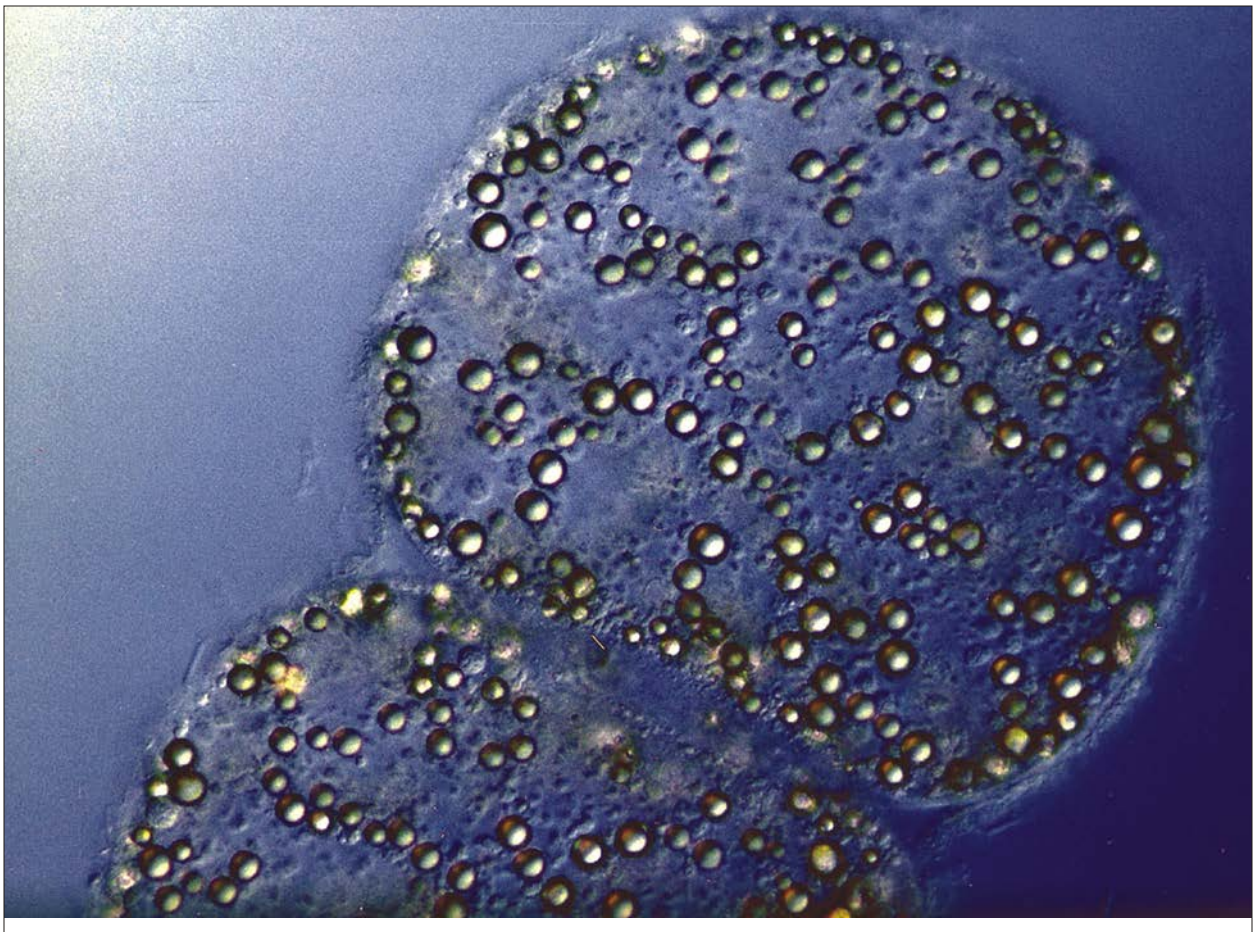

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Screening of Antimicrobial Activity of Wild Mushrooms from Khartoum State of Sudan

[1]Ehssan H.O. Moglad and [2]A.M. Saadabi
[1]Department of Microbiology and Parasitology, Medicinal and Aromatic Plants Research Institute, The National Center for Research, P.O. Box 2404, Khartoum, Sudan
[2]Department of Microbiology and Molecular Biology, Faculty of Science and Technology, Al Neelain University, P.O. Box 12702, Khartoum, Sudan

Corresponding Author: Ehssan H.O. Moglad, Department of Microbiology and Parasitology, Medicinal and Aromatic Plants Research Institute, The National Center for Research, P.O. Box 2404, Khartoum, Sudan Tel: 00249(9)22283316

ABSTRACT

A survey was conducted in Khartoum State to collect 150 samples of wild mushrooms from different areas. The identified specimens were *Agaricus bernardii*, *Agaricus arvensis*, *Agaricus bisporus*, *Agaricus porphyrocephalus*, *Agaricus silvicola*, *Coprinus comatus* and *Lepiota cristata*. Petroleum ether, ethanol and aqueous extracts of the mushrooms were screened for their antimicrobial activity against different pathogenic bacteria and fungi. These were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Aspergillus niger* and *Candida albicans* using the cup plate agar diffusion method. All of the ethanol extracts were exhibited inhibitory effects against three or more of the tested organisms. The Minimum Inhibitory Concentrations (MICs) of the most active ethanolic extracts of the six mushrooms against standard bacteria were also determined. All of the mushroom extracts were phytochemically screened and carbohydrates, reduced sugar, flavonoids, alkaloids, saponins, sterols, coumarins and triterpenes were present in all of the mushroom samples.

Key words: Antimicrobial activity, *Agaricus* sp., *Coprinus* sp., *Lepiota* sp., mushroom, phytochemicals, Sudan

INTRODUCTION

Mushrooms are useful, delicious and mysterious members of the biosphere and as such have been of interest to mankind for ages. Mushrooms have been shown to be rich sources of natural antibiotics (Kupra *et al*., 1979) and accumulate a variety of chemicals with strong anti-oxidant properties (Sun *et al*., 2007). Agrahar-Murugkar and Subbulakshmi (2005) noted that in addition to their pharmacological characteristics, mushrooms are becoming more and more important in our diet due to their high protein and low fat contents. Mushrooms are now being considered as an alternative food source to provide good nutrition for the world's growing population (Verma *et al*., 1987). Despite the need for new nutritional sources, the prevalence of drug resistant bacteria is becoming a worldwide problem with implications for treatment of patients (Donadio *et al*., 2002) and economics of health care systems (McGowan, 2001). Moreover, more effort should be made to seek antimicrobial agents effective against pathogenic microorganisms resistant to current treatment (Turkoglu *et al*., 2007). A significant amount of work has been carried out on the antimicrobial activities and chemical content of medicinal mushrooms including edible one but in

Sudan has not yet been explored. Therefore, this study seeks to identify and survey the properties of the species of wild mushrooms growing in Khartoum state. Specifically the antimicrobial potential of these mushrooms will be presented as well as phytochemical screening of the most active extracts.

MATERIALS AND METHODS

Collection and preparation of mushroom specimens: Macrofungi (*Agaricus*) specimens were collected from March to November 2010 from eight locations in Khartoum State. In order to have wide range of species as possible, specimens were collected from forests, fields and river banks. Identification of the mushrooms was done by comparing their morphological, anatomical and physiological traits with the standard description of Alexopolous *et al.* (1996). The mushrooms were dried in the shade to prevent mushroom cells from sun light which destroy the cell and ground to powder using mortar and pestle.

Extracts preparation: To perform antimicrobial activity three solvents were used for chemical extraction Petroleum ether, ethanol and water. Extraction was done by hot successive extraction in Soxhlet apparatus. For water extraction, 10 g of powder was immersed in 50 mL of hot water 60°C for 4 h before filtering. The dissolved extracts were concentrated under reduced pressure in a rotator evaporator before being transferred to petri dishes for complete evaporation of solvents.

Antimicrobial activity: The antimicrobial activities of each of the three extracts (aqueous, petroleum ether and ethanol) were tested against standard Gram positive bacteria (*Staphylococcus aureus* American Type Culture Collection, ATCC 25923), Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* National Culture Type Collection NCTC 8196) and fungi (*Aspergillus niger* ATCC 9763, *Candida albicans* ATCC 7596). The agar well diffusion method (NCCLS, 2000) was used here as it is the globally accepted testing method. Two replicates were performed for each extract against each of the tested organisms. Simultaneously, positive controls involving the addition of petroleum ether and methanol instead of the extracts were included. Upon the completion of incubation the diameter of the resultant inhibition zones were measured and tabulated as means.

Minimum inhibitory concentration (MIC): MICs were carried out according to the method described by Hirasawa *et al.* (1999). Different concentrations (2.5, 5, 10 and 20 mg mL^{-1}) were prepared using sterile distilled water as the diluents. Again, the agar well diffusion method was used. The test was carried out in duplicate and the mean recorded.

Phytochemical screening: The dried extracts were reconstituted in the solvent used for their extraction and subjected to qualitative chemical screening to identify the presence of a variety of phytoconstituents. The methods used have been described by Harborne (1998) and identified the following chemical classes: alkaloids, saponins, flavonoids, tannins, sterols, triterpenes, coumarins and cyanogenic glycosides. In addition the extracts were tested for the presence of carbohydrates and reducing sugars, again using standard tests.

RESULTS

One hundred and fifty samples of mushroom were obtained from different areas in Khartoum State and identified as *Agaricus bisporus* which was 42 samples (28%) followed by

Agaricus bernardii with 26 samples (17.3%). Then *Lepiota cristata* which was 3 samples (2%). Other species included *Agaricus arvensis*; 19 samples (12.7%), *Agaricus porphyrocephalus*; 25 samples (16.7), *Agaricus silvicola*; 5 samples (3.3%) and *Coprinus comatus*; 30 samples (20%). From the result of antimicrobial effects obtained, it could be observed that ethanol was the best solvent for extracting antimicrobial substances from these mushrooms (Table 1). This suggestion was based on the number of organisms inhibited and the diameter of inhibitory zones produced. It could also be seen from Table 1 that *Escherichia coli* the most sensitive microorganism inhibited by nine extracts (52.9%) and *Candida albicans* inhibited by seven extracts (41.2%). While *Aspergillus niger* was the most resistant organism inhibited by only two extracts (11.8%). Furthermore, all of the ethanolic extracts exhibited inhibitory activity against one or more of four bacteria i.e., *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* and two fungi (*Candida albicans* and *Aspergillus niger*) with inhibition zone of 11 to 19 mm. Ethanol extract of *Agaricus porphyrocephalus* was superior and active against all of the tested microorganisms, followed by ethanol extract of *Agaricus bisporus* and *Agaricus bernardii* which were active against all of the tested microorganisms except *Aspergillus niger* and *Proteus vulgaris* that were resistant. Ethanol extracts of *Lepiota cristata* and *Agaricus arvensis* were active against all tested organisms except *Aspergillus niger* and *Pseudomonas aeruginosa* which were resistant. The ethanol extract of *Coprinus comatus* was active against all of the tested microorganisms except *Aspergillus niger*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. Petroleum ether extracts of all mushroom species were found inactive against all of the tested microorganisms except extracts of *Agaricus porphyrocephalus*,

Table 1: Antimicrobial activity of mushrooms extracts against certain groups of microorganisms

		Test organisms used MDIZ*					
		Fungi			Bacteria		
Mushroom species	Solvent used	Ca.a	As.n	Pr.v	Ps.a	E.c	S.a
Agaricus bisporus (button mushroom)	Ethanol	18	-	-	15	14	12
	P. ether	-	-	-	-	-	-
	Water	-	-	-	-	-	-
	Ethanol	17	-	-	13	14	12
	P. ether	-	-	-	-	15	-
Agaricus bernardii	Water	-	-	-	-	-	-
Agaricus arvensis (horse mushroom)	Ethanol	15	-	12	-	13	12
	P. ether	-	-	-	-	-	-
	Water	-	-	-	-	-	-
Agaricus porphyrocephalus	Ethanol	18	19	12	11	17	11
	P. ether	-	-	-	-	17	-
	Water	12	15	-	-	-	13
Coprinus comatus (shaggy mane)	Ethanol	14	-	-	-	15	15
	P. ether	-	-	-	-	13	-
	Water	-	-	-	-	-	-
Lepiota cristata	Ethanol	15	-	18	-	16	18
	Water	-	-	-	-	-	-

S.a: *Staphylococcus aureus*, E.c: *Escherichia coli*, Ps.a: *Pseudomonas aeruginosa*, Pr.v: *Proteus vulgaris*, As.n: *Aspergillus niger* and Ca.a: *Candida albicans*. P. ether: Petroleum ether. MDIZ*: Mean diameter of growth inhibition zones, MDIZ>18: Sensitive, 14-18: Intermediate, <14: Resistant, -: No activity

Table 2: Phytochemical Screening of the different mushroom extracts

Mushroom names	Solvent	Tri	Gly	Rs	Car	Cou	Ste	Tan	Sap	Alk	Fla
Agaricus bisporus (button mushroom)	Ethanol	+	-	-	+	+	++	+	+	+	++
	P. ether	-	-	nd	nd	+-	+	nd	nd	-	+-
	Water	nd	-	+	+	-	nd	-	+	+	+
Agaricus bernardii	Ethanol	++	++	-	+	+	++	-	+	++	+
	P. ether	-	-	nd	nd	+	+	nd	nd	-	-
	Water	nd	-	+	+	+	nd	-	+	+	-
Agaricus arvensis (horse mushroom)	Ethanol	++	++	-	+	+	++	-	++	++	+
	P. ether	-	-	nd	nd	+	+	nd	nd	-	+-
	Water	nd	-	+	+	+	nd	-	+	-	-
Agaricus porphyrocephalus	Ethanol	++	++	-	+	+	++	-	++	+	+
	P. ether	-	-	nd	nd	-	+	nd	nd	-	-
	Water	nd	-	+	+	+	nd	-	+	-	-
Coprinus comatus (shaggy mane)	Ethanol	+	-	-	+	+	++	+	+	+	+
	P. ether	-	-	nd	nd	-	+	nd	nd	-	+
	Water	nd	-	+	+	+	nd	-	+	-	+
Lepiota cristata	Ethanol	+	-	-	+	+	+	+	++	++	++
	Water	nd	-	-	+	+	nd	-	+	-	-

Functional group; Fla: Flavonoids, Alk: Alkaloids, Sap: Saponins, Tan: Tannins, Ste: Sterols, Cou: Coumarins, Car: Carbohydrates, Rs: Reducing sugars, Gly: Cyanogenic glycosides, Tri: Triterpenes, P. ether: Petroleum ether, +: Positive reaction, + +: High concentration, -: Negative reaction, +-: Trace amount, nd: not done

Agaricus bernardii and *Coprinus comatus* that were found active against *Escherichia coli*. Water extracts on the other hand, were inactive against all tested bacteria and fungi except water extract of *Agaricus porphyrocephalus* mushroom which was found active against one bacterium (*Staphylococcus aureus*) and two fungi *Candida albicans* and *Aspergillus niger*.

The Minimum Inhibitory Concentrations (MICs) of the most active extracts were determined against reference organisms (*Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*) it was found that MICs a ranging between concentration 2.5-5 mg mL^{-1}.

The result of phytochemical screening, showed that carbohydrates, reduced sugar, flavonoids, alkaloids, saponins, sterols, triterpenes and coumarins were present in all of the mushrooms samples with different in concentration (Table 2).

DISCUSSION

The present study concluded that the predominant genus was *Agaricus* (117 samples). This genus was mostly found in dead decaying leave twigs and was grown in areas that contain manure of animals and close to the animals and humid places and the genus can also be found at the tropics of Africa and Asia (Singer, 1986). The genera collected were *Agaricus, Lepiota* and *Coprinus* are considered as cosmopolitan species (Singer, 1986).

Antibacterial activities showed by mushroom samples (Table 1), is due to the presence of bioactive substances in these fungi such as alkaloids, flavonoids, saponins, sterols, triterpenes, carbohydrates, reduced sugar, tannins, coumarins and cyanogenic glycosides and the solubility of those active compounds in the solvent used (Absolute ethanol). Mushrooms have been reported for its extensive use in medicine for curing variety of ailment or diseases (Stamets, 1993). The presence of bioactive substances in these mushrooms is in accordance with the work of Benedict and

Brady (1972) who reported isolation of bioactive bases from antibiotics producing mushrooms. It could also be seen from Table 1 that *Agaricus bernardii* and *Agaricus porphyrocephalus* extracts were different in their antimicrobial efficacy depending on the extractive solvent used. This result agrees favourably with the suggestion of Oloke and Kolawole (1998) that bioactive components of any medicinal plant may differ in their solubility depending on the extractive solvents used. Present study also conducted that water and petroleum ether were not good solvents of extracting mushrooms compared with the ethanol due to different in polarity.

Generally, the observed values for all extracts against fungi were low. This result supports the suggestion of Takazawa *et al.* (1982) that antifungal antibiotics are not common among basidiomycetes.

In conclusion, this study has shown that different genera of a wild mushrooms were growing in different areas throughout in Khartoum state with variable and complete difference in climatic condition and nutrition content of soil. Also this study has shown that different extracts (aqueous, petroleum ether and ethanol) have been used *in vitro* to inhibit the growth of some disease-causing bacteria and fungi. It can therefore be suggested that they are a promising antimicrobial agents and further investigation must be done to name the active compound.

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