms which are mostly cultivated. Ergocalciferol (vitamin D_2) is an active form of vitamin D which is available in plant sources mainly in mushrooms as ergosterol. Ergosterol is the main precursor sterol to convert from pre-vitamin D_2 vitamin D_2 by absorption of ultraviolet light. It also has potents of antibacterial, antioxidant, antifungal, antitumor, antiviral and other applications. The main aim of the study is to evaluate the antibacterial activity and the quality of ergocalciferol in lyophilized oyster mushroom powder and UV irradiated lyophilized oyster mushroom powder. Oyster mushrooms were lyophilized and powdered prior to extract. Extraction of oyster mushrooms was performed by ultrasonication bath using ethanol as solvent extract. Solvent extracts were qualitatively analysed by the HPTLC-method. Antibacterial activity of the extracts were performed by Kirby Bauer method using *E. coli* and *Staphylococcus aureus* as bacteria in agar medium. This study showed that the presence of ergocalciferol in the mushroom powder extracts and exhibited higher peak in the UV irradiated mushroom powder extract. The solvent extracts had the ability to inhibit bacterial growth. This study concluded that UV irradiated oyster mushroom powder extract was more capable of inhibiting bacteria and exhibited a higher peak in qualitative analysis of ergocalciferol.

1. Introduction

Mushrooms are highly nutritious fungi and also considered as nutraceuticals. The consumption of mushroom and cultivation has been increased presently because of its highly nutritional potential and nutraceutical properties. Currently, mushrooms have been promoted not only in the food industry but also in the pharmaceutical industry and cosmeceutical industry [1]. In individuals, vitamin D deficiency is globally occurring in all age groups, genders and regions. Consumption of vitamin D rich source is limited especially for vegans thus regular consumption of mushrooms can be an achievable alternative diet of fatty fishes and other natural food sources of vitamin D [2].

Oyster mushroom (*Pleurotus ostreatus)* is the world's second largest cultivation among all varieties of mushroom. The production size of *Pleurotus sp.* is increased to 6.1% in 2023 from 2022, China leads the production of *Pleurotus sp.* Globally [3]. Oyster mushroom is commonly referred to as Dhingri in India. Depending upon the species, the fleshy part of oyster mushroom has distinct shapes with different shades of white, grey, cream, yellow or light brown [4]. The fleshy part of oyster mushrooms contains approximately 100 bioactive compounds [5]. Bioactive compounds of several oyster mushrooms have potents of antibacterial, antiviral, antifungal, and antimicrobial activities [6]. Mushrooms are rich sources of protein, vitamin B, C, D

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and K, including minerals like phosphorous and potassium, appreciable amounts of dietary fibres and contain low fats [7]. Consumption and production of mushrooms have been increased because of highly demands due to their desirable aroma, nutritional benefits and unique taste [8]. According to the study findings from NHANES III, lower risk of total mortality rate was associated with consumption of mushrooms in US adults [9].

Oyster mushroom is a rich source of vitamin D_2 when exposed to sunlight or ultraviolet radiation. Mushrooms which are grown in dark places contain insignificant amounts of ergocalciferol while mushrooms exposed to sunlight enhance the level of ergocalciferol [10]. Dry oyster mushrooms contain 109 µg of ergocalciferol per 100 gram [11]. The main perspective of the study is consumption of mushrooms which are exposed to sunlight or ultraviolet irradiation, to provide feasible extraction of ergocalciferol for utilisation widely from naturally available sources which can promote the bioavailability of vitamin D as well as other essential nutrients to large population which could prevent deficiency diseases. The main aim of the study is to evaluate the antibacterial activity and the quality of ergocalciferol in lyophilized oyster mushroom powder and UV irradiated lyophilized oyster mushroom powder.

2. Materials and Methods

2.1 Sample selection

Fresh oyster mushrooms (*Pleurotus ostreatus*) were purchased from a local farm, Coimbatore, Tamil Nadu. It was grown in mushroom hut and harvested in June 2022.

2.2 Preparation of oyster mushroom powder

Oyster mushrooms were cleaned and washed thoroughly, taking the fruiting parts of the oyster mushroom. Sliced the mushrooms and kept for lyophilisation till moisture to be lost, at the temperature – 50±5 for 36 hours and vacuum pressure about 0.062 mbar. Papoutsis *et al.,* [12] reported that mushrooms which were lyophilized contained more vitamin D_2 when compared to other methods of drying. Freeze drying is one among the best methods of removal of moisture without changing colour and texture of food product by minimal volume change [13]. The lyophilized mushroom was finely powdered in a grinding machine and sieved through 0.5 mm pore size sieve and stored in an air tight container at temperature < 5℃ for further analysis.

2.3 Extraction of vitamin D_2 from oyster mushroom powder by Ultrasonication-assisted extraction (UAE)

Ultrasonication-assisted extraction (UAE) is a non-conventional method of solvent extract which has been employed to extract ergosterol and ergocalciferol from mushroom powder using ultrasound devices. UAE method of extraction was reported that the high yielding of ergosterol from mushroom in minimum time consumption than Soxhlet extraction method [12]. Patil *et al.,* [2] reported that continued exposure of mushroom powder to ultrasonication devices for one hour did not exhibit the loss of ergosterol. The protocol of extraction of vitamin D_2 was performed by the procedure of Patil *et al.,* (2018) with some modification.

1 gram of lyophilized powdered oyster mushroom was taken in 100 ml glass beaker and mixed with 20 ml of ethanol (HPLC grade) and labelled as sample 1. In another beaker, 1 gram of lyophilized powdered oyster mushroom was taken and mixed with 20 ml of ethanol, labelled as sample 2. Sample 2 was irradiated under UV-C lamp (Philips 11W UVC tube Jainsons light) for two hours which was kept at a distance of 10 cm from the beaker.

Both samples 1 and 2 were ultrasonicated (Labman digital ultrasonicator cleaner) for 30 minutes at temperature 50±5 ℃. After ultrasonication, extracts were centrifuged at 3500 rpm for 15 minutes. Separated the supernatant and filtered through 11µm whatman filter paper. Transferred both the samples to beakers and kept open to evaporate the solvent (5 days). Mixed the dried content with 10 ml of ethanol with a glass rod, covered with aluminum foil and kept overnight. The diluted mixture was filtered again through 11 µm whatman filter paper.

2.4 Antibacterial susceptibility of oyster mushroom extract

Antibacterial susceptibility was performed by the Kirby-Bauer method in accordance with the standards set by Advanced Research Laboratory, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. In brief, 4-5 ml of broth medium

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was incubated at 35 for 2-6 hours to exceed turbidity. The culture was diluted in sterile saline to 1 to 2 x 10^8 CFU/ml for *E.coli* and *Staphylococccus aureus.* The petri plates were placed inversely for complete diffusion and inhibition zones were examined by measuring the diameter (mm) formed around the well after 24 hours incubation at 37°C. The zones were measured by using standard (Hi-Media) scale [14].

2.5 Quality assessment of ergocalciferol in oyster mushroom extract

Quality assessment of ergocalciferol of oyster mushroom extract was performed by HPTLC method according to the protocol set by Advanced Research Laboratory, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. The volume of 2 µl, 4 µl, 6 µl, 8 µl and 10 µl of oyster mushroom extract of both the sample 1 and sample 2 were applied separately with reference to standard ergocalciferol (Sigma Brand) concentration of 2 µl/ml, 4 µl/ml, 6 µl/ml, 8 µl/ml and 10 µl/ml in silica gel 60 F 254, HPTLC plate size of 20×10 cm. The derivatization of the chromatogram was performed in the Twin Trough chamber (20×10 cm) at temperature of 60 for 5 minutes at a wavelength range from 230 nm to 280 nm. The peak height and area of the given samples were detected in CAMAG TLC scanner [15].

2.6 Statistical analysis

Diameter of inhibition against *Staphylococcus aureus* and *E.coli* in different concentrations of lyophilized oyster mushroom powder extract (S1) and UV irradiated lyophilized oyster mushroom powder extract (S2) were calculated and compared of both samples by one way ANOVA. Maximum height retained by S1 and S2 were analysed by student t- test, p-values were performed by Sigma Plot Software version 14.5.

3. **Results and Discussion**

3.1 ***Antibacterial susceptibility of oyster mushroom extract***

By the Kirby-Bauer method, lyophilized oyster mushroom powder extract (sample 1) and UV irradiated lyophilized oyster mushroom powder extract (sample 2) were performed for antibacterial susceptibility test. The result is given in Table 1, which shows the diameter of inhibition by sample 1 and sample 2 in different concentrations (10µ1, 20µ1, 30µ1, 40µ1 and 50µ1) against two bacteria namely *Staphylococcus aureus* and *E.coli* compared to standard (*kanamycin).* When compared to standard, the zone of inhibition against *Staphylococcus aureus* by sample 1 and sample 2 were less in inhibits bacterial growth. But comparing sample 1 and sample 2, in lower concentration sample 1 inhibited against *Staphylococcus aureus* than sample 2 while in higher concentration sample 2 extended zone of inhibition than sample 1. For the average concentration, the zone of inhibition against the bacteria was not significantly difference between both sample 1 and sample 2. In terms of the zone of inhibition against *E.coli*, both the samples inhibited bacterial growth more than standard. When comparing to sample 1 and sample 2, same as the above bacteria in lower concentration the zone of inhibition against *E.coli* by sample 1was greater than sample 2 but in higher concentration, the zone of inhibition against *E.coli* by sample 2 was greater than sample 1.

Figure 1a, 1b, 1c and 1d represent the diameter of inhibition from *Staphylococcus aureus* in different concentrations (10µl, 20µl, 30µl, 40µl and 50µl) of lyophilized oyster mushroom powder extract, diameter of inhibition from *Staphylococcus aureus* in different concentrations (10µl, 20µl, 30µl, 40µl and 50µl) of UV irradiated lyophilized oyster mushroom powder extract, diameter of inhibition from *E.coli* in different concentrations of lyophilized oyster mushroom powder extract and diameter of inhibition from *E.coli* in different concentrations of UV irradiated lyophilized oyster mushroom powder extract, respectively. The mean diameter of inhibition against bacteria by sample 1 and sample 2 are evaluated in table 1 and analysed by one way ANOVA. Sample 1 and sample 2 are not statistically significant when p value is >0.05 due to randomly select variability ie., concentrations of sample.

3.2 Quality assessment of ergocalciferol in oyster mushroom extract

Quality assessment of ergocalciferol of lyophilized oyster mushroom powder extract (sample 1) and UV irradiated lyophilized oyster mushroom powder extract (sample 2) were performed by HPTLC method using standard ergocalciferol (Sigma Aldrich brand). The result of quality analysis is given in table 2. This table

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illustrates the maximum retention factor, maximum height and maximum area retained by both sample 1 and sample 2 with reference to different concentration of both samples. From the table, in lower concentration of sample which was run in TLC, maximum height/peak and area was more in sample 1 than sample 2 while in higher concentration, maximum height/peak and area were shown to be greater in sample 2 when compared to sample 1. The overall average maximum peak was shown higher in UV irradiated lyophilized oyster mushroom powder extract than lyophilized oyster mushroom powder extract.

Figure 2 shows that the HPTLC chromatogram of lyophilized oyster mushroom powder extract (S1) and UV irradiated lyophilized oyster mushroom powder extract (S2). Figure 2A exhibits visualization the bands after development at 254 nm remission and figure 2B exhibits visualization the bands after development at 366 nm remission in HPTLC chromatogram. Track 1 to 5 exhibits the visualization of standard ergocalciferol in different concentrations (2µl, 4µl, 6µl, 8µl and 10µl), from track 6 to 10 exhibits the visualization of sample 1 in different concentrations and from track 11 to 15 exhibits the visualization of sample 2 in different concentrations. Both the samples revealed the visualization of bands after development of remission.

The maximum height/peak retained by sample1 and sample 2 were analysed by student t-test, one tail p-values are <0.05, there is statistically significant between them.

4. Conclusion

The present study concluded that antibacterial activity of both samples - lyophilized oyster mushroom powder extract (sample1) and UV irradiated lyophilized oyster mushroom powder extract (sample 2) had ability to inhibit the growth of *Staphylococcus aureus* in different concentrations but when compared to standard (*kanamycin*) they had less ability to inhibit. While both samples had more ability to inhibit the growth of *E. Coli* in different concentrations and also compared to standard. This study also concluded that quality assessment of ergocalciferol existed in sample 1 and sample 2, sample 2 was found to be higher ergocalciferol level with reference to maximum height/peak and area which was retained in HPTLC chromatogram. There was statistically significant (p-value:<0.05). From the FTIR spectra, this study also concluded that both the samples contained appreciable amount of aromatic groups, alkene groups and phenolic groups and there was no difference in sample 1 and sample 2. Hence it may be concluded that oyster mushroom extract which was lyophilised and UV irradiated exhibited more ergocalciferol content that those oyster mushroom extract which was lyophilised only. But there was no effect in bacterial inhibition and functional groups by UV irradiation of oyster mushroom. Therefore, consumption of oyster mushroom exposed to UV or sunlight is a boon for vegans.

CONFLICT OF INTEREST

No conflict of interest

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Table1. Diameter of inhibition from *Staphylococcus aureus* and *E.coli* in different concentrations of lyophilized oyster mushroom powder extract (S1) and UV irradiated lyophilized oyster mushroom powder extract (S2)

Concentration	**Zone of Inhibition (mm)**							
	Staphylococcus aureus				***E.coli***			
	Sample 1 (S1)		**Sample 2 (S2)**		**Sample 1 (S1)**		**Sample 2 (S2)**	
		Mean		Mean		Mean		Mean
10µ1	8mm	10.6 mm	0mm	10 mm	0mm	9.2 mm	0mm	7.8mm
20µ1	9mm		10mm		8mm		0mm	
30µ1	11mm		12mm		12mm		11mm	
40µ1	12mm		13mm		12mm		13mm	

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50µ1	13mm		15mm		14mm		15mm	
Standard (*kanamycin*)	25mm				14mm			

Table 2. A*ssessment of ergocalciferol by HPTLC method of* lyophilized oyster mushroom powder extract (S1) and UV irradiated lyophilized oyster mushroom powder extract (S2)

Sl. No.	Volume	Sample 1 (S1)					Sample 2 (S2)				
		Max Rf	Max height	Area	Mean±SD (Max Height)	P-value	Max Rf	Max height	Area	Mean±SD (Max Height)	P-value
1	2 µl	0.56	43.5	168.2			0.52	37	893.1		
2	4 µl	0.55	42.5	2080			0.52	65.2	1554		
3	6 µl	0.55	69	2464.8	61.52	<0.05	0.52	92	2146.3	83.94	<0.05
4	8 µl	0.55	71.9	3192.5	±17.5		0.57	70.5	2002.4	±39.6	
5	10 µl	0.55	80.7	2431.2			0.51	155	6526.4		

Note: Maximum height and maximum area retained by S1 and S2 in HPTLC, Rf- Retention factor.

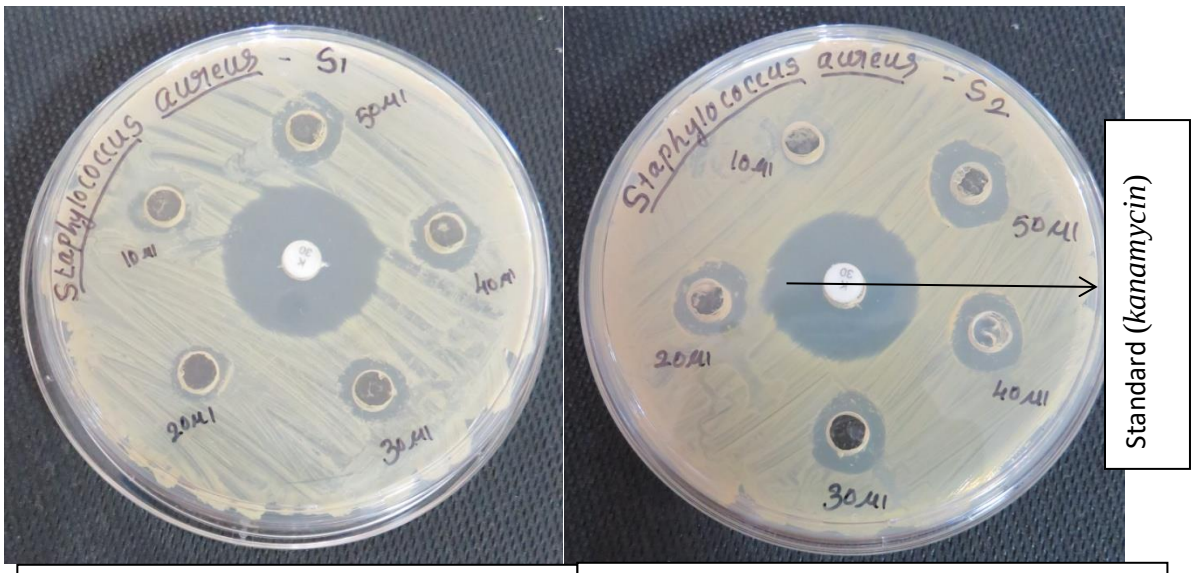

Figure 1a. Diameter of inhibition against *Staphylococcus aureus* in different concentrations of lyophilized oyster mushroom powder extract

Figure 1b. Diameter of inhibition against *Staphylococcus aureus* in different concentrations of UV irradiated lyophilized oyster mushroom powder extract

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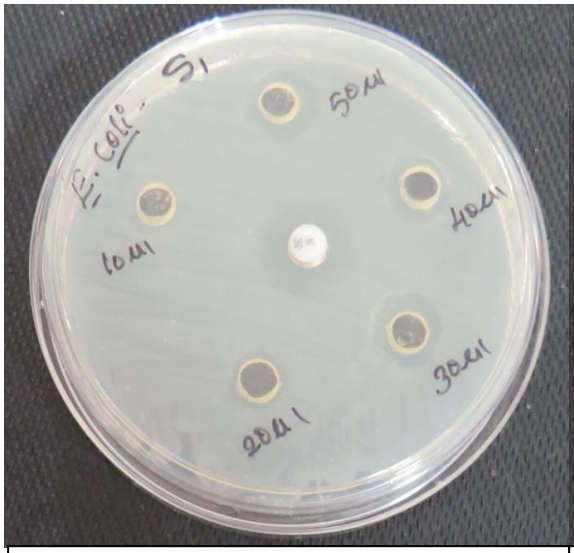


Figure 1c. Diameter of inhibition against *E.coli* in different concentrations of lyophilized oyster mushroom powder extract

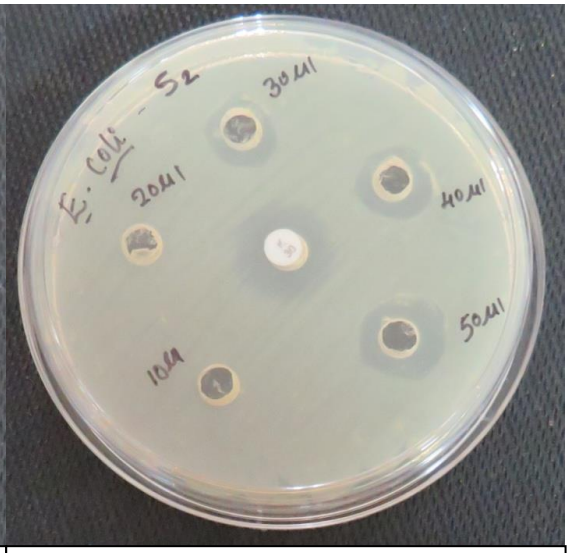
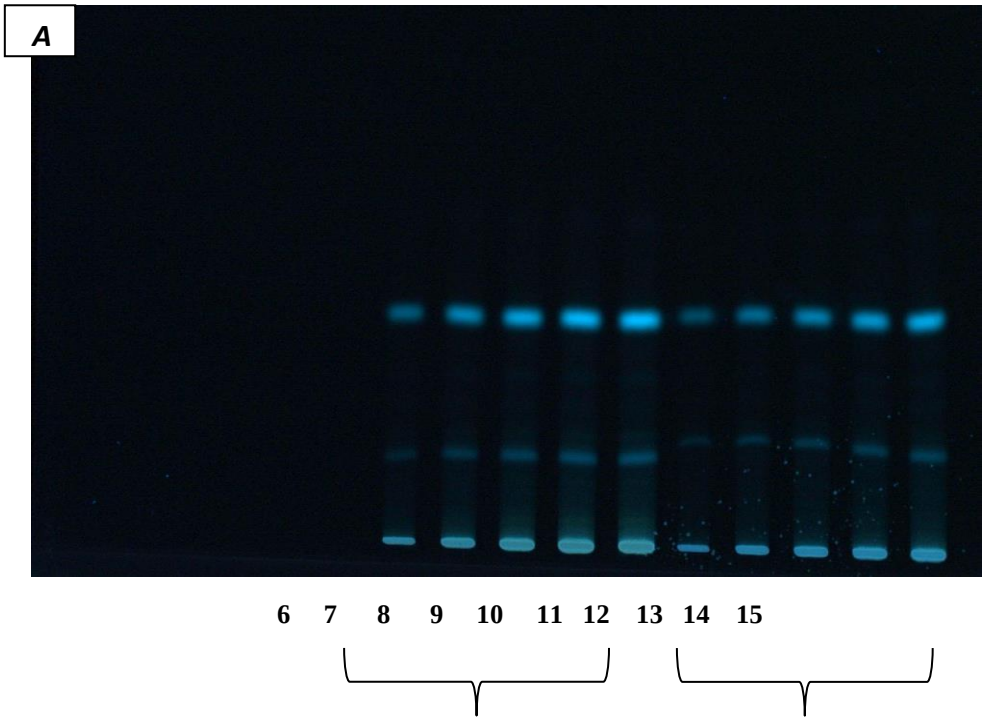


Figure 1d. Diameter of inhibition against *E.coli* in different concentrations of UV irradiated lyophilized oyster mushroom powder extract



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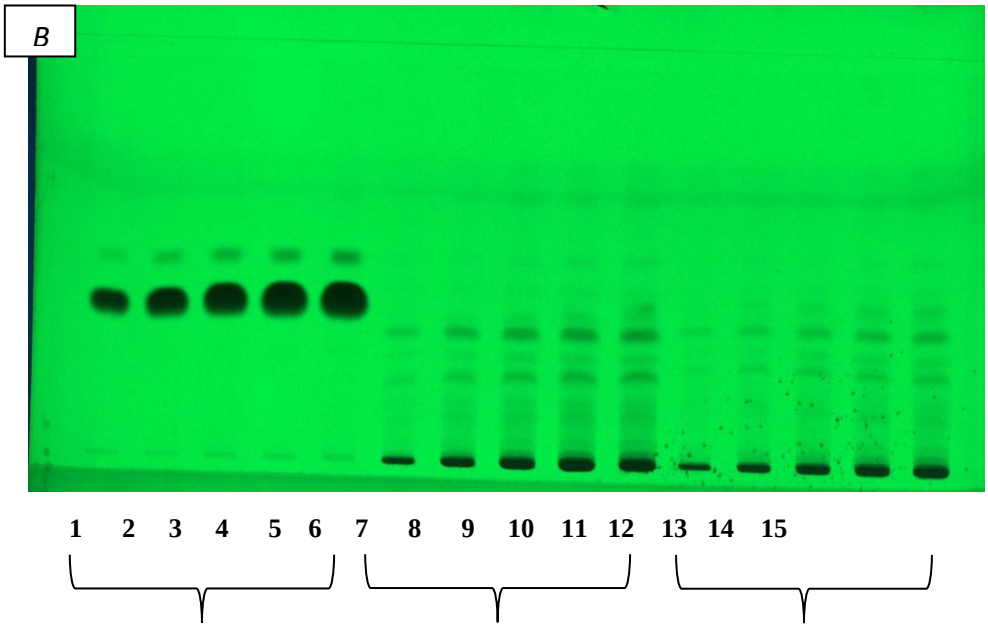

Figure 2. HPTLC chromatogram of lyophilized oyster mushroom powder extract (S1) and UV irradiated lyophilized oyster mushroom powder extract (S2): (A). Visualization after development at 254 nm remission and (B). Visualization after development at 366 nm remission.