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Antibacterial activity of Ethyl Acetate Extract of Agaricomycetes collected in Northeast Brazil

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Abstract

Agaricomycetes produce bioactive substances with antibiotic, antiallergic, anti–inflammatory, antioxidant, cytotoxic, antiatherogenic, hepatoprotective, hypoglycemic anti–immunoprotective properties. However, there have been few studies on material collected in Brazil. Thus, the current study aimed to improve the knowledge concerning the antibacterial potential of Agaricomycetes in the country by researching material collected in Northeast Brazil. Twenty one cultures deposited in Micoteca URM of UFPE and nine extracts from fresh basidiomata were tested. Extracts of the basidiomata and the cultures were obtained with ethyl acetate, which were solubilized at a concentration (1:10 v/v). The preliminary tests with the 30 samples (21 cultures and nine extracts of fresh basidiomata) were performed against eleven strains of *Staphylococcus aureus*, seven of which were resistant to oxacillin (ORSA), using block agar. Thirteen samples showed antibacterial activity, with inhibition between 9–24 mm and minimum inhibitory concentration (MIC) from 0.18–147 µg/mL, *Fomitopsis cupreorosea* (URM 6830), *Ganoderma multiplicatum* (URM 6975), *G. parvulum* (URM 2948), *G. orbiforme* (URM87741), *Grammothele lineata* (URM6827), *Rigidoporus lineatus* (URM 6828), *R. microporus* (URM 6878), *Stereum ostrea* (URM 6973, URM 87848) being the most significant.

Key words – antimicrobian – Fomitopsis – Ganoderma – Grammothele – Rigidoporus – Stereum

Introduction

Agaricomycetes is class of Basidiomycota that usually produces fruiting bodies (basidiomata) known as mushrooms, earth stars, bracket fungi, puffballs, clavarioid fungi, coralloid fungi, boletes, among others (Alexopoulos et al. 1996, Kirk et al. 2008).They usually produce enzymes that decompose plant organic material (mostly lignin and cellulose), which significantly contributes to carbon cycles in nature (Alexopoulos et al. 1996, Kendrick 2000, Deacon 2006). The use of species of Agaricomycetes as medicine has been reported for centuries and is considered an alternative in the search for new bioactive compounds, several of them of medicinal or pharmacological interest (De Silva et al. 2012a, 2012b, 2013, Wasser 2013, Giavasis 2014, De Mattos–Shipley et al. 2016). However, there is little information about substances extracted from neotropical specimens.

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Among the activities of medicinal and/or pharmacological interest, the antibacterial is one of the most searched. Infections caused by multi-resistant bacteria are more frequent in hospitals and are increasing in several countries, and the current antibiotics are becoming less effective (Cornejo–Juárez et al. 2015, Nowacka et al. 2015).

Thus, the aim of this work was to increase the knowledge about the antibacterial potential of neotropical fungi by testing the Agaricomycetes collected in Northeast Brazil against strains of *Staphylococcus aureus*, some of them resistant to oxacillin.

Materials & Methods

Microorganisms

Fungal cultures and fresh basidiomata

Thirty–one cultures of Agaricomycetes collected in Northeast Brazil, kept in tubes with Sabouraud agar, were deposited in URM Culture Collection (Universidade Federal de Pernambuco) in lyophilized oil (Table 1). Ten cultures could not be reactivated and were not used in antimicrobial activity tests (Table 1). The remnant cultures were sub–cultured in Petri dishes containing 10 ml of Sabouraud–agar medium (10 g/L peptone, 40 g/L glucose, 15 g/L agar). After growth, the cultures were transferred to Petri dishes with 10 ml of malt extract medium (MEA) (20 g/L malt extract, 20 g/L glucose, 1 g/L peptone, 20 g/L agar). The procedure was performed in triplicate. After inoculation, plates were incubated at 25 °C for about 14 to 30 days according to species. The culture fluid was separated from pellets by filtration.

In addition to the cultures, extracts of nine fresh basidiomata were used in the studies of antibacterial activity (Table 2).

Ethyl acetate extract

Pellets from the cultures and slices of basidiomata transferred into Erlenmeyer flasks (500 ml capacity) containing 100 ml of ethyl acetate for 24 hours (Table 2). After that, the liquid was separated from the fragments by filtration and the solvent evaporated in a rota evaporator at 45°C and 50 rpm. The residues were solubilized in dimethilsulfoxide (DMSO) (1:10).

Bacteria

Eleven strains of *Staphylococcus aureus* deposited in the Culture Collection of the Departamento of Antibiotico of Universidade Federal of Pernambuco and maintained in Mueller–Hinton (MH) broth (ATCC 01, 707 UFPEDA, UFPEDA 677* 676* UFPEDA, UFPEDA 691* UFPEDA 726, 728 UFPEDA* UFPEDA 729* 730* UFPEDA, UFPEDA 731, 733 UFPEDA*) were used, seven (*) of which resistant to oxacillin (ORSA). The bacteria were sub-cultured in Petri dishes containing 10 ml of MH broth and grown overnight 37 °C. Suspensions of *S. aureus* were prepared with sterile distilled water and standardized according to turbidity equivalent to 0.5 of the MacFarland scale.

Determination of antimicrobial activity

Fungal cultures

The evaluation of inhibitory activity of 21 cultures of Agaricomycetes was performed according to the modified method of Ichikawa et al. (1971).

The mycelial disks (5 mm diameter) of fungal strains were transferred into malt extract agar. After 10 days of incubation at 25 ºC, blocks of the 5 mm of fungal cultures were inoculated in Petri dishes with *S. aureus* in MH broth. The Petri dishes were incubated at 37 ºC for 18 hours. Antibacterial activity was determined by measuring the radius of the inhibition zone around each mycelial disk. Three replicates were made for each culture.

Table 1 Cultures Deposited in URM Culture Collection.

Species	Year of Deposit	State	Substrate	Culture collection URM	Situation
Antrodiella hydrophila (Berk. & M.A. Curtis) Ryvarden	2013	PB	Wood	6977	Reactivated
C. floccose (Jungh.) Ryvarden	2013	PB	Wood	6976	Reactivated
Clavulinopsis flavella (Berk. & M.A. Curtis) Corner	2013	PE	Wood	6972	Reactivated
Fomitopsis cupreorosea (Berk.) J. Carranza & Gilb.	2012	PE	Wood	6830	Reactivated
Ganoderma multiplicatum (Mont.) Pat.	2013	PB	Wood	6975	Reactivated
Ganoderma parvulum Murril	1988	PE	Wood	2948	Reactivated
Grammothele lineata Berk. & M.A. Curt.	2012	PE	Wood	6827	Reactivated
Polyporus adustus Willd. Fr.	1996	MA	Soil	2147	No reactivated
P. berkeleyi Fr.	1964	PE	Soil	1969	Reactivated
P. flabeliforme (Berk. & Br.) Sacc	1998	PE	No registered	4067	No reactivated
P. flabeliforme (Berk. & Br.) Sacc.	1996	PE	*Cocos nucifera*	3643	No reactivated
P. gilvus (Fr.) M.A. Souza	1980	PE	Wood	2559	No reactivated
P. gilvus (Fr.) M.A. Souza	1981	PE	Wood	2697	Reactivated
P. ostreatoroseus Singer	1998	PE	Wood	4073	No reactivated
P. ostreatoroseus Singer	1998	PE	Wood	4070	Reactivated
P. ostreatus roseus Singer	1996	PE	Wood	3642	No reactivated
P. rimosus (Berk.) Pilat	1980	PE	Wood	2565	Reactivated
P. zonatus Fr.	1972	PE	No registered	2355	Reactivated
Phellinus gilvus (Fr.) M.A. Souza	1980	PE	Wood	2560	No reactivated
Pleurotus atrocaeruleus Fr.	1958	BA	No registered	363	Reactivated
R. microporus(Sw.) Overeem	2013	PE	Wood	6978	Reactivated
Rigidoporus lineatus (Pers.) Ryvarden	1990	PE	Paraffin	3230	No reactivated
Rigidoporus lineatus(Pers.) Ryvarden	2012	PE	Wood	6828	Reactivated
Rigidoporus surinamensis (Mir) Murr	1960	PE	Wood	2562	No reactivated
Rigidoporus surinamensis (Mir) Murr.	1960	PE	Wood	2564	Reactivated
Scytinostroma duriusculum (Berk. & Broome) Donk	2013	PB	Wood	6974	Reactivated
S. cf. *duriusculum* (Berk. & Broome) Donk	2013	PB	Wood	6931	Reactivated
Stereopsis hiscens (Berk. & Ravenel) D.A. Reid	2013	PE	Wood	6970	Reactivated
Stereopsis hiscens (Berk. & Ravenel) D.A. Reid	2013	PE	Wood	6971	Reactivated
Stereum ostrea (Blume & Nees) Fr.	2013	PB	Wood	6973	Reactivated
Trechispora brinkmanii (Bres) Roger & Jackson	1961	PE	Ꜧ*bieslasiocarpa*	1714	No reactivated

MA= Maranhão, PE= Pernambuco, PB= Paraíba

Microdilution method

Analysis of the minimum inhibitory concentration (MIC) was carried in a 96–well microdilution plate according to the method described by National Centre for Clinical Laboratory Standards (NCCLS). In each well, 10 µl of bacterial suspension was added. A 100 µL aliquot of MH broth was used as inoculant for each well. The microplates were incubated at 37°C overnight. The MIC reading was performed by plaque turbidity, which indicated the presence or absence of bacterial growth. The MIC was considered the lowest concentration of the extracts that allowed bacterial growth. The tests were performed in triplicate for each extract. Solvent was used as negative control and bacterial suspension in MH broth with extract as positive control. The last well of the plate was filled with bacterial suspension in MH broth without extract.

Table 2 Basidiomata collected in the state of Pernambuco (PE) and deposited in Herbarium URM. Dry weight of basidiomata and extracts.

Species	Substrate	Herbarium URM	Dry weight (g)	Extract (mg)
Amauroderma sp.	Wood	87735	2.48	8.6
Datronia caperata (Berk.) Ryvarden	Wood	87737	2.17	25.0
Earliella scabrosa (Pers.) Gilb. & Ryvarden	Wood	87738	4.32	11.5
Ganoderma orbiforme (Fr.) Ryvarden	Wood	87741	8.36	15.8
Gloeophyllum striatum (Fr.) Murill	Wood	86483	1.30	14.7
Microporellus obovatus (Jungh.) Ryvarden	Wood	87739	1.85	21.0
Polyporus leprieurii Mont.	Wood	87736	0.72	4.6
Pycnoporus sanguineus (L.) Murrill	Wood	87740	0.91	2.1
Stereum ostrea (Blume & T. Nees) Fr.	Wood	87848	1.67	12.0

Results

Antibacterial activity of extracts

Thirty materials were tested against *S. aureus* (21 reactivated cultures and the extracts of nine fresh basidiomata) (Table 1, 2).

Of the 21 reactivated cultures, eight showed antibacterial activity, measured by the inhibition halo and by the minimum inhibitory concentration method (MIC) (Table 3, 4). Five extracts obtained from the fresh basidiomata also presented antibacterial activity determined by MIC (Table 4).

Discussion

Fomitopsis cupreorosea, *Ganoderma multiplicatum*, *G. orbiforme, Rigidoporus lineatus, R. microporus, Stereum ostrea* inhibited all eleven strains of *Staphylococcus aureus*, confirming the production of bioactive substances.

Of the tested species, there are no reports on the antibiotic potential of species of *Rigidoporus*, only on production of enzymes involved in decomposition of wood (Sridhar et al. 2013, Madushani et al. 2014).

Similarly, *F. cupreorosea* has never been tested for antibiotic activity before. However, chloroform from another species of the genus, *F. rosea*, showed antibiotic activity against *S. aureus* (Popova et al. 2009). Two new substances, lanostane (triterpenoid) and ergostane (steroid), isolated from *F. pinicola*, were tested against *Bacillus cereus*, with positive results. The extracts were obtained with dichloromethane (Liu et al. 2010). Positive results were also obtained with extracts of ethanol, hexane and water of *F. lilacinogilva* against *B. cereus*, *Escherichia coli* and *S. aureus* (Bala et al. 2011, 2012). Ethanolic compounds from *F. officinalis* exhibited bioactivity against *Mycobacterium tuberculosis* (Hwang et al. 2013).

Ganoderma species are widely studied for their antimicrobial activity and the positive results were expected. However, there are no reports of antimicrobial activity for *G. multiplicatum*, *G. parvulum* and *G. orbiforme*. In Brazil, the antibacterial activity of methanol extracts *G. australe*

Table 3 Antibacterial activity of species of Agaricomycetes extracts in ethyl acetate (diameter in mm of the zone of inhibition) against strains of *S. aureus*.

Species	Strains										
	707	**726**	**731**	**ATCC01**	**676***	**728***	**729***	**730***	**733***	**677***	**691***
***Fomitopsis cupreorosea* (URM 6830)**	23.9±0.26	23.7±0.06	23.7±0.15	22.9±0.15	20.3±0.42	20.6±0.15	20.15±0.44	20.4±0.12	20.7±0.95	20.2±0.06	20.4±0.17
***Ganoderma multiplicatum* (URM 6975)**	24.4±0.06	23.7±0.29	24.1±0.17	24.6±0.06	21.5±1.80	21.7±2.69	18.7±0.17	18.9±0.42	22.1±0.46	21.8±0.78	22.3±0.35
***G. parvulum* (URM 2948)**	19.0±0.26	19.7±0.17	20.1±0.26	-	-	-	16.1±0.26	16.3±0.36	-	-	17.5±0.35
***Grammothele lineata* (URM 6827)**	22.6±0.29	17.0±0.06	22.1±0.47	22.4±0.35	18.9±0.59	18.3±0.17	18.2±0.42	18.0±0.50	18.1±0.26	17.9±0.56	18.5±0.32
***Phellinus rimosus* (URM 2565)**	-	-	-	-	-	-	-	9.2±0.35	-	-	-
***Rigidoporus lineatus* (URM 6828)**	22.7±0.12	22.5±0.06	22.2±0.31	22.2±0.46	21.0±0.12	22.2±0.68	21.6±0.21	18.1±0.06	18.4±0.47	18.1±0.74	18.2±0.35
***R. microporus* (URM 6978)**	23.2±0.35	23.4±0.61	23.4±0.52	23.3±0.26	22.4±0.96	18.3±0.35	22.6±0.29	22.9±0	18.5±0.36	18.2±0.10	18.2±0.36
***Stereum ostrea* (URM 6973)**	12.4±0.31	17.3±0.70	21.3±0.55	9.2±0.10	9.2±0.17	9.3±0.25	21.3±0.06	9.0±0.40	9.2±0.15	9.0±0.17	21.3±1.33

* ORSA

Table 4 Antibacterial activity of species of Agaricomycetes extracts in ethyl acetate (µg/mL) against strains of *S. aureus*.

Species	Strains										
	676*	**707**	**726**	**728***	**729***	**730***	**731**	**733***	**677***	**691***	**ATCC01**
***Datronia caperata* (URM 87737)**	-	-	62.5	-	-	-	62.5	-	-	-	-
***Earliella scabrosa* (URM 87738)**	-	-	28.8	-	-	-	28.8	-	-	-	-
***Fomitopsis cupreorosea* (URM 6830)**	10.38	0.65	0.65	10.38	10.38	10.38	0.65	10.38	10.38	10.38	0.65
***Ganoderma multiplicatum* (URM 6975)**	12.38	3.10	3.10	3.10	12.38	12.38	3.10	3.10	3.10	3.10	3.10
***G. parvulum* (URM 2948)**	-	3.19	3.19	-	25.48	25.48	3.19	-	-	25.48	-
***Ganoderma orbiforme* (URM 87741)**	20.34	2.54	2.54	20.34	20.34	20.34	2.54	81.38	10.17	5.09	2.54
***Grammothele lineata* (URM 6827)**	10.59	0.66	10.59	10.59	10.59	10.59	0.66	10.59	10.59	10.59	0.66
***Microporellus obovatus* (URM 87739)**	-	-	26.25	-	-	-	-	-	-	-	-
***Phellinus rimosus* (URM 2565)**	-	-	-	-	-	58.35	-	-	-	-	-
***Rigidoporus lineatus* (URM 6828)**	2.94	1.47	1.47	2.94	2.94	5.88	1.47	5.88	5.88	5.88	1.47
***R. microporus* (URM 6978)**	2.04	1.02	1.02	4.07	2.04	2.04	1.02	4.07	4.07	4.07	1.02
***Stereum ostrea* (URM 6973)**	7.69	3.84	0.24	7.69	7.69	7.69	0.24	7.69	7.69	7.69	0.24
***Stereum ostrea* (URM 87848)**	11.75	2.93	0.18	23.50	23.50	23.50	0.18	23.50	23.50	23.50	0.18

* ORSA

against *B. cereus*, *E. coli*, *Pseudomonas aeruginosa* and *S. aureus* was tested, with positive results (Smânia et al. 2007). The MIC value observed for *G. australe* against *S. aureus* was 1 mg/Ml, while the MIC values observed in the present study were lower for *G. multiplicatum*, *G. parvulum* and *G. orbiforme*. In another study, chloroform extracts from the basidioma of *Ganoderma lucidum* were tested against Gram–positive and Gram–negative bacteria. The minimal inhibitory concentration for *S. aureus* was 8 mg/mL (Keypour et al. 2008). Quereshi et al. (2010) obtained MIC results of 20 mg/mL using acetone extracts tested against *S. aureus*. In other study, methalonic extracts *G. lucidum* inhibited the growth of *S. aureus* methyl–resistant (MRSA) (Shah et al. 2014).

The antimicrobial activity of species of *Stereum* has also been studied (Opatz et al. 2008). Tests with frustulosin, an active compound isolated from *Stereum frustulosum*, showed activity against *S. aureus*, *B. mycoides* and *B. subtilis* (Nair & Anchel 1975). Methanol extracts of *Stereum hirsutum* showed activity against *S. aureus*, while *Stereum insignitum* inhibited *B. subtilis* (Suay et al. 2000). While two new benzoates derivatives and three new sesquiterpenes from the same species inhibited the growth of *S. aureus* (Ma et al. 2014). Prust et al. (2014) tested acetone, ethanol and aqueous extracts proving the potential of species of *Stereum ostrea* in inhibiting bacterial growth of both Gram–positive and Gram–negative bacteria. Results showed a better activity for acetone extracts.

The ability of *P. sanguineus* and *Pleurotus* spp. and *Phellinus* spp., *D. caperata* and *E. scabrosa* to inhibit bacterial growth was reported in literature (Smânia et al. 1995, 1998, Gogavekar et al. 2014, Silva et al. 2009, Silva & Jorge 2011, Kalyoncu et al. 2010, Wisbeck & Robert 2002). However, the samples used in the current study showed weak or no inhibitory activity against the tested bacteria (Table 1, 4).

The present study provided new insights about the antimicrobial potential of Agaricomycetes, once species never before studied showed antibacterial properties. These species were collected in Brazil, where studies about fungal medicinal properties are usually neglected, contrasting to its endangered megadiversity.

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