STRUCTURE CHARACTERIZATION AND EVALUATION POTENTIAL OF ANTIMICROBIAL EXTRACTS FROM PHELLINUS LINTEUS AGAINST SKIN INFECTIOUS PATHOGENS, STAPHYLOCOCCUS EPIDERMIDIS ATCC12228 AND PROPIONIBACTERIUM ACNES DMST14916

SURACHAI TECHAOEI*, PATTARANUT EAKWAROPAS, KHEMJIRA JARMKOM, WARACHATE KHOBJAI
Thai Traditional Medicine College, Rajamangala University of Technology Thanyaburi, Pathum Thani 12130 Thailand
Email: surchai_te@rumut.ac.th

ABSTRACT

Objective: The objective of this study was to investigate the antimicrobial activity of Phellinus linteus against skin infectious pathogens, Staphylococcus epidermidis ATCC12228 and Propionibacterium acnes DMST 14916.

Methods: Fungal fruiting bodies were extracted with 95% ethanol and ethyl acetate, and then, vaporized. The antimicrobial activities were determined by the disc diffusion method against Propionibacterium acnes DMST 14916 and Staphylococcus epidermidis ATCC12228 skin infectious pathogens. A minimum inhibitory concentration (MIC) and a minimum bactericidal concentration (MBC) for those crude extracts were determined. Finally, the chemical profile of crude extract was determined by using thin layer chromatography and GC-MS.

Results: The result demonstrated that the ethanolic extraction had more active fractions with an MIC of 0.5 mg/ml against the growth of Propionibacterium acnes DMST 14916 and Staphylococcus epidermidis ATCC12228 and also showed a minimum inhibitory concentration (MBC) at a concentration of 1.0 mg/ml while ethyl acetate-based solvents failed to develop on TLC according to Retention factor (Rf) values of 0.71-0.76. The GC-MS was applied to investigate the chemical profile of crude extract of Phellinus linteus, revealing a component of hexadecanoic acid and 12-octadecadienoic acid.

Conclusion: Phellinus linteus fruiting body extracts have great potential as antimicrobial compounds against Propionibacterium acnes DMST 14916 and Staphylococcus epidermidis ATCC12228. Thus, they can be used in the treatment of infectious diseases caused by bacterial pathogens.

Keywords: Antimicrobial activity, Phellinus linteus, Staphylococcus epidermidis, Propionibacterium acnes, MIC, MBC

INTRODUCTION

In recent years, the increasing antimicrobial resistance called superbugs has driven a critical need to develop a novel antimicrobial agent [1]. The multi-drug resistant strains do not only occur through nosocomial infection, but also in the public condition [2] especially superbugs has driven a critical need to develop a novel antimicrobial agent. In recent years, the increasing antimicrobial resistance called superbugs has driven a critical need to develop a novel antimicrobial agent [1]. The multi-drug resistant strains do not only occur through nosocomial infection, but also in the public condition [2] especially superbugs has driven a critical need to develop a novel antimicrobial agent.

MATERIALS AND METHODS

Preparation of mushroom extract

The fruiting bodies of Phellinus linteus were obtained from a folk medicine company in Thailand. The extraction of the mushrooms was conducted by macerating the mushrooms in 95% ethanol and ethyl acetate at 1:20 (w/v) for 7 d. After the maceration, the extract was filtered through Whatman No. 1, and the filtrate was evaporated using an evaporator. The residue was extracted two times. Then, the extract was combined and evaporated to a constant weight. The crude extract was calculated for the percentage yield and kept at 20 °C for further investigation.

Preparation of inoculums

Both bacterial strains S. epidermidis ATCC12228 and P. acnes DMST 14916 were obtained from the National Institute of Health, Thailand. The tested bacteria were inoculated in nutrient broth and incubated for 16-18 h at 37 °C in aerobic condition for S. epidermidis ATCC12228 and in anaerobic condition for P. acnes DMST 14916. A 0.5 McFarland standard was used to adjust the turbidity corresponding to 10^8 CFU/ml.

Antimicrobial assay

The disc diffusion method was used to determine the antimicrobial activity of the P. linteus extracts by using nutrient-agar (NA). A fresh bacterial suspension with the turbidity corresponding to 10^8 CFU/ml was spread on NA plates with sterile cotton swabs. The disc diffusion assay was completed by adding the tested crude extract which dissolved in dimethyl sulfoxide (DMSO) solution at an initial concentration of 100 mg/ml onto the 6 mm disc. The Petri plates were then incubated at 37 °C for 24 h in an incubator. The experiment was conducted in triplicate and the mean diameter of...
the zone of inhibition was recorded in millimetres (mm). The results were represented by mean±standard deviation [13].

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

To assess the MIC and MBC of mushroom crude extracts, the serial two-fold dilution was carried out with the disc diffusion assay. The MIC represented the lowest concentration showing an inhibition zone; the MBC was determined by subculturing which showed no bacterial growth on the agar plates after incubated at 37 °C for 24 h in an incubator. The lowest concentration that did not show bacterial growth was defined as an MBC value. The experiment was conducted in triplicate, and the mean diameter of the zone of inhibition was recorded in millimetres (mm). Erythromycin was used as a positive control, and dimethyl sulfoxide (DMSO) solution was used as a negative one. All experiments were performed in triplicate. The results were represented by mean±standard deviation [14].

Thin layer chromatography analysis

TLC was carried out using aluminium silica gel 60GF254 of a thickness of 0.2 mm, (Germany). Standard chromatograms of Phellinus linteus extract were prepared by applying a 20 µl extract solution to a silica gel TLC plate and developed with chloroform/methanol (1:1; v/v) under saturated conditions. The chromatograms were detected by UV-light (254 nm and 365 nm) and the colour reaction with a 5% sulfuric acid-ethanol spraying solution after heating at 100 °C.

Gas chromatography-mass spectrometry analysis

GC-MS analysis tests for the fruiting body extract composition analysis were performed on an Agilent 789 GC system instrument equipped with HP-5MS (5% diphenyl 95% dimethylpolysiloxane) column (30 m x 0.25 mm, 0.25 µm) and interfaced to a 5975C inert XL MSD with Triple-Axis Detector. A volume injection of 2 µl was employed (a split ratio of 10:1) at an injector temperature of 250 °C. The column temperature was increased from 60 °C to 250 °C at a rate of 5 °C/min. The outlet temperature was 280 °C. Mass spectra were taken at 70 eV; and MS transfer line temperature of 250 °C. The components of the extract were identified by comparison of fragmentation patterns in mass spectra with those stored on the spectrometer database and reported in the literature. The relative percentage of the individual components was calculated from the GC peak areas [15].

Identification of bioactive constituents

Interpretation on Mass spectrum GC-MS was carried out by using the database of National Institute Standard and Technology (NIST) containing more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of the known compounds stored in the NIST library. The name, molecular formula, weight and chemical structure of the components of the test materials were ascertained [16].

Statistical analysis

The results of the data experiments were expressed in mean±SEM (Standard Error of Mean) for groups (n=3).

RESULTS AND DISCUSSION

Antibacterial activities

The antimicrobial activity results of Phellinus linteus fruiting body extracts are summarized by the paper-disc diffusion method as shown in table 1. The results of the disc diffusion method in terms of the size of inhibition zone (mm) for the extracts were compared against microorganisms studied. The highest inhibitory activity was determined against S. epidermidis ATCC12228 and P. acnes DMST14916 in a clearing zone of 15.67±1.15 and 12.67±1.53 mm, whereas ethyl acetate extract showed no inhibition zone.

In a previous study, numerous Phellinus mushroom extract was reported as having an antimicrobial activity against Gram-positive bacteria, B. cereus, B. subtilis and S. aureus [17]. The mushroom in the fraction of methanol extract demonstrated a good activity against MRSA [18, 19]. In addition, the sesquiterpenoid, a bioactive compound from Phellinus species such as P. fastuosus, P. merrillii, P. aureobruneus, P. crocatus, P. lloydii, and P. sublinteus had an effective antibacterial activity against different microorganisms such as Acinetobacter calcoaceticus NCIB2886, B. subtilis NCIM2010, Candida albicans MTCC 1637, C. albicans MTCC 3017, C. albicans ATCC 2991, Escherichia coli MTCC 724, E. coli MTCC 739, E. coli ATCC 2046, Klebsiella pneumonia MTCC 432, Proteus mirabilis MTCC1429, Pseudomonas aeruginosa ATCC2036, and S. aureus HAL 2079, respectively [20].

Table 1: Bacterial activities of the crude extract of Phellinus linteus

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Inhibition zone (mm)</th>
<th>EtOH</th>
<th>EtOAC</th>
<th>+ve</th>
<th>-ne</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis ATCC12228</td>
<td>15.33±0.58</td>
<td>-</td>
<td>22.67±0.58</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. acnes DMST14916</td>
<td>12.67±1.53</td>
<td>-</td>
<td>24.00±0.00</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ve: positive control (10.0 mg/ml erythromycin); -ne: negative control (10% DMSO); statistical analysis as mean±sd.

Bacteriostatic and bactericidal effect

In terms of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against S. epidermidis ATCC12228 and P. acnes DMST14916, a lower MIC was observed in Table 2. The MIC test indicated that the extract of the P. linteus fruiting body exhibited a minimal value of MIC (0.5 mg/ml) against S. epidermidis. MBC was found to have a concentration of 1.0 mg/ml with all strains.

According to a previous study, P. gilvus aqueous extract showed MIC against L. plantarum ACC14917, P. pneumonia ATCC 10031 and E. coli ATCC25922 at concentrations of 45.0, 90.0 and 360.0 mg/L, respectively, while those of MBC against them were 90, 180, and 720 mg/L, respectively [21].

Chemical characterization of Phellinus linteus

Thin layer chromatography analysis

Developed TLC plates with visualized spots were observed. There were dark blue spots of extracts under 365 nm and 254 nm UV light (Rf = 0.71-0.76). After colorized by 5% sulfuric acid-ethanol solution, polar spots appeared in the same UV light positions.

Gas chromatography-mass spectrometry analysis

The active principles with their retention time (RT), molecular formula, molecular weight (MW), concentration (Peak area %) and the chemical structure were analyzed.

Table 3 shows the components present in the unfractonated ethanolic extract as identified by GC-MS. Two components comprising about 98.50% of the total ethanolic extract were identified with hexadecanoic acid, ethyl ester (C16H33O2, 74.9%) forming the major constituent and 9,12-octadecadienoic acid, ethyl ester (C20H32O2, 23.6%), respectively.

In fig. 1, the peak at a retention time of 23.576 min has a mass spectrum data consistent with hexadecanoic acid, ethyl ester. The mass spectrum exhibited a parent ion at m/z 284 consistent with the molecular formula C16H33O2. In addition, in fig. 2, the peak at a retention time of 30.438 min has a mass spectrum data consistent with 9,12-octadecadienoic acid, ethyl ester. The mass spectrum exhibited a parent ion at m/z 308 consistent with the molecular formula C20H32O2.
Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Phellinus linteus to inhibit 100% of the bacterial growth

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Concentration (mg/ml)</th>
<th>MIC</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis ATCC12228</td>
<td>0.5±0.00</td>
<td>1.0±0.00</td>
<td></td>
</tr>
<tr>
<td>P. acnes DMST14916</td>
<td>0.5±0.00</td>
<td>1.0±0.00</td>
<td></td>
</tr>
</tbody>
</table>

MIC: the minimum inhibitory concentration; MBC: the minimum bactericidal concentration (mean±SD)

Table 3: Characterized chemical constituents of P. linteus extracts

<table>
<thead>
<tr>
<th>Compound</th>
<th>RT</th>
<th>PT</th>
<th>MW</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexadecanoic acid, Ethyl ester</td>
<td>23.57</td>
<td>74.90</td>
<td>284.27</td>
<td>C_{8}H_{36}O_{2}</td>
</tr>
<tr>
<td>9,12-Octadecadienoic acid, Ethyl ester</td>
<td>30.483</td>
<td>23.60</td>
<td>308.27</td>
<td>C_{20}H_{36}O_{2}</td>
</tr>
</tbody>
</table>

RT: retention time (min); PT: percentage (%); MW: molecular weight; MF: molecular formula

Previously, many species of Phellinus were reported as producing bioactive compounds like polysaccharides [22], flavones, triterpenes, aromatic acid, and amino acids [14]. These results were similar to those reported by Chinese scientists discovering active compounds from Phellinus species including palmitoleic acid, linoleic acid, oleic acid, hexadecanoic acid, and stearic acid [23].

CONCLUSION

According to the study, the results revealed that the ethanolic extract of Phellinus linteus fruiting body possessed measurable antimicrobial activities against both S. epidermidis ATCC12228 and P. acnes DMST14916. The results also showed MIC and MBC at concentrations of 0.5 mg/ml and 1.0 mg/ml, respectively. However, in order to use these mushrooms properly in medicine, more detailed studies need to be conducted.

ACKNOWLEDGEMENT

We would like to thank the Rajamangala University of Technology Thanyaburi for its financial support and Thai Traditional Medicine College, RMUTT for all facilities used in the conduct of this study.

CONFLICT OF INTERESTS

Declared none

REFERENCES


