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Antimicrobial activity of Phoma sp. URM 7221: An endophyte from Schinus terebinthifolius Raddi (Anacardiaceae)

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The discovery of new metabolites potentially bioactive against pathogenic microorganisms, mainly multidrug resistant, has aroused interest in endophytic fungi. The plant-associated microorganisms have been an important source for development of new compounds of biotechnological interest. This study aimed to investigate the antibacterial capacity of the endophytic fungus, Phoma sp. URM 7221 isolated from the medicinal plant Schinus terebinthifolius against human-pathogenic bacteria. An endophyte was isolated from S. terebinthifolius leaves. Phoma herbarum URM7221 was characterized morphologically and on the basis of ITS rDNA sequence. Primary antimicrobial activity was evaluated using the agar diffusion method and fermentation in liquid medium. Six different solvents were used to extract the active metabolites from fungal biomass and metabolic liquid. An antimicrobial activity test from the extract was carried out using a disk diffusion method with the endophytic extract containing the best antibacterial activity. Two tests were performed: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Partially purified secondary metabolite extracts were analysed by thin layer chromatography (TLC). Liquid metabolically bioactive compounds extracted with petroleum ether revealed a MIC of 25 and 500 μg.mL⁻¹ against S. aureus and MRSA, respectively. Ether and methanol extracts were assessed by chemical analyses and contained phenolic compounds, triterpenes, steroids, reducing sugars, mono- and sesquiterpenes. The thin layer chromatography assay showed the activity of different antimicrobial compounds produced by Phoma sp. URM7221. This endophyte (URM7221) could be efficiently used for production of bioactive metabolites against pathogenic microorganisms, with significant biotechnological potential.

Key words: Bioactive compounds, endophytic fungi, pathogenic bacteria, multidrug-resistance, antibacterial agents.
INTRODUCTION

The overuse of antibiotics in medical practice has contributed to the increase in antibiotic-resistant microorganisms (Paes et al., 2014). Infections caused by Gram-positive bacteria with antibiotic resistance are one of the main reasons for the high mortality and morbidity rates reported for patients, resulting in costly treatment (Woodford and Livermore, 2009). Staphylococcus aureus is one of the most common pathogen in a diverse number of infectious diseases, including simple skin infections and invasive diseases that lead to bacteremia and sepsis (Kim et al., 2012; Rocha et al., 2015). This bacterium requires attention due to emergence of resistant strains to the primary antibiotics used in their treatment, such as methicillin. Methicillin-resistant S. aureus (MRSA) is a major cause of hospital morbidity and mortality (Siqueira et al., 2011). Similar to S. aureus, the genus Enterococcus includes some of the most important opportunistic pathogens. Their variable genome has influence on their adaptation to different environments (Arias and Murray, 2012).

The race to reduce the impact of infections caused by multidrug resistant bacteria, has reinforced the need to discover new antimicrobial substances. Studies have researched new compounds in plants and microorganisms that live inside vegetal tissues, such as endophytes. Endophytes are microorganisms that live in association with plant tissue (Kusari et al., 2012) and many species are known to produce bioactive secondary metabolites with medicinal potential (Kusari et al., 2012). This suggests alternative paths to discovery of new bioactive substances, leading to development of new drugs derived from compounds produced by endophytes species (Kumar et al., 2011).

Several studies have demonstrated that endophytic fungi can produce numerous compounds with biological activities of interest, such as antitumor (Jin-long et al., 2011; Chandra, 2012), antimicrobial (Siqueira et al., 2011; Tayung et al., 2012; Bagchi and Banerjee, 2013; Pinheiro et al., 2013; Orlandelli et al., 2015), enzymes (Chandra, 2012), plant growth hormones (Hwang et al., 2011), leishmanicidal (Santiago et al., 2012), cytotoxic compounds (Li et al., 2011) and compounds with industrial and pharmaceutical potential (Meng et al., 2011; Wang and Dai, 2011), reinforcing the broad biotechnological potential of these microorganisms.

Schinus terebinthifolius Raddi belongs to Anacardiaceae family and is largely found in the coastal region of Brazil (Carvalho et al., 2013). This plant has used in traditional medicine as antipyretic, analgesic and in the treatment of urogenital diseases (Carvalho et al., 2013). Several studies have shown that S. terebinthifolius produces pharmacologically important substances, such as anticancer (Bendaoud et al., 2010; Matsuo et al., 2011), antimicrobial (Silva et al., 2010; Pereira et al., 2011), antioxidant (Bendaoud et al., 2010), healing purposes (Estevão et al., 2013) and antiproliferative (Queires et al., 2013).

Considering the impact on health human and the necessity of more studies verifying the biotechnological properties of endophytic fungi, the present study aims to 1) evaluate the antibacterial potential of endophytic fungi against Gram-positive pathogenic bacteria and 2) identify the class of bioactive metabolites produced by Phoma sp. (URM 7221) by thin layer chromatography (TLC).

MATERIALS AND METHODS

Endophytic fungi: Isolation and identification

S. terebinthifolius Raddi leaves were collected from different plants on the campus of Federal University of Pernambuco, Recife city, Pernambuco state, Brazil (08°03’07”S 34°56’59”O). For endophytic isolation, healthy leaves were selected as described by McInroy et al. (1995). The leaves were cut into 180 fragments of approximately 1 cm² and were cultured for up to 20 days at 30°C on Sabouraud agar and potato dextrose agar (PDA) media containing chloramphenicol (100 mg/L).

For morphological identification of endophytic fungi, the fungi were cultured on Synthetic Nutrient-poor Agar (SNA), malt extract agar (MEA) and potato dextrose agar (PDA) media for 10 days in the dark at 28°C and for 10 days under continuous UVA light. After this period of cultivation macro and microscopic characteristics of colonies were observed according to Boerema et al. (2004). For molecular analysis, 7-day old culture on PDA was used for DNA extraction with an UltraClean TM Microbial Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer’s instructions.

Polymerase chain reaction for the part of ITS region (first and second internal transcribed spacer regions and intervening 5.8S nrDNA) amplification was performed with 50 µL volume, using Taq® DNA polymerase 1X buffer, 1.5 mM MgCl₂, 0.4 µM ITS1 and ITS4 primers (White et al., 1990), 0.2 mM dNTPs, 0.2 Taq® DNA polymerase and 25ng DNA. Amplification was done in a thermal cycler with the following conditions: 5 min at 95°C (1 cycle), 30 s at 95°C (30 cycles), 1 min at 62°C (annealing), 2 min at 72°C (extension) and 5 min at 72°C (final extension). Amplification products were separated by electrophoresis on a 1% agarose gel, colored with GelRed® and visualized with UV light using molecular weight marker 1 kb plus (Fermentas®). PureLink PCR Purification Kit (Invitrogen®) was used according to manufacturer’s recommendation. Amplification products were sequenced and electropherograms were edited by Staden Package software. The consensus sequence was blasted against sequences on GenBank.

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using BLASTn. Sequences with the highest similarity percentage (99-100%) in comparison with the studied sequence were analysed.

**Antimicrobial activity: Solid medium**

Forty endophytic fungi isolated with distinct morphological characteristics were selected for antimicrobial activity. Four microorganisms were tested using an agar block test as previously described by Ichikawa et al. (1971). The four bacteria species used for the test were: S. aureus Rosenbach (UPPDEA02), MRSA (UFPEDA663), Enterococcus faecalis (Andrewes & Horder) Schleifer & Klipper-Bätz (UFPEDA138), and Bacillus subtilis (Ehrenberg) Cohn (UFPEDA86). The inhibition zones obtained were compared with control information from the table of the Clinical and Laboratory Standards Institute (CLSI, 2013).

**Fermentation in liquid medium**

The endophytic fungus (URM 7221) that demonstrated the best antimicrobial activity using solid medium was selected to test in liquid medium for extraction of active metabolites. After growth, fragments of 10 mm diameter of the endophytes were transferred to Erlenmeyer flasks (250 mL) with 50 mL of the liquid medium MPE (Hamada et al., 1974), Caspeck and M1 (20 g/L glucose, 200 g/L peptone) incubated for 2 days, at 30°C at 120 rpm. After this period, 10 mL of each pre-inoculum was transferred to flasks (500 mL) containing 90 mL of the same liquid medium and incubated in the same conditions. Every 24 h (during 120 h), 1 mL of fermentation broth was removed from the flask and centrifuged at 10,000 rpm for 3 min. From the supernatant fluid, 50 μL was used to perform the disk diffusion antimicrobial test (Kirby et al., 1966).

**Endophytic extracts**

After fermentation of endophytic fungus (URM 7221) for 48 h in MPE medium, the fungal biomass was separated from the metabolic liquid using Whatman filter paper 4 and centrifuged at 10,000 rpm for 3 min. Ethanol, methanol and acetone (1:10 g/mL) at pH 2.0, 7.0 and 9.0, respectively, were used to extract the active metabolites from the biomass. To extract the active substances present in the metabolic liquid petroleum ether, ethyl acetate and chloroform (2:1) were used. The pH of the metabolic liquid was adjusted to 2.0, 7.0 and 9.0, respectively. After 1 h of shaking, the samples were centrifuged at 10,000 rpm for 3 min and the solvent containing the extract was evaporated using a rotary evaporator at 55°C (Lyra et al., 1964).

**Antimicrobial activity: Endophytic extract**

The antimicrobial activity test of the endophytic extract was performed using a disk diffusion method, with sterile paper discs impregnated with 50 μL of each extract. The pH of the endophytic extract was adjusted to 7.0, to avoid interference. The diameter of the inhibition zones around the discs was measured and was assessed using the same microorganisms tests as described in “Antimicrobial activity: solid medium” section. Chloramphenicol 1 mg/mL was used as a positive control, methanol was applied as solvent control, and the depleted liquid and water were used as negatives controls (Lyra et al., 1964).

**Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)**

Two tests: MIC and MBC were used to assay the endophytic extract with the best antibacterial activity. A broth microdilution assay using 96-well microplate, performed according to the Clinical and Laboratory Standards Institute (CLSI, 2013) was used. All determinations were executed in triplicate.

**Plant tissue extraction**

For extraction of compounds with no antimicrobial activity, leaves of S. terebenthifolius (1 g) were immersed in 10 mL of hexane for two hours. The leaves were then immersed in 10 mL of ethyl acetate for two hours to extract bioactive compounds. Solvent was evaporated using a rotary evaporator at 55°C (Ceruks et al., 2007).

**Secondary metabolic analyses**

For partial purification of secondary metabolites, 10 μL aliquots of biomass extract (methanol) and metabolic fluid extract (ether) of Phoma sp. (URM 7221) were analysed by thin layer chromatography (TLC) on silica gel F254 (Merck®). Several mobile phases, such as ethyl acetate : acetic acid : formic acid : water (100:11:11:26, v/v), toluene : ethyl acetate (80:20; 97:3, v/v), acetone : n-butanol : phosphate buffer pH 5.0 (5:4:1, v/v ), and specific visualization reagents were used (Robertson et al., 1956; Metz, 1961; Sharma and Darwra, 1991; Wagner and Bladt, 1996; Harborne, 1998). The chromatograms were run in saturated chambers.

The bioactive extracts obtained from Phoma sp. (URM 7221) were compared with compounds present in crude ethyl acetate extract of leaves by TLC using an agar overlay method (Rodrigues et al., 2009). Ethyl acetate: methanol (9:1) solution was used as mobile phase. An aqueous solution of 2,3,5-triphenyltetrazolium chloride (20 mg/mL) (Rodrigues et al., 2009) was used for visualization. Biological activity was determined by the formation of white and well defined inhibition zones against a red-purple background. Retention factor (Rf) values were measured and compared (Homans and Fuchs, 1970). All tests were performed in triplicate.

**RESULTS**

**Endophyte isolation, identification and antimicrobial activity**

Using culture media for isolation of endophytes, 220 endophytic fungi were isolated from leaves of S. terebenthifolius. Among these, 137 endophytes (64.62%) were obtained using Sabouraud agar medium and 75 (35.37%) using PDA medium.

The endophyte that showed the best antibacterial activity was identified. Morphological analysis was performed after 20 days culture on SNA, MEA and PDA media. The fungus grew on three different media with similar macroscopic characteristics (velvety-powdered texture, colony color ranging between light brown and dark pink, reverse light brown to reddish brown). Pycnidia
Fermentation and endophytic extracts antimicrobial activity

Fermentation tests revealed that MPE medium was the most efficient and only Czapeck medium did not stimulate the production of active metabolites against the four bacteria tested. *E. faecalis* (UFPEDA138) was resistant to all endophytic extracts tested. The most efficient extract (using MPE medium) gave the best production of the bioactive principle and the largest inhibition zones against *S. aureus* (31 ± 0.47 mm after 48 h), MRSA (26.4 ± 0.81 mm, 48 h) and *B. subtilis* (25 ± 0.94 mm, 72 h). These results indicate that the production of bioactive metabolites with antimicrobial activity changes during incubation time (Table 2).

Antibacterial activity of biomass and metabolic liquid extracts obtained from *Phoma* sp. (URM 7221) and from leaves of *S. terebinthifolius* was evaluated (Table 3). The endophyte was subjected to liquid fermentation in MPE medium (pH 7.0) at 48 h. Petroleum ether (pH 7.0) was the most efficient solvent because it extracted bioactive metabolites active against *S. aureus* and MRSA, with inhibition zones reaching up to 23 mm. Ethyl acetate was able to partially extract bioactive compounds against the test microorganisms with inhibition zones between 10-18.6 mm. MIC and MBC values of petroleum ether extract were, respectively, 125 and 250 mg L⁻¹ against *S. aureus*, and 500 and 1000 mg L⁻¹ against MRSA.

Ethanol and methanol were used to extract active metabolites from fungal biomass and were effective against *S. aureus* and MRSA. Methanol (pH 2.0 and 7.0) showed inhibition zones ranging from 12 to 15 mm, with MIC and MBC of 250 and 500 mg L⁻¹, for *S. aureus* and 1000 mg L⁻¹ and >1000 mg L⁻¹ against MRSA. Acetone extracts were only active against *S. aureus*.

### Table 1. Antibacterial activity of endophytic fungi isolated from leaves of *S. terebinthifolius* against human pathogenic bacteria.

<table>
<thead>
<tr>
<th>Test microorganism</th>
<th>F-10</th>
<th>F-61 (URM 7221)</th>
<th>F-100</th>
<th>F-105</th>
<th>F-169</th>
<th>F-178</th>
<th>F-188</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em> UFPEDA86</td>
<td>20 ± 0.0</td>
<td>35 ± 0.0</td>
<td>16.4 ± 0.94</td>
<td>16 ± 0.8</td>
<td>-</td>
<td>-</td>
<td>&gt;10 ± 0.0</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> UFPEDA138</td>
<td>17 ± 0.0</td>
<td>23.7 ± 0.47</td>
<td>16.7 ± 0.47</td>
<td>&gt;10 ± 0.0</td>
<td>-</td>
<td>&gt;10 ± 0.0</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> UFPEDA02</td>
<td>-</td>
<td>35 ± 0.0</td>
<td>-</td>
<td>&gt;10 ± 0.0</td>
<td>19 ± 0.0</td>
<td>&gt;10 ± 0.0</td>
<td>&gt;10 ± 0.0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (MRSA) UFPEDA663</td>
<td>16 ± 0.86</td>
<td>22.4 ± 0.47</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&gt;10 ± 0.0</td>
</tr>
</tbody>
</table>

F: Endophytic isolates; (MRSA): Methicillin-resistant *Staphylococcus aureus* resistant; (-): no activity.

### Table 2. Susceptibility profile of test microorganisms to endophyte *Phoma* sp. (URM 7221) grown in MPE or M1 liquid fermentation.

<table>
<thead>
<tr>
<th>Test Microorganisms</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
<th>120 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MPE</td>
<td>M1</td>
<td>MPE</td>
<td>M1</td>
<td>MPE</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>14 ± 0.0</td>
<td>-</td>
<td>16.3 ± 0.94</td>
<td>17 ± 0.81</td>
<td>25 ± 0.94</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>15 ± 0.0</td>
<td>-</td>
<td>31.3 ± 0.47</td>
<td>21 ± 0.47</td>
<td>29.3 ± 0.47</td>
</tr>
<tr>
<td><em>S. aureus</em> MRSA</td>
<td>15 ± 0.0</td>
<td>-</td>
<td>26.4 ± 0.81</td>
<td>-</td>
<td>15 ± 0.81</td>
</tr>
</tbody>
</table>

- No activity.

(91-111 × 58-203 μm) and conidia (4-5.5 × 2-2.5 μm) were formed only on SNA medium. Using ITS rDNA sequence to a megablast search of the NCBI GenBank nucleotide database, our sequence (KP966098) has high identity (98 to 100%) to sequences deposited as *Phoma* sp. and *P. herbarum* (HQ630963, Shrestha et al., 2011; KJ188712, Luo et al., 2014), among others. The morphological characters described above match the *P. herbarum* description by Boerema (2004). However, because the new publications (Chen et al., 2015) using multigene phylogenetic analyses on the taxonomy of this genus, the authors decided to identify the isolate only as *Phoma* sp. (URM 7221).

Forty endophytes were tested in the primary assay, and only seven (17.5%) expressed antimicrobial activity. The endophyte identified as *Phoma* sp. (URM 7221) showed the greatest activity against *B. subtilis* (inhibition zone of 35 mm), *E. faecalis* (23.7 mm), *S. aureus* (35 mm) and MRSA (22.4 mm) (Table 1).
Table 3. Antimicrobial activity of crude extracts of _Phoma_ sp. (URM 7221) obtained from liquid fermentation. MLE: Metabolic liquid exhausted; EXT: extract.

<table>
<thead>
<tr>
<th>Solvents</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus (UFPEDA 02)</td>
<td>MRSA (UFPEDA 663)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH2</td>
<td>pH7</td>
<td>pH9</td>
<td>pH2</td>
<td>pH7</td>
</tr>
<tr>
<td><strong>Biomass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>EXT</td>
<td>21.6 ± 0.47</td>
<td>25 ± 0.0</td>
<td>22 ± 0.81</td>
<td>15 ± 0.0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>EXT</td>
<td>23 ± 0.0</td>
<td>24 ± 0.0</td>
<td>12 ± 0.0</td>
<td>13.6 ± 0.47</td>
</tr>
<tr>
<td>Methanol</td>
<td>EXT</td>
<td>21.3 ± 0.94</td>
<td>21.6 ± 1.2</td>
<td>21 ± 0.0</td>
<td>17 ± 0.0</td>
</tr>
<tr>
<td><strong>Metabolic liquid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>EXT</td>
<td>27.6 ± 0.94</td>
<td>28 ± 0.81</td>
<td>27 ± 0.81</td>
<td>21.6 ± 0.47</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>EXT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MLE</td>
<td>11.6 ± 0.47</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MLE</td>
<td>24 ± 0.0</td>
<td>24 ± 0.0</td>
<td>23.6 ± 0.47</td>
<td>19 ± 0.0</td>
</tr>
</tbody>
</table>

**Chemical comparison of active compounds**

Analysis of metabolic liquid by TLC suggested the presence of phenolic compounds, triterpenes, steroids, mono and sesquiterpenes. Analysis of fungal biomass showed reducing sugars and phenolic compounds. Using bioautographic assay, both extracts showed antimicrobial activity against _S. aureus_ and MRSA. Petroleum ether extract showed Rf values of 0.65 and 0.6 for _S. aureus_ and MRSA, respectively. Similar Rf values were observed for methanol extracts (0.125 and 0.15).

**DISCUSSION**

Studies of medicinal plants have received wide attention in recent years due to the large diversity of endophytic microorganisms that inhabit plant tissues (Siqueira et al., 2011). New species, useful for biotechnology applications, have been reported to produce extracellular active metabolites with pharmacological interest (Bagchi and Banerjee, 2013; Pinheiro et al., 2013). These microorganisms can also protect plants against phytopathogens, temperature, drought and world weather changes (Gundel et al., 2010). This research demonstrates the diversity of endophytic fungi living in leaves of _S. terebinthifolius_ and the capacity of these endophytes to produce molecules with biotechnological importance. In this study, a unique endophytic species identified as _Phoma_ sp. (URM 7221) produced more efficiently bioactive compounds against four Gram-negative bacteria species.

The genus _Phoma_ comprises several species and varieties that are recognized as producers of antimicrobial compounds (Bezerra et al., 2015). Researchers have reported that _Phoma_ species can be isolated from many plants, such as _Laguncularia racemosa_ (Costa et al., 2012), _Amaranthus cruentus_ (Pusz et al., 2015), _Taraxacum mongolicum_ (Zhang et al., 2013a) and _Mitrajyna javanica_ (Pharamat et al., 2013). An important taxonomic paper from CBS-KNAW (The Netherlands) highlights the importance of a polyphasic approach to characterise _Phoma_ species and similar genera, and it presented an overview of the phytopathological importance of this genus (Aveskamp, 2010). This antimicrobial potential can be associated with production of phenolic secondary metabolites and steroids (Hwang et al., 2011). Similar to our results, these compounds have been identified by chemical prospecting, which might be related to antimicrobial activity presented by _Phoma_ sp. (URM 7221).

According to Strobel et al. (2011), species of the genus _Phoma_ can produce organic volatile compounds, of which sesquiterpenes are the most prominent. Hamayun et al. (2009) described that _P. herbarum_ produced several metabolic compounds, because it is adaptable to many environmental conditions. These compounds can play a role in immunomodulation (Zhang et al., 2013b; Shen et al., 2014) and antitumor activity (Fang et al., 2011; Pharamat et al., 2013), which reinforces the importance of new surveys involving this fungal genus.

Studies in different countries have tested _Phoma_ spp. antimicrobial activity against Gram-positive bacteria. Some authors studying this genus reported similar results. For example, Kumar et al. (2010) tested culture filtrate with the capacity to inhibit _S. aureus_ (11 to 19 mm). In this study, primary assays proved antimicrobial activity of _Phoma_ sp. (URM 7221), efficiently inhibiting _S. aureus_ (11 to 35 mm) and MRSA (15 to 26.4 mm). In addition to the initial tests that showed activity against _B._
subtilis (12 - 35 mm) and E. faecalis (23.7 ± 0.47 mm), other experiments demonstrated that Phoma sp. (URM 7221) extracts were not able to suppress the growth of these bacteria. Shukla et al. (2014) also demonstrated antimicrobial potential of this endophyte against S. aureus (18.3 ± 0.10 mm). Similar results were obtained by Zhang et al. (2013a) in a study of fungi associated with Taraxacum mongolicum, and the authors also found positive results against S. aureus (40 mm). Shen et al. (2012) has isolated endophytes from branches of Phyllostachys edulis and tested P. herbarum extracts against bacteria; it was not able to inhibit S. aureus and B. subtilis. The same fact occurred according to Pharamat et al. (2013) when evaluating fungal endophytes species obtained from Mitrajyna javanica.

**Conclusions**

Study of endophytic fungi from leaves of S. terebinthifolius revealed that different plants are capable of harboring endophytes, such as Phoma sp. (URM 7221) that demonstrated the most efficient antibacterial activity among other endophytes.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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