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Antibacterial Activity of Extracts of Selected Indigenous Edible and Medicinal Mushrooms of Eastern Uganda

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Abstract: Antibacterial resistance is a world wide growing problem. Isolation of microbial agents less susceptible to regular antibiotics and recovery of resistant isolates during antibacterial therapy is increasing throughout the world. One of the measures to combat the increasing rate of resistance is to have a continuous investigation for new, safe and effective antimicrobials as alternative agents to substitute with no-effective ones. In this study the efficacy of selected mushrooms of Teso region (Kumi District) against common bacteria was investigated. Antibacterial assays were done using methanol and petroleum ether extracts from mushrooms and Agar Well Diffusion Method and study Disc Methods were used to demonstrate activity. It was noted that *E. coli* was resistant to most petroleum ether and methanol extracts. Also *S. aureus* was significantly inhibited by petroleum ether extracts while *P. aeruginosa*, a very resistant bacterium always, was significantly inhibited by the methanol extracts. These activities might be of some practical importance if the chemical compounds inducing activity are known. Further studies should therefore be done to confirm and extend the present findings, working on a broader range of species.

Key words: Antibacterial, mushroom extracts, edible and medicinal mushrooms, *S. aureus, E. coli* and *P. aeruginosa*

INTRODUCTION

Natural resources especially plants, mushrooms and microorganisms have been used as potent candidates for new, safe and effective antimicrobials. Usage of plants and mushrooms in curing illnesses has deep roots in man's history since plants and mushrooms are sources of many life sustaining metabolites[1]. Traditional curative medicine of most nations is based upon plants and mushrooms. Ethnobotanical uses of mushrooms prevail among many local communities[2,3] including the people of Teso. They use mushrooms in treating measles, general body pains, stomach up sets and cough. A large number of mushroom species elsewhere are not only edible but also possess tonic and medicinal qualities[4-6]. Mushrooms have been shown to produce several biologically active compounds that are usually associated with the cell wall and these have been suggested to contribute to immune enhancing and tumour retarding effects[7-9]. It has been suggested that the anti tumour and anti cancer effects of the polysaccharides are based on enhancement of the bodies immune systems rather than the direct cytocidal effects. Among the local communities, mushrooms may represent a potential source of antibacterial drugs since earlier screening for antibiotics started with basidiomycetes (mushrooms) and proved successful[10-13]. The beliefs that mushrooms treat illnesses was investigated and hence presented here is the evaluation of antibacterial properties of selected edible and medicinal mushroom extracts of the Teso region in Eastern Uganda.

MATERIALS AND METHODS

Mushroom handling and extraction procedures: Fresh mushroom samples were obtained from four microhabitat sites (cultivated area, overgrazed area, less grazed area and woodland). Collected samples were then sun dried for two to four days as a means of preservation. The fresh mushrooms to be used for comparisons were kept in a refrigerator. The sun dried mushrooms were stored in a cool dry and air tight container to avoid growth of other

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fungi. Prior to pounding, the dried mushrooms were warmed in the sun to enable easy pounding. The warmed mushrooms were powdered with a mortar and pestle to finer flour to increase surface area during extraction. The fresh frozen mushrooms were thawed and pasted using a motor and pestle.

A known weight of the prepared mushroom samples were obtained and placed in a flask. A known volume of the solvents (petroleum ether or methanol) was added to the mushroom samples. The flasks were covered using black polythene bags to avoid light interference. The methanol or petroleum ether extracts were prepared by maceration of the mushroom with either methanol or petroleum ether for four days at room temperatures. The respective extracts were filtered using whatman filter papers and dried under reduced pressure at a temperature below 60°C to yield a dense residue. Samples were transferred to universal bottles and kept in a fridge for 3-4 weeks before use

Antibacterial studies

Test organisms and media: The mushroom extracts were assayed for antibacterial activity against three registered bacterial isolates which were obtained from the American Type Culture Collection at the Faculty of Veterinary Medicine, Makerere University. The bacteria included two gram negative bacterial isolates: *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and one gram positive bacterium: *Staphylococcus aureus* (ATCC 25923). The bacteria were rejuvenated on Mueller Hinton Agar medium and sub cultured as needed. Other medium used was the Brain heart infusion broth.

Agar well diffusion assays: Wells were made on Muller Hinton agar plates using a sterile borer. Two to five hour broth cultures of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were centrifuged at 1956.5 g for 15 min and reconstituted in 1% Gelatin in Normal Saline (GNS). The turbidity of the reconstituted organisms was adjusted to that of turbidity standard (prepared by adding 0.5 mL of 1% $BaCl_2$ to 99.5 mL of 0.36N H_2SO_4). Both the standards and bacterial suspensions were agitated in a vortex mixer immediately prior to use. A sterile cotton swab (on a wooden applicator stick) was dipped into standardized bacterial suspension. Broth was expressed from swab by pressure and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then evenly streaked in three directions over the entire surface of the agar plate to obtain uniform inoculums; a final sweep of the agar rim was made with the cotton swab. The plates were allowed to dry for 3-5 min after which test samples and the controls were dispensed into each well. The concentration of the test samples were 0.5 g mL^{-1} for methanol extracts and 0.2 g mL^{-1} for petroleum ether extracts. The plates were inoculated at 37°C for at least 16 h during which activity was evident by presence of a zone of inhibition surrounding the well. Diameter of Inhibition Zones (DIZ) were measured in millimeters and compared to standard tetracycline or gentamycin as positive control and Dimethylsulphoxide 100%(DMSO) as negative control.

Filter paper disc assays: The procedure for inoculation of the plates was essentially as given in the agar well diffusion assays. However instead of drilling wells on the agar, filter paper discs (Whatmann No. 1) impregnated with mushroom extracts were applied on the surface of the agar and the plate incubated overnight. Diameter of Inhibition Zones (DIZ) were measured in millimeters and compared to standard tetracycline or gentamycin as positive control and DMSO as negative control.

Determination of Minimum Inhibitory Concentrations (MIC): The Minimum Inhibition Concentration (MIC) of the effective extracts was then determined by broth dilution method. Brain heart infusion broth was prepared by mixing 120 g in 1000 mL of distilled water and 1 mL (for methanol extract) or 0.5 mL (for petroleum ether extract) of the broth were put in test tubes and equal amounts of the extracts were added to the first tube and serial dilution done with the last 1 mL or 0.5 mL being discarded. To complete the test, 0.1ml (for methanol extracts) or 0.05 mL (for petroleum ether extracts) of the organisms suspended in peptone water and incubated overnight was added to all the tubes and then incubated overnight. The growth of bacteria was confirmed by a milky or turbid colouration while inhibited growth showed clear solutions. The concentrations used to determine MIC of methanol extracts were 0.5, 0.25, 0.125, 0.063, 0.031, 0.016 and 0.08 g mL^{-1} and for petroleum ether extracts were 0.2, 0.1, 0.05, 0.025 and 0.0125 g mL^{-1}. The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth.

RESULTS

Extraction solvents and extracts: Table 1 summarizes the different extraction out put of mushrooms using methanol or petroleum ether solvents. For the sun dried samples, it was demonstrated that equal volumes of both methanol and petroleum ether could be used for similar weights except slight differences shown by *Odilit (Termitomyces* sp*)* and *Eguti (Termitomyces aurantiacus)*. The fresh samples required varying volumes of solvents for similar weights. The methanol solvent gave much extracts than the petroleum ether solvent.

Table 1: Extraction solvents (mL) and extracts (g)

Species	Trt	Vol. M (mL)	Vol. P (mL)	Wt.MS (g)	Wt. PS (g)	Wt. M. Ex (g)	Wt. P. Ex (g)
Eswei (*Termitomyces microcarpus*)	dry	400	400	200	200	5.6000	2.6204
Echoroi (*Agaricus sp.*)	dry	500	500	200	200	5.0724	0.4019
Otulelut (*Agaricus sp.*)	dry	500	500	200	200	4.7263	3.1773
Oujoi (*Lepiota sp.*)	dry	800	800	200	200	17.0599	0.1900
Esiara (*Tricholoma sp.*)	dry	400	400	200	200	8.3595	1.2986
Odilit (*Termitomyces sp*)	dry	750	600	200	200	6.8565	0.78533
Eburukunyu (*Agaricus sp.*)	dry	700	700	200	200	6.9612	0.4033
Erusurus (Not identified)	dry	450	500	166	200	7.7697	0.5954
Eguti (*Termitomyces aurantiacus*)	dry	700	650	200	200	14.1344	0.9908
Opungrei (Not identified)	dry	500	-	216	-	4.6658	-
Etimijaka (*Agaricus species*)	Fresh	400	450	248	257	5.5673	1.4398
Eguti (*Termitomyces aurantiacus*)	Fresh	800	350	619	391	22.9970	1.3458
Echoroi (*Agaricus sp.*)	Fresh	900	-	678	-	11.4115	-
Otulelut (*Agaricus sp.*)	Fresh	700	-	443	-	8.2755	-
Esiara (*Tricholoma sp.*)	Fresh	300	-	183	-	5.4220	-
Oujoi (*Lepiota species*)	Fresh	350	-	144	-	2.9797	-

Key: - = Not done,Vol. =Volume of, Wt. = Weight of, M = Methanol, P = Petroleum ether, S = Sample, Ex = Extract and Trt = Treatment

Antibacterial activity for methanol mushroom extracts and respective MIC: From the Table 2a Agar Well Diffusion Method (AWDM) was found to demonstrate activity better than the Paper Disc Method (PDM). Most activities on bacteria were shown in AWDM and only one showed activity with PDM. *P. aeroginasa* was the most susceptible of the 3 bacteria and *E. coli* being the least. The Table also shows that from extracts of 11 species (both sun dried and fresh), only extracts of 3 species showed activity against *Escherichia coli* at 0.5 g mL^{-1} concentration. It also shows that from the 3 species with activity *E. coli*, only extracts of 2 species (*Echoroi (Agaricus* sp.*)* and *Otulelut (Agaricus* sp.) were active when fresh, both with DIZ of 10±00 mm. The most active sun dried mushroom methanol extract on *E. coli* was *Otulelut (Agaricus* sp*)* (23.5±0.5) followed by *Echoroi (Agaricus* sp*)* (13.5±0.5) extract and least in *Esiara (Tricholoma* sp*)* (11.5±1.5). *Otulelut (Agaricus* sp*)* showed inhibition at very low concentrations (0.016 g mL^{-1}) as compared with the other two species with MIC of 0.125 g mL^{-1}.

The Table 2a also shows that from extracts of 11 species, only extracts of 4 species did not show activity against *Staphylococcus aureus* at 0.5 g mL^{-1} concentration. It also showed that from the extracts of species with activity against *Staphylococcus aureus,* only 1 species (*Etimijaka(Agaricus* sp) was not active when fresh. The most active sun dried mushroom methanol extract on *Staphylococcus aureus* was *Otulelut [Agaricus* sp.*]* (21.5±1.0) followed by *Eburukunyu (Agaricus* sp.*), Echoroi (Agaricus* sp.*), Esiara (Tricholoma* sp.*), Eswei (Termitomyces microcapus)* and least in *Odilit (Termitomyces* sp*)* and *Opugurei [Not identified]* (7.00±00). *Otulelut (Agaricus* sp*)* showed inhibition at very low concentrations (0.016 g mL^{-1}) as compared with the other species with MIC ranging from 0.25 g mL^{-1} in fresh *Oujoi (Lepiota* sp*)* to 0.031 g mL^{-1} in sun dry *Echoroi (Agaricus* sp*)* and fresh *Eguti (Termitomyces aurantiacus).*

The Table 2a further shows that from extracts of 11 species, extracts of only 2 species did not show activity against *Pseudomonas aeroginosa* at 0.5 g mL^{-1} concentration. It also shows that from the extracts with activity against *Pseudomonas aeroginosa* activity all extracts of species studied when fresh were active. The most active sun dried mushroom methanol extract on *Pseudomonas aeroginosa* was *Otulelut (Agaricus* sp.*)* (21.5±0.5) followed by *Eburukunyu (Agaricus* sp*), Eswei (Termitomyces microcarpus), Echoroi (Agaricus* sp*), Esiara (Tricholoma* sp.*), Oujoi (Lepiota* sp.*)* and least in *Odilit (Termitomyces* sp.*)* and *Eguti [Termitomyces aurantiacus]* (9.5±0.5). Fresh samples showed best activity with *Etimijaka (Agaricus* sp*)* (13.51.5) followed by *Oujoi (Lepiota* sp*), Otulelut (Agaricus* sp.*), Echoroi (Agaricus* sp. *Esiara (Tricholoma* sp.*)* and least in *Eguti (Termitomyces aurantiacus)* (7.5±0.7). *Otulelut (Agaricus* sp.*)* (sun dried) and *Eguti (Termitomyces aurantiacus)* (fresh) showed inhibition at lower concentrations (0.031 g mL^{-1}) compared to the other species with MIC ranging from 0.25 g mL^{-1} in fresh *Oujoi (Lepiota* sp.*)* to 0.031 g mL^{-1} in sun dry *Otulelut (Agaricus* sp.*)* and fresh *Eguti (Termitomyces aurantiacus).*

From the Table 2b, Agar Well Diffusion Method (AWDM) was the only method that demonstrated activity since the Paper Disc Method (PDM) demonstrated no activity. It is also noted that *S. aureus* was the most susceptible bacteria to petroleum ether extracts of the 3 bacteria used. Only 3 mushroom extracts were active on either *E. coli* or *P. aeroginosa* each while all extracts were active on *S. aureus.* The table also shows that from extracts of 10 species (both sun dried and fresh), only 3 extracts showed activity against *Escherichia coli* activity at 0.2 g mL^{-1} concentration. The most active petroleum

Table 2a: Zones of growth inhibition (mm) showing antibacterial activity for selected methanol mushroom extracts and respective MIC on *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeroginosa* (ATCC 27853)

Extracts		*Escherichia coli*			*Staphylococcus aureus*			*Pseudomonas aeroginosa*		
sp.	Trt	AWDM	PDM	MIC	AWDM	PDM	MIC	AWDM	PDM	MIC
Eswei (*T. microcarpus*)	dry	-	-	-	8.50±1.5	-	0.125	14.0±1.0	-	0.063
Echoroi (*Agaricus sp*)	dry	13.5±0.5	-	0.125	10.5±1.5	-	0.031	13.0±1.0	-	0.125
Otulelut (*Agaricus sp*)	dry	23.5±0.5	-	0.016	21.0±1.0	7.5±0.5	0.016	21.5±0.5	-	0.031
Oujoi (*Lepiota species*)	dry	-	-	-	-	-	-	10.5±0.5	-	0.063
Esiara (*Tricholoma sp*)	dry	11.5±1.5	-	0.125	10.0±00	-	0.125	12.0±00	-	0.125
Odilit (*Termitomyces sp*	dry	-	-	-	7.00±00	-	0.05	9.50±0.5	-	0.05
Eburukunyu *Agaricus s*	dry	-	-	-	10.5±0.5	-	0.063	14.0±1.0	-	0.063
Erusurus (Not Id.)	dry	-	-	-	-	-	-	-	-	-
Eguti (*T. aurantiacus*)	dry	-	-	-	-	-	-	9.50±0.5	-	0.063
Opungrei (Not Id.)	dry	-	-	-	7.00±00	-	0.063	-	-	-
Etimijaka (*Agaricus sp*)	Fresh	-	-	-	-	-	-	13.5±1.5	-	0.125
Eguti (*T. aurantiacus*)	Fresh	-	-	-	9.50±0.5	-	0.031	7.50±0.5	-	0.031
Echoroi (*Agaricus sp*)	Fresh	10.0±00	-	0.125	9.00±00	-	0.125	10.0±1.0	-	0.125
Otulelut (*Agaricus sp*)	Fresh	10.0±00	-	0.250	9.50±0.5	-	0.063	10.0±1.0	-	0.125
Esiara (*Tricholoma sp*)	Fresh	-	-	-	12.5±0.5	-	0.125	9.50±2.5	-	0.125
Oujoi (Lepiota species)	Fresh	-	-	-	10.0±00	-	0.25	11.5±0.5	-	0.25
Gentamycin	+ve	ND	21±00	ND	ND	25±1.0	ND	ND	17±1.0	ND
Tetracyclin	+ve	ND	16±00	ND	ND	00±00	ND	ND	00±00	ND
DMSO	-ve	00±00	00±00	ND	00±00	00±00	ND	00±00	00±00	ND

Key: (-) = No inhibition, ND = Not done, Trt = Treatment, AWDM = Agar Well Diffusion Method, Paper Disc Method, MIC = Minimum inhibition concentration. Zones for growth inhibition are presented as mean ± SEM (Standard Error of Mean) and MIC in g/ml, -Ve = Negative control, +Ve = Positive control, well diameter 6mm and disc diameter 5mm, ATCC = American type culture collection, T = Termitomyces, Id. Identified.

Table 2b: Zones of growth inhibition (mm) showing antibacterial activity for selected Petroleum ether mushroom extracts and respective MIC on *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeroginosa* (ATCC 27853)

Extracts		*Escherichia coli*			*Staphylococcus aureus*			*Pseudomonas aeroginosa*		
Sp.	Trt	AWDM	PDM	MIC	AWDM	PDM	MIC	AWDM	PDM	MIC
Eswei (*T. microcarpus*)	dry	-	-	-	9.50±0.5	-	0.100	-	-	-
Echoroi (*Agaricus sp*)	dry	-	-	-	7.50±0.5	-	0.200	-	-	-
Otulelut (*Agaricus sp*)	dry	-	-	-	7.50±0.5	-	0.200	8.0±00	-	0.200
Oujoi (*Lepiota species*)	dry	-	-	-	8.00±00	-	0.020	-	-	-
Esiara (*Tricholoma sp*)	dry	-	-	-	9.50±0.5	-	0.100	-	-	-
Odilit (*Termitomyces sp*)	dry	-	-	-	9.50±0.5	-	0.100	-	-	-
Eburukunyu *Agaricus sp*	dry	10.0±00	-	0.100	8.00±00	-	0.100	7.0±00	-	0.100
Erusurus (Not Identified	dry	-	-	-	9.00±00	-	0.100	9.0±2.0	-	0.100
Eguti (*T. aurantiacus*)	dry	9.0±00	-	0.100	13.0±1.0	-	0.100	-	-	-
Opungrei (Not identified	dry	ND	ND	ND	ND	ND	ND	ND	ND	ND
Etimijaka (*Agaricus sp*)	Fresh	9.0±00	-	0.100	10.5±0.5	-	0.100	-	-	-
Eguti (*T. aurantiacus*)	Fresh	-	-	-	10.5±0.5	-	ND	-	-	-
Echoroi (*Agaricus sp*)	Fresh	ND	ND	ND	ND	ND	ND	ND	ND	ND
Otulelut (*Agaricus sp*)	Fresh	ND	ND	ND	ND	ND	ND	ND	ND	ND
Esiara (*Tricholoma sp*)	Fresh	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oujoi (Lepiota species)	Fresh	ND	ND	ND	ND	ND	ND	ND	ND	ND
Gentamycin	+ve	ND	21±00	ND	ND	25±1.0	ND	ND	17±1.0	ND
Tetracyclin	+ve	ND	16±00	ND	ND	00±00	ND	ND	00±00	ND
DMSO	-ve	00±00	00±00	ND	00±00	00±00	ND	00±00	00±00	ND

Key: (-) = No inhibition, ND = Not done, Trt = Treatment, AWDM = Agar Well Diffusion Method, Paper Disc Method, MIC = Minimum inhibition concentration. Zones for growth inhibition are presented as mean ± SEM (Standard Error of Mean) and MIC in g/ml, -Ve = Negative control, +Ve = Positive control, well diameter 6mm and disc diameter 5mm, ATCC = American type culture collection, T= Termitomyces

ether extract on *E. coli* was *Eburukunyu [Agaricus sp]* (10±00) followed by *Eguti (Termitomyces aurantiacus) and Etimijaka [Agaricus sp]* (9±00). All the three showed inhibition at lower concentrations (0.1 g mL^{-1}) as MIC.

The Table 2b also shows that from extracts of the 10 species, all showed showed activity against *Staphylococcus aureus* activity at 0.2 g mL^{-1} concentration. It also shows that from the species with activity against *Staphylococcus aureus*, all were active when fresh with both *Etimijaka (Agaricus sp)* and *Eguti (Termitomyces aurantiacus)* with DIZ of 10.5±0.5. The most active sun dried mushroom extract on *Staphylococcus aureus* was from *Eguti (Termitomyces aurantiacus)* (13±1.0) followed by *Esiara (Tricholoma* sp.*), Odilit (Termitomyces* sp.*), Eswei (Termitomyces microcarpus), Erusurus (Not identified), Eburukunyu (Agaricus* sp.*), Oujoi (Lepiota* sp.*)* and least in *Echoroi (Agaricus* sp.*)* and *Otulelut (Agaricus* sp.*)* (7.50±0.5).

Most extracts showed inhibition at lower concentrations (0.1 g mL^{-1}) except *Echoroi (Agaricus* sp.*)*, *Otulelut (Agaricus* sp.*)* and *Oujoi Lepiota* sp.*)* with MIC of 0.2 g mL^{-1}.

The Table 2b also shows that from extracts of the 10 species, extracts of only 3 species showed activity against *Pseudomonas aeroginosa* at 0.2 g mL concentration. It also showed that from these species with activity against *Pseudomonas aeroginosa*, none of the species studied when fresh was active. The most active sun dried mushroom extract on *Pseudomonas aeroginosa* was from *Erusurus(Not identified)* (9.0±2.0) followed by *Otulelut (Agaricus* sp*)* and least in *Eburukunyu (Agaricus* sp.*)* (7.00±00). *Erusurus* (Not identified) and *Eburukunyu (Agaricus* sp.*)* showed inhibition at lower concentrations (0.1 g mL^{-1}) as compared with *Otulelut (Agaricus* sp.*)* with MIC of 0.2 g mL^{-1}.

DISCUSSION

The amount of extracts obtained with use of a particular solvent would be expected to relate to the respective composition of the mushrooms, structural composition of the constituent components of the mushrooms and their functional groups. The mushroom species studied could therefore be having more methanolic compounds than for petroleum ether Table 1 since better extraction was demonstrated with the use of methanol solvent. Most mushroom extracts were bactericidal although it was not possible to demonstrate this using paper disc assays. This was probably because the paper disc retained the active compounds and did not allow them to diffuse into the Mueller Hinton Agar except for one mushroom species. In the agar well assay, the methanol extracts showed better activity (MIC) as compared to the petroleum extracts. This is probably because the Mueller Hinton agar dissolved methanol extracts better and therefore enabled easier diffusion and hence agar well assays can be better for demonstrating activity of mushroom extracts. It is also probable that the active ingredients in petroleum extracts are in very low quantities compared to the methanol extracts and therefore require use of larger quantities of the crude extracts.

The results also showed that *P. aeroginasa* was the most susceptible to methanol extract followed by *S. aureus* and *E. coli* being the least. It was also noted that *S. aureus* was the most susceptible bacteria to petroleum ether extracts of the 3 bacteria used. This is probably because the mushroom extracts had chemical compounds that inhibited the growth of both *P. aeroginosa* and *S. aureus* effectively than *E. coli*. This susceptibility difference could have also been that the compounds that could inhibit the growth of *E. coli* were in very meager quantities and therefore needed larger amounts of the crude extracts. It should also be noted that *E.coli* show differences in sensitivity patterns even within strains[14] and therefore the strain used could have been the one which is very resistant to the mushroom extracts.

The mechanisms by which microorganisms generally survive the action of antibacterial agents are poorly understood and remain debatable. This is true for *E. coli* (a gram negative bacterium) also because the resistance to both petroleum ether and methanol mushroom extracts could be attributed to genetic factors or to cell membrane permeability[15]. The fact that that *S. aureus* which is gram positive was significantly inhibited by petroleum ether extracts and contrasting results exhibited in *E. coli* and *P. aeruginosa* which are all gram negative is very interesting. This further suggests that petroleum ether extracts could be active on most gram positive bacteria. Therefore more investigations should be done using bacteria that are gram positive to find the range of activity of these mushroom extracts.

It was also noted that the methanol extracts demonstrated most activity on *P. aeruginosa* compared to the other two bacteria. This could be a better avenue to developing stronger antibiotics since most reports show that this bacterium is resistant to most natural extracts and antibiotics and yet significantly inhibited by the mushroom extracts. The result of this study therefore is contrary to earlier reports of less activity of natural products against *P. aeruginosa*[15,16].

Comparison of results in this study with the previous published results is problematic. First the composition of mushrooms and extracts is known to vary according to local climatic and environmental conditions[16]. Secondly the methods used to demonstrate antibacterial activity and the choice of test organism(s) vary between publications[16]. Although a method commonly used to screen extracts for antimicrobial activity is agar well diffusion method, the usefulness of this method is limited to generation of preliminary qualitative data only. The hydrophobic nature of some extracts prevents the uniform diffusion of these substances through the agar medium[16]. the results may differ between these methods as many factors vary between methods. These include differences in microbial growth, exposure of microorganisms to extract and the use of quantity of emulsifier. These and other elements may account the large differences in MIC's obtained by the agar and broth dilution method.

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