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Screening of *Lignosus rhinocerus* Extracts as Antimicrobial Agents against Selected Human Pathogens

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Abstract:

Over the decades, natural source has become an important essence to discover new antimicrobial compounds to control the dramatic increase of infectious disease. In this present study, petroleum ether, chloroform, methanol, and water extracts of *Lignosus rhinocerus* sclerotium were obtained. All the extracts were screened for their antibacterial and antifungal activities against gram positive bacteria such as *Staphylococcus aureus, Corynebacterium diphtheriae, Bacillus cereus, Stapyhlococcus epidermidis, Streptococcus pyogenes, Strepococcus viridans, Micrococcus luteus,* gram negative bacteria such as *Klebsiella pneumoniae, Salmonella typhi, Entorobacter aerogenes, Vibro cholera, Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, Proteus hauseri* and fungi such as *Candida albicans, Candida tropicalis, Candida krusei and Mucor racemosus* by disc diffusion method. Antibacterial and antifungal activities of these extracts were assessed by measuring diameter of zone of inhibition and the results were compared with standard antibiotics, Amoxicillin (10µg/disc) and Fluconazole (30µg/disc) respectively. From these findings, the extracts of *Lignosus rhinocerus* indicated that plant possess significant antimicrobial activity against tested microbes, which could be a potential source to modern medicine to treat microbial infections.

Keywords: *Lignosus rhinocerus,* Mushroom, Antibacterial, Antifungal, Disc diffusion.

Introduction:

Mushrooms have their own established uses and been consumed widely by many natives due to its valuable nutrient content and pharmacological properties [1,2]. It is well recognized that mushroom extracts contain a range of compounds such as protein, fibre, lectins, polysaccharides and polyphenols, where each of which may have its own therapeutic effects [3]. In general, mushrooms which have been used as therapeutic agents are known as medicinal mushrooms. Mushroom species have been reported to have antagonist activity against bacteria, fungi, viruses and cancer [4].

Lignosus rhinocerus is an exclusive medicinal mushroom found in Malaysia that belongs to Polyporaceae family. In Malaysia, the mushroom is locally well-known as "cendawan susu rimau" or "mushroom of tiger's milk". The tuber is the part which comprised with medicinal value compounds. The aboriginal population in Peninsular Malaysia uses it extensively to treat many diseases such as fever, cough, asthma, breast cancer, stomach cancer, food poisoning and healing wound [5]. It is also used traditionally by Chinese physicians to treat liver cancer, chronic hepatitis, and gastric ulcers in China [6]. The sclerotial polysaccharides from *L. rhinoceros* reported for its antiproliferative [7-9] and immunomodulatory effects [10]. The antiproliferative activity of *L. rhinocerus*, positively identified by genetic marker on human breast and lung cancer cell lines while its cytotoxicity on normal breast and lung cell lines [8].

Since there is no scientific report on *L. rhinocerus* for its antimicrobial activity, the present study was undertaken to determine the antimicrobial property on microbes responsible for wound since *L. rhinocerus* is traditionally used to heal wounds caused by various microbes [11].

Materials and methods:

Collection and Authentication of Material

Mushrooms were collected from Lata Iskandar, Tapah aborigines, Perak, Malaysia on October 2011 and authenticated by Ms. Thi Bee Kin, Research Officer, Forest Health and Conservation Programme, Forest Biodiversity Division, Forest Research Institute Malaysia (FRIM), Malaysia.

Preparation of Extracts:

The collected mushrooms, *Lignosus rhinocerus* were shade dried, pulverized into coarse powder and divided equally into four separate portions. The mushroom sample (100g) was extracted by stirring with 500 ml of petroleum ether, chloroform, methanol and water separately at 30°C at 150rpm for 24h and filtered through Whatmann filter paper [12]. The extracts were collected separately and concentrated using rotary vacuum evaporator under reduced pressure. The color, consistency and percentage yield of various extracts of *L. rhinocerus* was tabulated in ***Table 1.*** All the extracts were stored in dessicator until further use.

Preliminary Phytochemical Tests:

The qualitative preliminary phytochemical analysis of various extracts of *L. rhinocerus* was carried out to investigate phytoconstituents such as alkaloids, carbohydrates and glycosides, fixed oils and fats, flavonoids, mucilages, phenolic compounds and tannins, proteins, saponins, sterols and triterpenoids [13] present in the mushroom extracts. The results were recorded in ***Table 2.***

Screening of Antimicrobial activity: Collection of Microorganisms:

All the microbe cultures were obtained from microbiology lab, Masterskill University College of Health Sciences, Cheras, Selangor, Malaysia. Gram positive bacteria such as *Staphylococcus aureus* (ATCC 25923), *Corynebacterium diphtheria* (ATCC 27010), *Bacillus cereus* (ATCC 13061), *Stapyhlococcus epidermidis* (ATCC 49461), *Streptococcus pyogenes* (ATCC 12384), *Strepococcus viridians* (ATCC 10551), *Micrococcus luteus* (ATCC 10240), gram negative bacteria such as *Klebsiella pneumonia* (ATCC 13883), *Salmonella typhi* (ATCC 14901), *Entorobacter aerogenes* (ATCC 13048), *Vibro cholera* (ATCC14033), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Serratia marcescens* (ATCC 13880), *Proteus hauseri* (ATCC 13315) and fungi such as *Candida albicans* (ATCC 14053), *Candida tropicalis* (ATCC 13803), *Candida krusei* (ATCC 34135) *and Mucor racemosus* (ATCC 7935) were used for this study.

Antibacterial activity:

The antibacterial assay for this study was examined using disc diffusion method described by Ejikeme [14]. All the four extracts were diluted separately with distilled water to get concentration of 30mg/ml. Muller Hinton agar was used to culture *S. aureus*, *B. cereus*, *S. epidermidis*, *M. luteus*, *S. typhi*, *E. aerogenes*, *V. cholera*, *E. coli*, *P. aeruginosa* and *S. marcescens*. Blood agar was used to culture *C. diphtheria*, *S. pyogenes*, *S. viridians*, *K. pneumonia and P. hauseri* [14]. Suspension of inoculum (100µl) containing 10^5 colony forming units per ml (cfu.ml^{-1}) of bacteria was swabbed on respective agar plates for uniform distribution of colonies. Whatmann filter paper with 6mm in diameter were made, sterilized and wetted with 10µl of each extracts separately and allowed to dry. The discs and standard antibacterial agent, amoxicillin (10µg/disc) were placed firmly on the surface of cultured agar plate. All the plates were incubated at 37°C for 24h. The diameter of zone of inhibition (mm) was measured to assess the antibacterial activity and the results were tabulated in ***Table 3.*** All experiments were performed in triplicate.

Antifungal activity:

The antibacterial assay for this study was examined by using disc diffusion method described by Ejikeme [14]. All the four extracts were diluted separately with distilled water to get concentration of 30mg/ml. Sabouraud dextrose agar was used as medium for fungi such as *C. albicans*, *C. tropicalis*, *C. krusei and M. racemosus.* Suspension of inoculum (100µl) containing 10^5 colony forming units per ml (cfu.ml^{-1}) of fungi was swabbed on respective agar plates for uniform distribution of colonies. Whatmann Filter paper were cut into circular disc shape size 6mm each and sterilized in autoclave. The disc 6mm then wetted in the each extracts (10µl) separately and allowed to dry. The discs and standard antifungal agent, fluconazole (30µg/disc) were impregnated on the cultured agar plate. All the plates were incubated at 37°C for 72h. The diameter of zone of inhibition (mm) was measured to establish antifungal activity and the results were tabulated in ***Table 3***. All experiments were performed in triplicate.

Statistical Analysis:

Data are expressed as mean ± SEM. The antimicrobial activities of the extracts against the standard antimicrobial agents were compared using Student's t-test. The statistical analysis was conducted with SPSS software (v.19, SPSS, USA) at significant levels of 0.05 and 0.01 [15].

Table 3: Antibacterial and Antifungal activities of various extracts of *Lignosus rhinocerus*

Test microorganisms	Diameter of zone of inhibition (mm) Mean ± SEM				
	PE	CE	ME	AE	Standard
S. aureus	12.33±1.2**	12.00±1.00**	13.00±0.58**	17.67 ± 0.33**	16.00±0.00** (A)
C. diphtheria	7.67±0.88	8.67±0.67	17.00±0.58*	17.67 ± 0.33*	30.00±0.00** (A)
B. cereus	13.00±0.58*	8.33±0.33	13.00±1.00*	16.33 ± 0.33*	29.67±0.33** (A)
S. epidermidis	8.3±0.33*	9.00±1.00*	13.33±1.2**	13.33 ± 0.67**	18.33±0.33** (A)
S. pyogenes	7.33±1.2	8.67±0.67*	8.67±1.2*	13.33 ± 0.67*	26.00±0.00** (A)
S. viridians	8.67±0.33**	10.00±1.53**	15.67±1.45**	15.33 ± 0.88**	18.33±0.33** (A)
M .luteus	10.00±0.00	10.67±0.88	18.00±0.58*	18.33 ± 0.33*	37.00±1.00** (A)
K. pneumonia	10.33±0.33**	12.67±0.67**	16.67±0.67**	13.33 ± 0.88**	0.00 ± 0.00(A)
S. typhi	9.00±0.58*	9.00±0.00*	9.00±1.00*	11.33 ± 0.33*	22.33±0.33**(A)
E. aerogenes	7.33±0.88*	9.00±1.00*	14.00±0.00**	10.33 ± 0.33*	18.00±0.00**(A)
V. cholera	9.00±1.00*	11.33±0.33*	14.33±0.88*	11.67 ± 0.33*	24.00±0.58**(A)
E. coli	8.00±0.00*	7.67±0.33	17.00±0.58**	14.33 ± 0.67**	21.00±0.58**(A)
P. aeruginosa	7.00±1.15*	7.00±0.58*	10.67±0.88**	9.00±0.00**	11.67±0.33**(A)
S. marcescens	7.33±1.2*	7.67±0.88*	8.33±0.33*	8.67±0.67*	18.00±0.58**(A)

P. hauseri	6.67±1.2	9.67±0.88*	12.67±0.33**	13.00±0.00**	15.67±0.33**(A)
C. albicans	8.67±0.33*	9.00±1.15*	12.00±0.00*	10.00±0.58*	19.67±0.33**(F)
C. tropicalis	10.33±0.67*	12.00±1.15*	12.00±0.00*	16.33±0.33**	20.00±0.00**(F)
C. krusei	7.67±0.88*	9.67±1.45*	11.33±0.88*	16.00±0.00**	20.00±0.00**(F)
M. racemosus	10.00±0.00**	10.33±1.2**	15.67±1.2**	12.33±0.67**	0.00 ± 0.00 (F)

PE - Pet. ether Extract. ME - Methanol extract, CE - Chloroform extract, AE - Aqueous extract, (A) - Amoxicillin, (F) – Fluconazole.
Values are mean ± SEM. *P<0.05, **P<0.01, diameter of zone of inhibition (mm) of extracts against bacteria and fungus vs. zone of inhibition of standard drugs.
(n = 3)

Results and discussion:

The color, consistency and the percentage yield of petroleum ether, chloroform, methanol and aqueous extract of *L. rhinoceros* were recorded ***(Table 1).*** The percentage yield of aqueous extract was higher than other extracts. The antimicrobial activity of four different extracts of *L. rhinocerus* was investigated using disc diffusion method against fifteen bacteria and four fungi. The zone of inhibition in diameter (mm) of the extracts (30mg/ml) against the selected microorganisms revealed that *L. rhinocerus* extracts possesses mild to moderate antibacterial and antifungal activities which was well compared with standard antibacterial agent, Amoxicillin (10µg/disc) and antifungal agent, Fluconazole (30µg/disc) respectively. The results exhibited that the aqueous and the methanol extract (30mg/ml) illustrated maximum zone of inhibition against the pathogenic organisms selected except *S. pyogenes* and *S. marcescens*. The aqueous extract showed highly significant (p<0.01) antimicrobial activity than the methanol extract (p<0.05) against most of the selected pathogens. The qualitative preliminary phytochemical analysis showed the presence of phytochemical constituents such as alkaloids, protein, gums and mucilage, and flavonoids ***(Table 2).*** The presences of various phytochemical constituents in the extracts might be responsible for the antimicrobial activity. The earlier studies for antibacterial activity stated that the presence of alkaloids and flavonoids [16] and proteins [17] supported the antibacterial activity of *L. rhinocerus.* Antibacterial and antifungal activities of gums and mucilage [18] were also supported by the results of the present study of *L. rhinocerus*.

Table 1: The color, consistency and percentage yield of various extracts of *Lignosus rhinoceros*

Extracts	Colour	Consistency	% Yield
Pet. Ether	Yellowish white	Powder	1.16
Chloroform	Pale greenish yellow	Powder	2.84
Methanol	Greenish yellow	Powder	9.40
Aqueous	White	Powder	15.4

Table2: Preliminary phytochemical analysis of various extracts of *Lignosus rhinocerus*

Chemical constituents	Pet. ether extract	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids	+	+	+	+
Carbohydrates and glycosides	+	+	+	+
Proteins and amino acids	-	-	+	+
Sterols	-	-	-	-
Fixed oils and fats	-	-	-	-
Tannins-phenolic compounds	-	-	-	-
Triterpenoids	-	-	-	-
Saponins	-	-	-	-
Gum and mucilage	+	+	+	+
Flavones and flavanones	+	+	+	+

+ = present, - = absent

Conclusion:

The findings from the results exhibited various spectrum of antimicrobial activity of *Lignosus rhinocerus* of which might be due to the presence of bioconstituents in the extracts. Hence, the present study has scientifically proven that the selected mushroom, *L. rhinocerus* has an effective antimicrobial activity against the selected pathogenic bacteria and fungi which are responsible for wound. As *Lignosus rhinocerus* could be a crucial source to develop new antimicrobial agents, a further work will be required to isolate the secondary metabolite and to specify its possible mechanism for antibacterial and antifungal activities.

References:

1.Chang ST, Miles PG. Edible mushrooms and their cultivation, CRC Press: Boca Raton, Florida, USA; 1989.
2.Lindequist U, Niedermeyer THJ, Julich WD. The pharmacological potential of mushrooms-A Review. eCAM. 2005; 2(3):285–99.
3.Smiderle FR, Sassaki GL, Van Arkel J, Iacomini M, Wichers HJ, Van Griensven LJ. High molecular weight glucan of the culinary medicinal mushroom *Agaricus bisporus* is an α-glucan that forms complexes with low molecular weight galactan. Molecules. 2010; 15(8):5818–30.
4.Jonathan GS, Ishola OF. Antimicrobial activities of some selected nigerian mushrooms. Afr J Biomed Res. 2005;8(2):83-7.
5.Lee SS, Tan NH, Fung SY, Pailoor J, Sim SM. Evaluation of the sub-acute toxicity of the sclerotium of *Lignosus rhinocerus* (Cooke), the tiger milk mushroom. J Ethnopharmacol. 2011; 138(1):192-200.

6.Lee SS, Chang YS, Noraswati MNR. Utilization of macrofungi by some indigenous communities for food and medicine in Peninsular Malaysia. Forest Ecol Manag. 2009; 257(10):2062–65.
7.Tan CS. Setting-up pilot-plant for up-scaling production of tiger-milk mushroom as dietary functional food. Tech. Rep. MOA TF0109M004: Government of Malaysia; 2009.
8.Lai CKM, WongKH, Cheung PCK. Antiproliferative effects of sclerotial polysaccharides from *Polyporus rhinocerus* cooke (Aphyllophoromycetideae) on different kinds of leukemic cells. Int J Med Mushrooms. 2008; 10(3):255–64.
9.Lee ML, Tan NH, Fung SY, Tan CS, Ng ST. The Antiproliferative activity of sclerotia of *Lignosus rhinocerus* (Tiger Milk Mushroom). eCAM. 2012; 2012(2012):5.
10.Wong KH, Lai CKM, Peter CCK. Immunomodulatory activities of mushroom sclerotial polysaccharides. Food Hydrocoll. 2010; 25(2):150-58.
11.Lee-Fang Eik, Murali Naidu, Pamela David, Kah-Hui Wong, Yee-Shin Tan, Vikineswary Sabaratnam. Lignosus rhinocerus (Cooke) Ryvarden: A Medicinal Mushroom That Stimulates Neurite Outgrowth in PC-12 Cells. Evidence-Based Complementary and Alternative Medicine. 2011; 2012 (2012):7.
12.Aziz T, Mehmet ED, Nazime M. Antioxidant and antimicrobial activity of *Russula delica* Fr: An edidle wild mushroom. Eurasian J Anal Chem. 2007;2(1):54-67.
13.Anshurathi RV, Khatoon S, Mehrotra S. Ethnopharmacological evaluation of *Peristrophe bicalyculata* Nees for anti-inflammatory and analgesic activity. Nat Prod Sci. 2003; 9:195-9.
14.Ejikeme N, Henrietta UO. Antimicrobial activity of some local mushrooms on pathogenic isolates. J Med Plant Res. 2010;4(23):2460–65.
15.Anandarajagopal K, Anbu JSJ, Promwichit P. *Bombax ceiba* Linn. bark extracts shows anti-microbial activity. Int J Pharm Res. 2011;1(3):24-6.
16.Iwalokun BA, Usen UA, Otunba AA, Olukoya DK. Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus.* Afr J Biotech. 2007;6(15):1732-9.
17.Karaket P, Rakrudee S, Sompong T, Pasakorn B, Saranyu K. Antibacterial activity of *Bauhinia acuminata* L. seed protein extract with low hemolytic activity against human erythrocytes. Chiang Mai J Sci. 2011;38(2):242-51.
18.Benarji PT, Hariprasad A. Preliminary phytochemical and antimicrobial activity of *Agaricus bisporus.* Asian J Biochem Pharm Res. 2011;2(1):102-5.

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