Int.J.Curr.Res.Aca.Rev.2023; 11(5): 8-14

EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 (Online) Volume 11 Number 5 (May-2023)

Journal homepage: http://www.ijcrar.com

doi: https://doi.org/10.20546/ijcrar.2023.1105.002

Antibiotic Property of Edible and Non-Edible Wild Mushrooms from Menengai Crater in Kenya

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Abstract

Antibiotic resistance is a global challenge of our times. To avert the problem, search for alternative antibiotics from natural sources is inevitable. This study sought to extract antibiotics from edible and non-edible wild mushrooms from Menengai crater. The mushroom samples were collected in new khaki bags from Menengai crater and transported to the laboratory. The samples were dried under room temperature (25±2^{o}C). Extraction of the crude extracts was carried out using ethyl acetate, methanol, ethanol and acetone. Antibiotic bioassay was done using Kirby Bauer disc diffusion technique against *Enterococcus faecalis* (ATCC 29212), *Staphilococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 70063), *Aspergillus flavus* (ATCC 9170), *Aspergillus fumigates* (96918) and *Candida albicans* (10231). The yield of crude extract obtained using ethyl acetate, methanol, ethanol and acetone varied significantly (P=0.009). Likewise, the total phenolic content varied significantly among the extracts (P= 0.000105). However, the zones of inhibition did not vary significantly among the bacterial (P=0.98) and fungal pathogens (P=0.61). The selected mushroom samples produced bioactive compounds that inhibited growth of the selected pathogenic microorganisms. There is need to characterize the active compounds in the crude extracts obtained from the mushroom samples.

Article Info

Received: 08 March 2023
Accepted:20 April 2023
Available Online: 20 May 2023

Keywords

Antibiotic, Extracts, Menengai, Mushroom, Solvents, fatty acid content.

Introduction

Human dietary requirements are changing with changing lifestyle all over the world (New and Zin, 2020). The demand for alternative healthy sources of nutrients and medicine is on the increase. This can be partly attributed to increase in antibiotic resistance (Shen *et al.*, 2017). Microbial products are a major constituent of new drug molecules. Researchers are searching for novel antimicrobial agents of biological origin including fungi (Waithaka *et al.*, 2017).

More than 2000 species of mushroom exist in nature of which 25 are used as food while a few are commercially cultivated. They are of importance nutritionally because of their organoleptic merit, medicinal properties and economic significance (Uddin *et al.*, 2015).

Mushrooms are of high nutritional value (Rezaeian and Pourianfar, 2016). They are rich in protein and fibre while their fat content is low but with incomparable fatty acid content. Besides, they provide vitamin B complex, C, D and E (Mohamed and Farghaly, 2014). Mushrooms

have extensively been used in traditional medicine in curing intestinal disorders, bleeding, high blood pressure and various bacterial infection (Mirfat *et al.,* 2014). The Metabolites from mushroom have been shown to have antimicrobial, antioxidant and anti-inflammatory properties (Alves *et al.,* 2012). Besides, antibiotics from mushrooms have anti-obesity, anti-diabetic and anti-cancer properties (Shikongo *et al.,* 2013). Their antibiotic properties are attributed to procession of bioactive compounds such as terpenoids, flavonoids, tannins, alkaloids, tannins, alkaloids and polysaccharides (Christiane *et al.,* 2016).

Ethyl acetate, ethanol, methanol and water have previously been used in extracting antibiotics from mushrooms (Thillaimahanan *et al.,* 2016). Water is usually used because of its high polarity. The extraction capacity of water can be increased though increasing its temperature (Aina *et al.,* 2012). However, high temperature may lead to degradation of antimicrobials that are heat sensitive (Celik *et al.,* 2014). This study sought to investigate the antibiotic properties of wild mushrooms from Menengai crater.

Materials and Methods

Sample collection

Two edible (*Lactarius kabansus* and *Boletus edulis*)and two non-edible (*Coprinus comatus* and *Ganoderma lucidum*) wild mushrooms were collected from Menengai crater and transported to the Department of Biological Sciences Laboratory in Egerton University. The samples were identified using morphological characteristics and identification keys (Khan and Tania, 2012).

Extraction of crude extracts

The samples were sliced into thin strips and dried for 7d under room temperature (25±2^{o}C). The samples were separately ground to powder using an electrical grinder (Siebtechnik steel pulverizer 2, 376, GmbH). Briefly, 100g of the powder was separately mixed with 100mL of ethyl acetate, methanol, ethanol and acetone and shaken using an orbital shaker at 150 rpm for 72h (Sagar *et al.,* 2022).

The extracts were centrifuged at 3000 rpm for 15 min. Filtration was carried out using Whatman No.1 filter paper. The extracts were concentrated using a rotary evaporator at 50◦C, freeze dried and weights determined (Hussein *et al.,* 2018).

Test pathogenic Microorganisms

The bacterial test pathogens were *Enterococcus faecalis* (ATCC 29212), *Staphilococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 70063). The fungi used as test organisms were: *Aspergillus flavus* (ATCC 9170), *Aspergillus fumigates* (96918) and *Candida albicans* (10231).

Determination of total phenolic content

Total phenolic content in each mushroom extract was determined using the Folin and Ciocalteu (FC) reagent method (Padmavathy *et al.,* 2014). Briefly, 40µl of each sample was diluted to 200µl using distilled water and mixed with 200µl of Folin and Ciocalteu's phenol reagent, diluted in the ratio of 1:9 mL using distilled water. After 6 min, 200µl of 7.5% sodium carbonate was added to the mixture and adjusted to 2mL with distilled water and stored in the dark for 60 min. The absorbance was measured at 725 nm using a spectrophotometer (SpectronicR) (Deepalakshmi and Mirunalini, 2014).

Antibiotic bioassay

Antimicrobial activity of the crude extracts was carried out using Kirby Bauer disc diffusion bioassay. Briefly, each extract was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 200 mg/mL and sterilized by passage through a ChromafilXtra PTFE-45 filter. Sterile Mueller Hinton Agar, was dispensed into 90mm Petri dishes. Whatmann No:1 paper was used to prepare 6mm discs which were sterilized using a hot air oven at 150^{o}C for 1h (Woldegiorgis *et al.,* 2014). Test microorganism cultures incubated for 24h were adjusted to a cell density of 1.5x10^{8} CFU/mL as per 0.5 McFarland Standard.

A micropipette was used to dispense 100µl on Mueller Hinton agar and spread using an L shaped glass rod. The Discs were aseptically dipped into 10µl of each extract. The discs were dried under room temperature (25±2^{o}C) before placement at certain points within the Petri dish.

Distilled water was used as negative control while ampicillin 10µg for bacteria and nystatin 10µgfor fungi disc were used as positive control. The Petri dishes were stored at 4°C for 2 h to allow absorption of the extracts and then incubated at 37°C for 24 h for bacterial and 5d at 25^{o}C for fungal pathogens. The diameter of zones of inhibition were measured in millimeters.

Statistical analyses

All the statistical analyses were carried out using SPSS version 25.0 software. All values are expressed as mean + SD of three parallel measurements.

Results and Discussion

Yield of extracts

The percentage yield in ethyl acetate varied from4.7±0.01 to 6.5±0.02, methanol (2.5±0.03-3.4±0.02), ethanol (3.0±0.03-3.5±0.03) and acetone (4.1±0.02-4.7±0.03) (Table 1). The crude extracts yield obtained from the mushroom samples using the selected extracts varied significantly (P=0.009).

Total phenolic content

The total phenolic content of the crude extracts obtained using ethyl acetate ranged from 75±0.01 to 87±0.03 mg GAE/ 100g, methanol (47±0.01-69±0.02 mg GAE/ 100g), ethanol (45±0.02- 58±0.01 mg GAE/ 100g) and acetone (77±0.02- 86±0.02 mg GAE/ 100g) (Table 2). The total phenolic content in the extracts varied significantly (P= 0.000105).

Growth inhibition of bacterial pathogens

Growth inhibition of *Enterococcus faecalis* varied from 13±0.01-19±0.02 mm, *Staphylococcus aureus* (13±0.01-19±0.01mm), *Pseudomonas aeruginosa* (10±0.02-15±0.03mm) and *Klebsiella pneumoniae* (10±0.01-15±0.03mm) (Table 3). However, the zones of inhibition did not vary significantly (P=0.98) among the test bacterial pathogens.

Growth inhibition of fungal pathogens

The zones of inhibition in *Aspergillus flavus* ranged from10±0.01 to 13±0.03mm, *Aspergillus fumigates* (10±0.03-15±0.01mm) and *Candida albicans* (14±0.03-18±0.01mm). The zones of inhibition obtained did not vary significantly (P=0.61) between *Aspergillus flavus*, *Aspergillus fumigates* and *Candida albicans.* However, the zones of inhibition obtained in *Candida albicans* were higher than in *Aspergillus flavus* and *Aspergillus fumigatus.*

The crude extracts obtained using ethyl acetate was higher than in methanol, ethanol and acetone. This may be attributed to differences in polarity of the solvents (Chowdhury *et al.,* 2015). A previous study on Antioxidant profile of four selected wild edible mushrooms in Nigeria obtained similar results (Ejelonu *et al.,* 2013). However, the % yield of the crude extracts in the present study was higher than in a study carried out by Özcan and Ertan, (2018). The difference could be attributed to differences in the environmental conditions in which the samples were growing (Pathirage and Yunman, 2011).

In addition, non-edible (*Coprinus comatus* and *Ganoderma lucidum*) mushrooms produced higher crude extracts than the edible (*Lactarius kabansus* and *Boletus edulis*) mushrooms. This concurred with a study on antimicrobial activity of crude ethanolic extracts from some medicinal mushrooms carried out by Lukáš *et al.*, (2016). Differences in biosynthesis pathways between the edible and non-edible mushrooms may have contributed to the results (Gan *et al.,* 2013).

The results on total phenolic content obtained in the current study were higher than in a previous study carried out by Sun *et al.,* (2014). The difference could be attributed to the geographical conditions in which the sample mushrooms were obtained (Gan *et al.,* 2013).

According to Sagar *et al.,* (2015), the temperature of the area in which mushrooms grow significantly determines their level of crude extracts. In addition, Asri *et al.*, (2019) maintained that the type of substrate on which mushrooms grow determines their phenolic content.

The zones of inhibition of the selected bacterial pathogens by crude extracts from the sampled mushroom obtained in the current study were larger than those obtained in a previous study by Chowdhury *et al.*, (2015). Differences in the bioactive compounds present in the crude extracts could be a contributing factor (Geethangili *et al.,* 2013). The zones of inhibition obtained in the Gram positive bacteria were bigger than those from Gram negative bacteria. Roy *et al.,* (2016) maintained that differences in the structure of the cellular components in bacterial pathogens result in larger zones of inhibition in Gram positive than in Gram negative bacteria.

Table.1 Yield (%) of the crude extracts from the mushroom samples

Mushroom	**Solvent**			
	Ethyl acetate	Methanol	Ethanol	Acetone
Lactarius kabansus	5.7±0.02	2.7±0.01	3.0±0.03	4.3±0.01
Boletus edulis	4.7±0.01	2.5±0.03	3.2±0.01	4.1±0.02
Coprinus comatus	6.0±0.03	3.4±0.02	3.5±0.03	4.7±0.03
Ganoderma lucidum	6.5±0.02	3.2±0.01	3.4±0.02	4.6±0.01

Table.2 Total phenolic content (mg GAE/ 100 g) of mushrooms extracted using different solvents.

Mushroom extract	**Total phenolic content**			
	Ethyl acetate	Methanol	Ethanol	Acetone
Lactarius kabansus	78±0.01	59±0.02	47±0.03	77±0.02
Boletus edulis	75±0.01	47±0.01	45±0.02	79±0.01
Coprinus comatus	90±0.02	67±0.01	57±0.01	86±0.02
Ganoderma lucidum	87±0.03	69±0.02	58±0.01	85±0.01

Table.3 Zones of inhibition (mm) of the test bacterial pathogens by the crude extracts from the selected mushroom samples

Pathogen	**Zone of inhibition**			
Enterococcus faecalis	**Ethyl acetate**	**Methanol**	**Ethanol**	**Acetone**
Lactarius kabansus	14±0.01	14±0.02	13±0.03	15±0.02
Boletus edulis	15±0.01	13±0.01	15±0.02	13±0.01
Coprinus comatus	18±0.02	17±0.01	17±0.01	17±0.02
Ganoderma lucidum	17±0.03	19±0.02	18±0.01	19±0.01
Staphylococcus aureus				
Lactarius kabansus	13±0.01	15±0.02	14±0.03	13±0.03
Boletus edulis	14±0.02	13±0.02	15±0.02	14±0.02
Coprinus comatus	17±0.02	18±0.01	15±0.01	16±0.02
Ganoderma lucidum	16±0.03	19±0.01	17±0.02	18±0.02
Pseudomonas aeruginosa				
Lactarius kabansus	10±0.02	12±0.01	11±0.03	10±0.02
Boletus edulis	11±0.01	10±0.02	10±0.02	12±0.01
Coprinus comatus	13±0.02	14±0.01	15±0.03	14±0.01
Ganoderma lucidum	15±0.01	14±0.01	13±0.01	13±0.02
Klebsiella pneumoniae				
Lactarius kabansus	12±0.03	10±0.02	11±0.01	10±0.03
Boletus edulis	10±0.01	11±0.02	11±0.01	12±0.01
Coprinus comatus	14±0.02	15±0.01	15±0.03	14±0.02
Ganoderma lucidum	13±0.01	14±0.01	13±0.02	15±0.01

Table.4 Zones of inhibition (mm) of the test fungal pathogens by the crude extracts from the selected mushroom samples

Pathogen	Zone of inhibition			
Aspergillus flavus	**Ethyl acetate**	**Methanol**	**Ethanol**	**Acetone**
Lactarius kabansus	11±0.02	13±0.03	12±0.01	13±0.03
Boletus edulis	12±0.01	10±0.01	13±0.03	11±0.01
Coprinus comatus	10±0.01	11±0.02	11±0.03	10±0.02
Ganoderma lucidum	13±0.03	12±0.01	12±0.02	12±0.01
Aspergillus fumigatus				
Lactarius kabansus	13±0.02	10±0.03	12±0.02	11±0.01
Boletus edulis	14±0.03	12±0.02	11±0.01	13±0.02
Coprinus comatus	15±0.01	11±0.02	13±0.02	12±0.01
Ganoderma lucidum	14±0.01	13±0.01	11±0.01	14±0.01
Candida albicans				
Lactarius kabansus	18±0.01	15±0.02	17±0.03	18±0.01
Boletus edulis	16±0.02	14±0.03	18±0.01	17±0.02
Coprinus comatus	16±0.01	16±0.01	17±0.02	16±0.02
Ganoderma lucidum	17±0.03	16±0.02	16±0.02	18±0.01

Candida albicans presented larger zones of inhibition than *Aspergillus flavus* and *Aspergillus fumigatus.* Nedelkoska *et al.,* (2013) asserted than moulds are more resistant to crude extracts than in unicellular fungi. Besides, the ability of moulds to produce resistant spores accords them the ability to resist inhibition by crude extracts (Tsungai *et al.,* 2016).

The results of the present study concurred with previous studies carried out elsewhere (Hleba *et al.,* 2016; Reid *et al.,* 2016; Roy *et al.,* 2016). This may be attributed to production of similar active ingredients by the sample mushrooms (Krishnaveni and Manikandan, 2014).

Ethyl acetate, methanol, ethanol and acetone extracted bioactive materials from the selected mushroom samples. Ethyl acetate was the best solvent for recovering crude extracts from mushroom samples. Polarity of the solvent is key in the extraction process. The crude extracts from the mushroom samples contained phenolic compounds. In addition, the crude extracts inhibited growth of the selected bacterial and fungal pathogens

Recommendations

There is need to characterize the crude extracts from the selected mushroom samples. The crude extracts obtained in this study need to be tested against other microbial pathogens. Extraction of crude extracts from other mushroom samples need to be carried out.

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How to cite this article:

Paul Njenga Waithaka and Githaiga Benson Muriuki. 2023. Antibiotic Property of Edible and Non-Edible Wild Mushrooms from Menengai Crater in Kenya. *Int.J.Curr.Res.Aca.Rev.* 11(05), 8-14.
doi: https://doi.org/10.20546/ijcrar.2023.1105.002