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TLC and FTIR Analyses of *Hypsizygus ulmarius* (Bull.) Fruiting Bodies

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ABSTRACT

Mushrooms are known to contain many compounds that can be used for medicinal purposes. The present study evaluates the phytochemical constituents of methanolic extract, petroleum ether fraction and ethyl acetate fraction of *Hypsizygus ulmarius (BULL.)* fruiting bodies by thin-layer chromatography using various solvent systems and FTIR spectra. TLC test showed the presence of many compounds such as coumarins, anthraglycosides, arbutin, flavonoids, saponins, Furano- and pyranocoumarins. FTIR spectroscopic studies revealed characteristic peak values with various functional groups for compounds in the methanolic extract and its fractions.

INTRODUCTION

Ethnobotanically, mushrooms have been used by ancient people as a source of food and also for the treatment of many diseases (1-2). Mushrooms are rich in nutritional compounds such as proteins, carbohydrates, vitamins, minerals, etc. They considered a good source for bioactive compounds that can be used in the treatment of many diseases such as microbial infections, diabetes, cancer, inflammation, low immunity, high cholesterol, etc. (3-5).

Hypsizygus ulmarius (BULL.), Elm oyster or Blue oyster, is one of the most popular mushrooms in Japan, China, and many other Asian countries. It contains 52.4% carbohydrates, 23.6% proteins, 12.9% fiber and 2.2% fat. From the medical view, it contains phytochemicals which can be considered as a good source for many pharmaceutical compounds. It is known for its anti-cancer, anti-diabetic, anti-inflammation and antioxidant (6-8). The present study was carried out to screen the phytochemical constituents using TLC and functional groups using FTIR analyses of *Hypsizygus ulmarius (BULL.)* fruiting bodies extracts.

MATERIALS AND METHODS

Sample collection

Hypsizygus ulmarius fruiting bodies have been collected from "S" Mushroom Agritech, Hyderabad, Telangana state, India. (In dry form). The fruiting bodies were crushed into powder using an electronic grinder.

Preparation and fractionation of the extract

Powder sample of *Hypsizygus ulmarius* fruiting bodies (650 g) was extracted by Soxhlet apparatus using methanol (14 cycles) (9). The methanolic extract was evaporated using a rotary evaporator to yield a viscous brown extract (133 g). Part of Methanolic extract (90 g) had been fractionated by petroleum ether and ethyl acetate, respectively. Both solvents were evaporated using a rotary evaporator to yield a sticky brown extract (8 g for petroleum ether) and waxy brown extract (4 g for ethyl acetate).

Qualitative Phytochemical analysis

Thin-layer chromatography (TLC)

Methanolic extract and its fractions were analyzed by thin-layer chromatography on TLC pre-coated silica gel plate with a fluorescent indicator ($60GF_{254}$) from Merck $PSGF_{254}$. A small drop of samples was spotted about 1.0 cm from the bottom of the TLC plate by using a capillary tube. When the samples had dried, the TLC plate was placed into a TLC developing tank filled with an appropriate solvent system (seven different solvent systems were used) which listed in Table 1. The TLC plate was removed from the developing tank when it was fully developed to the solvent front. Then it left to dry at room temperature and was visualized in UV light (254 nm and 365 nm) and again in visible light and 365 nm after spraying with different reagents (Table 1) (10-12).

Table No. 1: TLC solvent systems and spray reagents

Sr. No.	Solvents	Solvent Proportions (by volume)	Spray reagents
1	**Chloroform: Methanol: Ammonia**	8:4:0.2	Anisaldehyde sulfuric acid (ASA)
2	**Chloroform: Ethyl acetate**	6: 4	Vanillin sulfuric acid (VSA)
3	**Ethyl acetate: Glacial acetic acid: Formic acid: Water**	10: 1.1:1.1:2.6	Potassium hydroxide (KOH)
4	**Ethyl acetate: Methanol**	9:1	Potassium hydroxide
5	**Ethyl acetate: Methanol: Water**	10:1.3:1	1-Vanillin sulfuric acid 2-Potassium hydroxide
6	**Toluene: Ethyl acetate**	7:3	1-Vanillin sulfuric acid 2-Concentrated sulfuric acid
7	**Petroleum ether: Ethyl acetate***	2:8	1-Anisaldehyde sulfuric acid 2-Vanillin sulfuric acid

Note: * solvent system used for petroleum ether and ethyl acetate fractions only

Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier Transform Infrared spectrophotometer (FTIR) is a tool used to identify the types of chemical bonds (functional groups) present in compounds. Methanolic extract, petroleum

ether fraction and ethyl acetate fraction were analyzed using the attenuated total reflection (ATR-FTIR) method. Alpha BRUKER FTIR spectrometer from 400–4000 cm^{-1} was used (13).

RESULTS AND DISCUSSION

This is the first study conducted to investigate phytochemicals in *H. ulmarius* fruiting bodies using the TLC technique and functional groups by ATR-FTIR analysis.

Phytochemical analysis by TLC

Results of TLC analysis of methanolic extract (M), petroleum ether fraction (P) and ethyl acetate fraction (E) are presented in Figures.1-7.

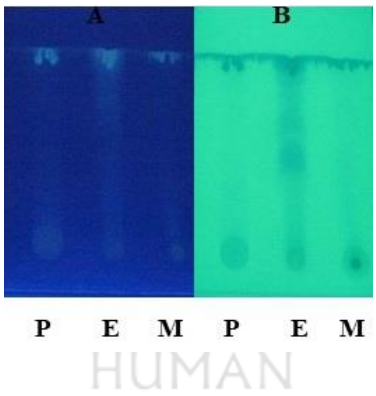

Figure No. 1: TLC analysis of methanol extract and its fractions. TLC plate solvent system was chloroform: ethanol: ammonia (8:4:0.2) and sprayed with ASA and seen under (A) UV light (365nm) and (B) UV light (254nm).

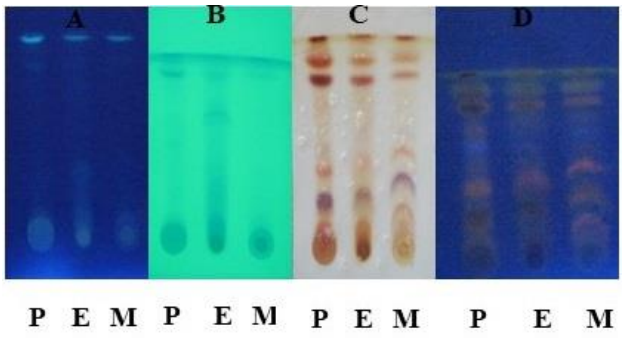

Figure No. 2: TLC analysis of methanol extract and its fractions. The TLC plate solvent system was chloroform: ethyl acetate (6:4). TLC plate under (A) UV light (365nm) and (B) UV light (254nm). TLC plate sprayed with VSA (C) and seen under UV light (365nm) (D).

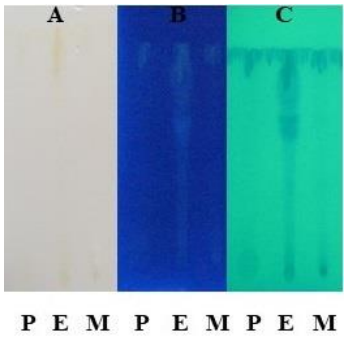

Figure No. 3: TLC analysis of methanol extract and its fractions. TLC plate solvent system was ethyl acetate: glacial acetic acid: formic acid: water (10: 1.1:1.1:2.6). TLC plate sprayed with KOH (A) and seen under UV light (365nm) (B) and UV light (254nm) (C).

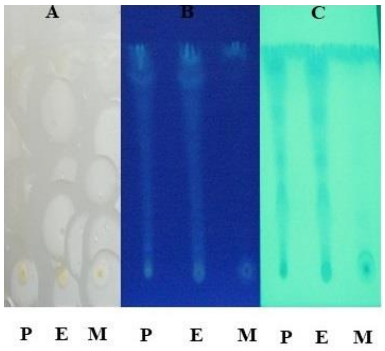

Figure No. 4: TLC analysis of methanol extract and its fractions. The TLC plate solvent system was ethyl acetate: methanol (9:1). TLC plate sprayed with KOH (A) and seen under UV light (365nm) (B) and UV light (254nm) (C).

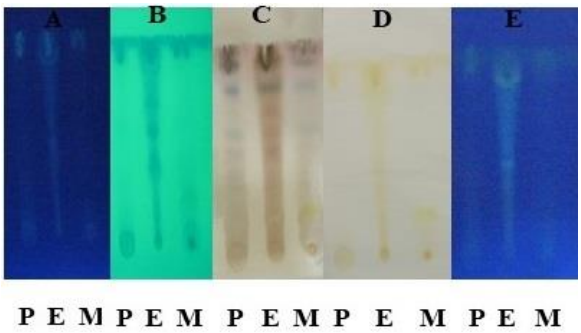

Figure No. 5: TLC analysis of methanol extract and its fractions. TLC plate solvent system was ethyl acetate: methanol: water (10:1.3:1). TLC plate under (A) UV light (365nm) and (B) UV light (254nm). TLC plate sprayed with VSA (C) and seen in visible light. TLC plate sprayed with KOH (D) and seen under UV light (365nm) (E)

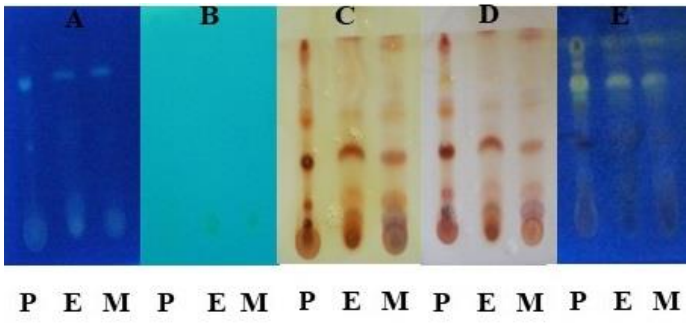

Figure No. 6: TLC analysis of methanol extract and its fractions. TLC plate solvent system was toluene: ethyl acetate (7:3). TLC plate under (A) UV light (365nm) and (B) UV light (254nm). TLC plate sprayed with VSA and seen in visible light (C). TLC plate sprayed with Conc. sulfuric acid (D) and seen under UV light (365nm) (E)

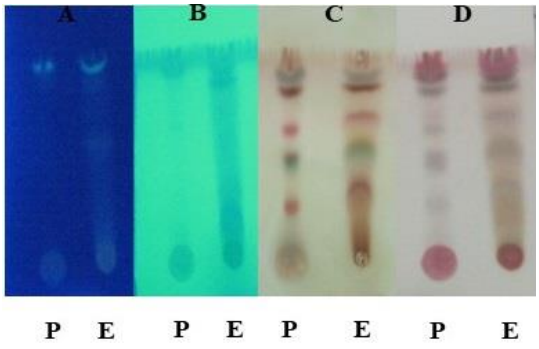

Figure No. 7: TLC analysis of petroleum ether and ethyl acetate fractions. TLC plate solvent system was petroleum ether: ethyl acetate (2:8). TLC plate under (A) UV light (365nm) and (B) UV light (254nm). TLC plate sprayed with ASA and seen in visible light (C). TLC plate sprayed with VSA and seen in visible light (D).

TLC was performed to separate and identify the different components in the methanolic extract and its fractions. As shown in Figures 1-7, By using different solvent systems and different spray reagents, similar chromatographic results were detected in which bright blue fluorescence zones in UV-365nm may be due to coumarins (e.g. scopoletin, umbelliferone) (Figures 1,2 (A), 3,4 (B), 5,6,7(A)). Quenching spots may be due to all compounds with conjugated double bonds e.g. anthraglycosides, arbutin, coumarins and flavonoids (UV light (254nm) (Figures 1,2 (B), 3,4(C), 5,7(B). Saponins from colored (vis.) zones with VSA reagent (Figures 2,5,6,7 (C) and 7(D). Colored fluorescent zones may be due to all anthraglycosides, coumarins, and flavonoids (Figures 2,5 (D). Green-blue, yellow, yellow-brown in UV-365 nm is due to Furano- and pyranocoumarins (Figure 5(E)). Most

constituents react with VSA and H_2SO_4 reagents with colored zones in vis. Both reagents are sufficient to detect bitter principles and saponins (Figure 6(D, E)) (12).

FTIR spectra of methanolic extract and its fractions

FTIR analysis of the methanolic extract and its fractions showed the presence of similar functional groups (14).

Methanolic extract

The result of FTIR analysis showed the absorbance bands at 718.50 cm^{-1} and 970.92 cm^{-1} revealed the presence of a C-H bond for aromatic and alkene. The band at 1046.77 cm^{-1}, 1172.05 cm^{-1}, and 1234.43 cm^{-1} indicate the presence of a C-O bond for ether, ester, anhydride, and alcohol. The absorption band at 1373.85 cm^{-1} and 1454.72 cm^{-1} indicate the presence of a C-H bond for alkane ($-CH_3$) and band at 2857.27 cm^{-1} showed the presence of C-H bond for alkane (stretch). The band at 1720.21 cm^{-1} indicates the presence of a C=O bond for aldehyde. The band at 2669.66 cm^{-1} and 2923.21 cm^{-1} represent the O-H bond for Carboxylic acid and band 3373.79 cm^{-1} showed the presence of O-H bond for alcohol, phenol (Table 2, Fig.8).

Table No. 2: FTIR spectral peak values and functional groups for methanolic extract of *H. ulmarius* fruiting bodies.

Sr. No.	Peak value (cm^{-1})	Bond	Functional Group
1	718.50	C-H group (out-of-plane bend)	Aromatic
2	970.92	C-H group (out-of-plane bend)	Alkene
3	1046.77	C-O group	Ether, ester
4	1172.05	C-O group	Anhydride
5	1234.43	C-O group	Alcohol
6	1373.85	C-H bending	Alkane ($-CH_3$)
7	1454.72	C-H bending	Alkane ($-CH_3$)
8	1720.21	C=O group	Aldehyde
9	2669.66	O-H group	Carboxylic acid
10	2857.27	C-H stretching	Alkane
11	2923.21	O-H group	Carboxylic acid
12	3373.79	O-H group	Alcohol, phenol

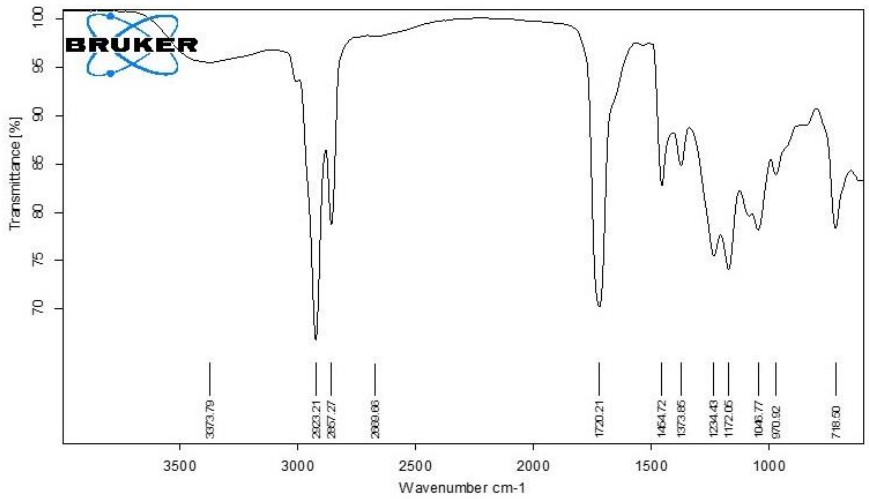

Figure No. 8: FTIR spectrum of methanolic extract.

Petroleum ether fraction

The FTIR spectrum showed the presence of absorbance bands at 715.44 cm^{-1} and 818.26 cm^{-1} indicate the presence of a C-H bond for aromatic and alkene. The band at 1050.20 cm^{-1} and 1181.38 cm^{-1} showed the presence of a C-O bond for ether, ester, and anhydride. The absorption band at 1373.49 cm^{-1} and 1454.78 cm^{-1} showed the presence of a C-H bond for alkane ($-CH_3$) and band at 1653.87 cm^{-1} and 1731.12 cm^{-1} showed the presence of C=O bond for amide and aldehyde. Absorbance band at 2856.99 cm^{-1} and 3007.10 cm^{-1} indicate the presence of a C-H (stretch) bond for alkane and alkene. The band at 2922.90 cm^{-1} and 3335.59 cm^{-1} represent the O-H bond for carboxylic acid and alcohol, phenol, respectively (Table 2, Fig.9).

Table No. 3: FTIR spectral peak values and functional groups for petroleum ether fraction of *H. ulmarius* fruiting bodies.

S. No.	Peak value(cm^{-1})	Bond	Functional Group
1	715.44	C-H group (out-of-plane bend)	Aromatic
2	818.26	C-H group (out-of-plane bend)	Alkene
3	1050.20	C-O group	Ether, ester
4	1181.38	C-O group	Anhydride
5	1373.49	C-H bending	Alkane ($-CH_3$)
6	1454.78	C-H bending	Alkane ($-CH_3$)
7	1653.87	C=O group	Amide
8	1731.12	C=O group	Aldehyde
9	2856.99	C-H stretching	Alkane
10	2922.90	O-H group	Carboxylic acid
11	3007.10	C-H stretching	Alkene
12	3335.59	O-H group	Alcohol, phenol

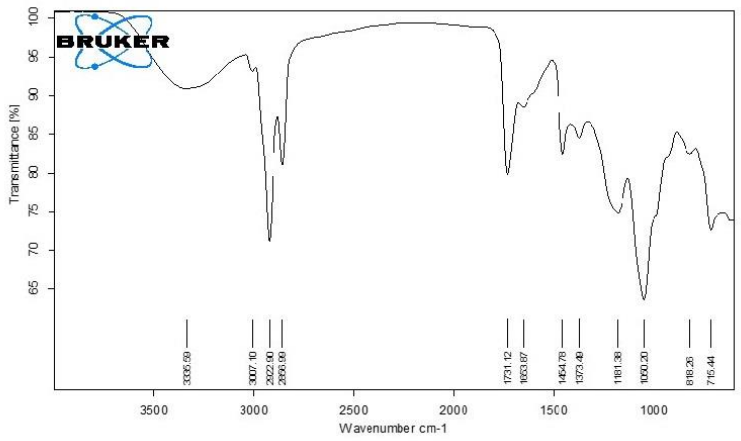

Figure No. 9: FTIR spectrum of petroleum ether fraction.

Ethyl acetate fraction

FTIR analysis indicates the presence of the absorbance band at 790.98 cm^{-1} showed the presence of a C-H bond for aromatic. Absorbance band at 837.67 cm^{-1}, 939.49 cm^{-1}, and

988.59 cm^{-1} indicate the presence of a C-H bond for alkene. The band at 1032.95 cm^{-1} and 1226.31 cm^{-1} indicate the presence of a C-O bond for ether, ester, and alcohol. The absorption band at 1395.13 cm^{-1} showed the presence of a C-H bond for alkane ($-CH_3$) and band at 1578.39 cm^{-1} showed the presence of an N-H bond for primary and secondary amines and amides. Absorbance band at 2861.67 cm^{-1} and 2925.50 cm^{-1} indicate the presence of a C-H (stretch) bond for an alkane. The band at 3271.07 cm^{-1} represents the O-H (stretch) bond for alcohol, phenol (Table 4, Fig.10).

Table No. 4: FTIR spectral peak values and functional groups for ethyl acetate fraction of *H. ulmarius* fruiting bodies.

Sr. No.	Peak value(cm^{-1})	Bond	Functional Group
1	790.98	C-H group (out-of-plane bend)	Aromatic
2	837.67	C-H group (out-of-plane bend)	Alkene
3	939.49	C-H group (out-of-plane bend)	Alkene
4	988.59	C-H group (out-of-plane bend)	Alkene
5	1032.95	C-O group	Ether, ester
6	1226.31	C-O group	Alcohol
7	1395.13	C-H bending	Alkane ($-CH_3$)
8	1578.39	N-H stretching	Primary and secondary amines and amides
9	2861.67	C-H stretching	Alkane
10	2925.50	C-H stretching	Alkane
11	3271.07	O-H stretching	Alcohol, phenol

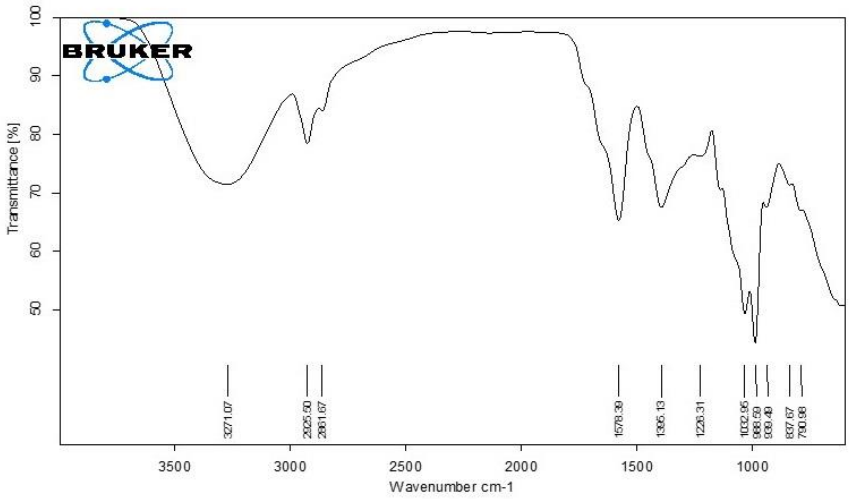

Figure No. 10: FTIR spectrum of ethyl acetate fraction.

CONCLUSION

The results of the present study confirmed that *H. ulmarius* fruiting bodies may be a good source of phytoconstituents which can be isolated and examined for further pharmacological activities. Further studies are required to evaluate the phytochemical compounds by TLC using many different solvent systems and also using specific spray reagents.

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