IJBPAS, May, 2020, 9(5): 938-945

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EVALUATION OF ANTIMICROBIAL ACTIVITY OF *HYPSIZYGUS ULMARIUS* (*BULL.*)

AL-FAQEEH LAS[*1], NASER R[2] AND KAGNE SR[3]

1: Research Scholar in Microbiology, Department of Botany, Maulana Azad College of Arts, Science and Commerce, Dr. Rafiq Zakaria Campus, Rauza Bagh, Aurangabad, M.S., India

2: Department of Botany, Maulana Azad College of Arts, Science and Commerce, Dr. Rafiq Zakaria Campus, Rauza Bagh, Aurangabad, M.S., India

3: Department of Microbiology, Badrinarayan Barwale Mahavidyalaya College, Jalna, M.S., India

***Corresponding Author: E Mail: lenaalfaqeeh8@gmail.com**

Received 27th Oct. 2019; Revised 19th Nov. 2019; Accepted 28th Dec. 2019; Available online 1st May 2020

https://doi.org/10.31032/IJBPAS/2020/9.5.5029

ABSTRACT

Mushrooms are known for their nutritional and medical values. The present study aimed to investigate antimicrobial activity of methanolic extract and its fractionation of *Hypsizygus ulmarius (Bull.)* fruiting bodies. Agar well diffusion method was used. The tested bacteria included *Staphylococcus aureus* (*S. aureus*), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (*S. epidermidis*) and one fungal species *Candida albicans* (*C. albicans*). Methanolic extract showed activity to *S. aureus*, MRSA and *C. albicans* while its fractions showed activity to *S. aureus* (petroleum ether fraction), *S. aureus* and MRSA (ethyl acetate fraction).

Keywords: Antimicrobial, Methanolic extract, Petroleum ether fraction, Ethyl acetate fraction, MIC, MBC, MFC

INTRODUCTION

Resistance to antibiotics become a serious concern in recent years **[1]**. Many pathogenic microorganisms exhibited a resistance against chemotherapeutic compounds and antibiotics, which lead to increase screening of alternative sources for their potential antifungal and antibacterial activity. These alternative sources include fungi, herbs, prokaryotic, animals and plants **[2]**. In the last few years edible mushrooms become one of the important sources of bioactive compounds that have been used in therapeutic application. They have many activities against cancer, bacteria, fungi, viruses, inflammation and high cholesterol **[3, 6]**. Among several numbers of edible mushrooms, *Hypsizygus ulmarius (BULL.)*, commonly called 'Elm oyster' or; Blue oyster' is a species with very large fruiting bodies. It's one of the most popular mushrooms in the world especially in China, Japan and other Asian countries **[7]**. From medical view it can be considered as a good source for active compounds. Phytochemical compounds and medical properties of *Hypsizygus ulmarius (BULL.)* have been reported **[8]**. The aim of this study was to investigate *In vitro* antimicrobial activity of crude methanolic extract and its fractionation from *Hypsizygus ulmarius (BULL.)* fruiting bodies.

MATERIAL AND METHODES

Mushroom

Dried *Hypsizygus ulmarius* fruiting bodies were collected from "S'' Mushroom Agritech, Hyderabad, Telangana state, India. Mushroom had been grown at 25 ℃ and dried by solar method.

Preparation of mushroom for extraction

Dried mushroom fruiting bodies have been blended to form a fine powder.

Extraction

Powdered mushroom sample (650g) was extracted with methanol (5L, 40-50℃), using Soxhlet apparatus. After extraction extract have been evaporated by rotary evaporator to produce a viscous residue **[9]**.

Fractionation of methanolic extract by different solvents

Part of methanolic extract had been fractionated using petroleum ether and ethyl acetate, respectively. Fractionated extracts were evaporated using rotary evaporator to produce a viscous (petroleum ether fraction) and a waxy residue (ethyl acetate fraction).

Phytochemical analysis and FTIR

Phytochemical analysis using TLC and FTIR analysis of methanolic extract and its

fractions were characterized by previous study **[10]**.

Test microorganisms

Four Gram - positive bacteria *Staphylococcus aureus* (clinical sample), *Staphylococcus aureus* (ATCC 25923), Methicillin-resistant *Staphylococcus aureus* (ATCC 43300) and *Staphylococcus epidermidis* (ATCC 14990) and one fungal species *Candida albicans* (ATCC 10231) were collected from Microbiology Department, Medical College, Aurangabad, India

Antimicrobial activity of methanolic extract and its fractionation

'Agar well diffusion method' was employed to evaluate antimicrobial activity of methanolic extract and its fractions **[11, 12]**. Bacteria were maintained on nutrient broth and fungus was maintained on potato dextrose agar at 37°C. Bacterial and fungal suspensions were prepared and adjusted to match 0.5 McFarland turbidity standard. Methanolic extract, petroleum ether and ethyl acetate fractions were dissolved in 1% dimethyl sulfoxide (DMSO) to final concentrations of 100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml. 0.1 ml of bacterial and fungal suspensions were spread over nutrient agar and potato dextrose agar plates using sterile cotton swab. Wells (8 mm diameter) were made in each of these plates using sterile cork borer. 100 µl of different concentrations of methanolic extract, petroleum ether and ethyl acetate fractions were added in to the wells and allowed to diffuse for 15 minutes at room temperature. Plates were incubated at 37°C for 18-24 hours. DMSO was used as a negative control, streptomycin (1mg/ml) and fluconazole (1mg /ml) as a positive control for bacteria and fungi. Activity was evaluated by measuring inhibition zone in millimeter. Studies were performed in triplicate.

Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

MIC, MBC and MFC were determined by classical method **[13, 14]**. A serious of double dilution of methanolic extract, petroleum ether and ethyl acetate fractions, ranging from 3.125 to 100 mg/ml were prepared in 1 ml of nutrient broth. 10 µl of bacterial culture and fungal culture, which adjusted to 0.5 McFarland standard, were inoculated in to test tube containing 1 ml of nutrient broth mixture and potato dextrose broth mixture, respectively. The tubes were incubated at 37°C for 18-24 hours and thereafter observed for turbidity or growth.

MBC

A loopful of nutrient broth from each test tube not showing growth was inoculated on to a sterile nutrient agar plates and an equal volume of sterile nutrient broth were added in to test tube cultures and incubated at 37℃ for 18-24 hours. Then, plates and tubes were examined for growth or turbidity using naked eye.

MFC

MFC was determined by inoculating a loopful of Potato dextrose broth tubes which showed no growth on to a fresh Potato dextrose agar plates and also equal volume of fresh sterile Potato dextrose broth were added in to test tube cultures and incubated for 18-24 hours at 37℃. Then, plates and tubes were examined for growth or turbidity using naked eye **[15]**.

Statistical Analysis

Experimental data are expressed as means ± stander error. Statistical analyses were performed by one-way ANOVA. This analysis was done using SPSS ver. 20.0 software

RESULTS AND DISCUSSION

Yield of the Methanolic extract and its fractions

The yield of methanolic extract after extraction from fruiting bodies of *H. ulmarius* was 133 g (Dark brown, semisolid and viscous). From this 133g only 90g have been fractionated by petroleum ether and ethyl acetate to yield 8g (Dark brown, semisolid and sticky) and 4g (Dark brown and waxy), respectively. This result is similar to Shivashankar M and Premkumari B findings, where methanolic extract of *H.ulmarius* fruiting bodies was brown, semisolid and sticky **[11, 16]**.

Antimicrobial activity

The results of antimicrobial activities of methanolic extract and its fractions at various concentrations are shown in **Table 1, 2 and 3 and Figure 1, 2 and 3**.

Methanolic extract and its fractions were tested against 4 bacterial species and one fungal species in which different activity appears. During the screening step, methanolic extract was more active in compared to its fractions. All concentrations of methanolic extract were active against *S. aureus*, MRSA and *C. albicans* only **(Table 1, Figure 1)**. The highest activity was noticed against *S. aureus* and MRSA at concentration of 400 mg/ mL in which inhibition zone was 21.67mm and 21.0 mm, respectively. The activity of petroleum ether fraction was shown in **Table 2**. It was only active against *S. aureus* and at concentration of 400mg/ml, the inhibition zone was 18.67 mm **(Figure 2)**. The ethyl acetate fraction

showed inhibition activity only on *S. aureus* and MRSA **(Table 3, Figure 3)**. The highest inhibition zone was 21.0 mm at concentration 400 mg/ml for *S. aureus* while at concentrations 100 mg/ml and 200 mg/ml there is no difference in activity (at both concentrations inhibition zone was 17.67 mm). Methanolic extract and its fractions were not active against *S. aureus* (ATCC 25923) and *S. epidermidis*. Antimicrobial activity increases gradually as dose is increased **(Table 1, 2, 3)**. The commercial antibiotic and antifungal were more effective in their antimicrobial activity.

In previous study antibacterial activity of methanolic extract and its fractions against Gram - negative and Gram - positive bacteria was reported **[17]**. In which, methanolic extract showed activity against *P. aeruginosa, P. mirabilis, K. pneumoniae, E. faecalis* (ATCC 29212) and *K. pneumoniae* (ATCC 700603). Petroleum ether fraction showed activity on *P. aeruginosa, P. mirabilis, K. pneumoniae* and *E. coli* (ATCC25922), while ethyl acetate fraction has activity against *P. aeruginosa* and *K. pneumoniae*.

The minimum inhibitory concentration (MIC) values which ranged from 3.125 to 100 mg/ml, minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) are represented in **Table 4**.

The Lowest MIC was noticed in methanolic extract against *S. aureus* (3.125mg/ml) while for the rest of microorganisms it was 6.25 mg/ml. MBC and MFC were varying from 6.25mg/ml to 12.5mg/ml.

Table 1: Antimicrobial activity of methanol extract

Microorganisms	Methanolic extract (Inhibition zone (mm))				Streptomycin	Fluconazole
	100 mg/ml	200mg/ml	300 mg/ml	400 mg/ml	1mg/ml	1mg/ml
S. aureus	16.67±0.33	17.67±0.33	20.67±0.33	21.67±0.33	28.00±0.58	-
MRSA (ATCC 43300)	14.67±0.33	15.67±0.33	19.67±0.33	21.00 ± 0.58	27.33±0.33	-
***C. Albicans* (ATCC 10231)**	14.67±0.33	15.67±0.33	17.00 ± 0.58	19.33±0.67	-	23.67 ±0.88

All values are expressed as Mean ± SEM (n = 3).

Table 2: Antibacterial activity of petroleum ether fraction

Microorganisms	Petroleum ether fraction (Inhibition zone (mm))				Streptomycin
	100 mg/ml	200mg/ml	300 mg/ml	400 mg/ml	1mg/ml
S. aureus	11.67± 0.33	13.67 ± 0.33	15.33± 0.33	18.67 ± 0.88	29.33± 0.33

All values are expressed as Mean ± SEM (n = 3).

Table 3: Antibacterial activity of ethyl acetate fraction

Microorganisms	Ethyl acetate fraction (Inhibition zone (mm))				Streptomycin
	100 mg/ml	200mg/ml	300 mg/ml	400 mg/ml	1mg/ml
S. aureus	17.67± 0.88	17.67±0.88	19.67± 0.33	21.00 ± 0.58	25.33± 0.33
MRSA (ATCC 43300)	15.00 ± 0.58	16.33±0.33	16.67 ± 0.33	19.00 ± 0.58	24.33± 0.33

All values are expressed as Mean ± SEM (n = 3).

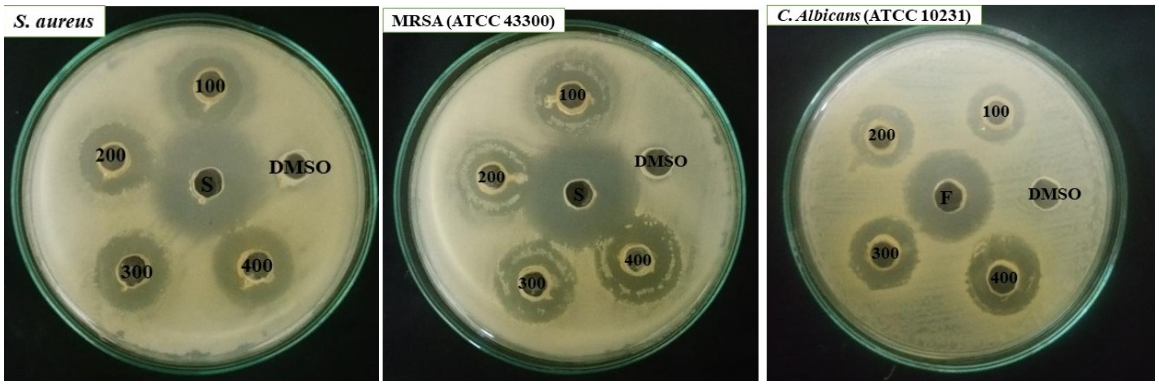

Figure 1: Antimicrobial activity of methanol extract against *S. aureus*, MRSA and *C. albicans*.

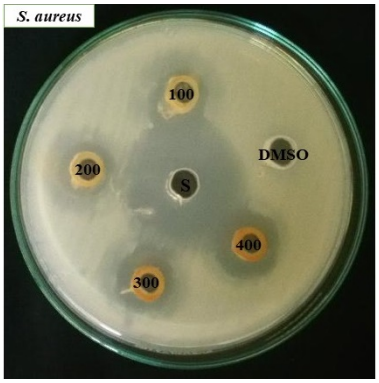

Figure 2: Antibacterial activity of petroleum ether fraction against *S. aureus*

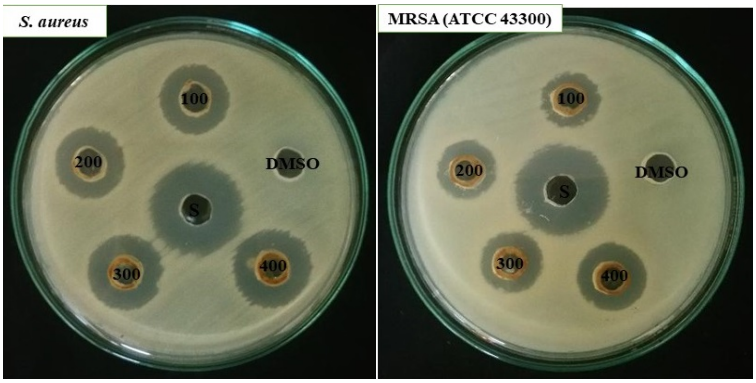

Figure 3: Antibacterial activity of ethyl acetate fraction against *S. aureus* and MRSA

Table 4: MIC, MBC, MFC of methanolic extract and its fractions

Microorganisms	Methanolic extract			Petroleum ether fraction		Ethyl acetate fraction	
	MIC mg/ml	MBC mg/ml	MFC mg/ml	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml
S. aureus	3.125	6.25	-	6.25	12.5	6.25	6.25
MRSA (ATCC 43300)	6.25	12.5	-	-	-	6.25	12.5
C. Albicans (ATCC 10231)	6.25	-	12.5	-	-	-	-

Note: (-) not tested

CONCLUSION

This is the first study that conducted to evaluate antimicrobial activity of *H. ulmarius* fruiting bodies. We found that methanolic extract have a good activity against selected Gram-positive bacteria and *candida albicans* in compare to its fractions. Further studies are required to evaluate the extract activity against different microorganisms.

REFERENCES

[1] Bush K. why it is important to continue antibacterial drug discovery. AMS News. 2004; 70:282-287.

[2] Fallarro A, Hanski L, Vuorela P. How to translate a bioassay into a screening assay for natural products: general considerations and implementation of antimicrobial screens. Planta Med. 2014; 80:1182-1192.

[3] Aletor VA. Compositional Studies on edible tropical specials of mushrooms. Food Chem, 1995; 54: 256 –268

[4] Fasidi IO. Studies on Volvariella esculenta mass singer, Cultivation on agricultural wastes and proximate composition of stored mushrooms. Food Chem, 1996; 55: 161 –163.

[5] Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, Baptista P. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem, 2007; 103: 413–9.

[6] Oyetayo VO. Free radical scavenging and antimicrobial properties of extracts of wild mushrooms. Braz J Microbiol, 2009; 40: 380–6.

[7] Chang ST. World production of cultivated edible and medicinal mushroom in 1997 with emphasis on *Lentinus edodes (Berk)* Sing. in China. Int. J. Med Mushroom, 1999; 1: 291- 300

[8] Lena Ahmed Saleh Al-Faqeeh, Suresh R. Kagne, Mohammad Rafiuddin Naser. Medical Properties of *Hypsizygus ulmarius (Bull.)*. WJPR. 2018; 7 (10): 1077-1085.

[9] Shirmila Jose, Radhamany P M. In Vitro Antioxidant Activities, Total Phenolics and Flavonoid of Wild Edible Mushroom *Macrolepiota mastoidea (FR.)* singer. Int J Pharm Pharm Sci. 2015; 5 (2):161-166.

[10] Lena Ahmed Saleh Al-Faqeeh, Rafiuddin Naser, Kagne SR, Subur W. Khan. TLC and FTIR Analyses of *Hypsizygus ulmarius* (Bull.) Fruiting Bodies. Ijppr. Human,

2019; 17(1): 60-71.

[11] Shivashankar M and Premkumari B. Preliminary Qualitative Phytochemical Screening of Edible Mushroom *Hypsizygus ulmarius*. Sci. Technol. Arts Res. J .2014; 3 (1): 122-126.

[12] Esimore CO, Adikwu MU, Okonta JM. Preliminary antimicrobial screening of the ethanolic extract from the lichen *usnea subfloridans*. J Pherm Res Dev. 1998; 3(2): 99-100.

[13] Toki T, Atsuko O, Naoya N, Makiko S, Masanobu I. Characterization and antifungal activity of Gazyumani (*Ficus microcarpa*) latex chitinase: Both the chitin – binding and antifungal activities of class I chitinase are reinforced with increasing ionic strength. Biosci Biotechnol Biochem. 2005; 69(4): 811-818.

[14] CLSI. Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved stander. 9th ed, clinical and laboratory standers institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.2012.

[15] Arikan S. Current status of antifungal susceptibility testing methods. Med Mycol .2007; 45: 569-587.

[16] Shivashankar M, Premkumari B. Study on invitro free radical scavenging activity of *Hypsizygus ulmarius* mushroom. Jocpr. 2014; 6(6): 501-507.

[17] Lena Ahmed Saleh Al-Faqeeh, Rafiuddin Naser, Kagne SR. *In Vitro* Antibacterial Activity of *Hypsizygus ulmarius* (Bull.) Fruiting Bodies. Ijsrm. Human. 2019; 14 (2): 39-47.