

# Ethno Mycological Survey and Biological Activities of Mushroom Extracts Against *Escherichia Coli* and *Staphylococcus Epidermidis* From Papua New Guinea

John K Nema<sup>1</sup>, Stewart Wossa<sup>2</sup>, Edwin Costillo<sup>2</sup>, Basil Marasinghe<sup>1</sup>, Jeyarathan Pooranalingam<sup>3</sup> and Russell Barrow<sup>2</sup>

1. School of Science, University of Goroka, Papua New Guinea.

2. Research School of Chemistry, Australian National University, Australia.

3. GAJ Medical Pty Ltd, New South Wales, Australia.

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**Abstract:** An ethno - mycological survey of more than 60 edible and non edible mushroom species was undertaken in the Siane area of Simbu Province in Papua New Guinea to establish the scientific merits of the ethno mycological knowledge of the Waefo tribe. Ethno mycological information obtained was used as a guide to prepare mushroom extracts of the *Polyporaceae* family. The concentrated mushroom extracts were tested for their antibacterial properties against *Escherichia coli* and *Staphylococcus epidermidis* using a 96 well plate method at the Research School of Chemistry Australian National University. The results showed that the extracts (*KMsp001* and *KMsp002*) were proven to be moderately active against *S.epidermidis* with a growth inhibition of 34 and 47 percent respectively and *KMSp 002* was also active against *E.coli* with growth inhibition of 26 percent.

**Keywords:** Ethno-mycology, growth inhibition, Polypore extracts, *S.epidermidis*, *E.coli*

## 1. Introduction

Papua New Guinea (PNG) has a diverse culture. Associated with such unique cultural backgrounds, indigenous tribal people used their traditional knowledge to identify materials of fungal origins that were used as traditional medicine to heal diseases and as food for a long time. Our recent study is focused on traditional knowledge (ethnology) guided collection and extraction of bioactive chemical constituents from fungal fruiting bodies (mushrooms). Mushrooms are scientifically classified under the Fungi Kingdom and in the phylum Basidiomycota. Chang and Miles [1] defined mushrooms as macro-fungus with a distinctive fruiting body which can be hypogeous (grows underground) or epigeous (grows above the ground) and large enough to be seen by the eye and picked by hand. The crude extracts containing secondary metabolites from the mushrooms were tested against *S.epidermidis* and *E.coli* to establish their antibacterial activities.

Mycological research on antibacterial properties of polypores was done in many parts of the world including USA [2], India [3] and PNG on taxonomic details [4]. The recent work on antibacterial activities of PNG mushrooms was done

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### Corresponding author:

John K Nema, School of Science, University of Goroka, Papua New Guinea. Email: nemaj@uog.ac.pg.

by Wossa *et al.*, [5]. They have reported bioactive compounds from Bankeraceae family and a more recent report on Amanitaceae family [6] and our research has reported the presence of bioactive compounds in Polyporaceae family.

## **2. Materials & Methods**

### **2.1 Mushroom Ethnology**

The established mushroom team from the local village who are between the ages of 50 to 80 years were interviewed for their ethno-mycological knowledge. Sample collection was done in collaboration with the focus group whose ethno-mycological knowledge was documented and fully utilized as guide during this study. All ethno-mycological information in the field were recorded using an interview questionnaire and mushroom sample descriptions in the field were also recorded using field data instrument for mushroom-like fungi developed by the UOG – ANU mushroom team.

#### **2.11 Sample Collection**

Mushroom samples were collected from the local forest at a GPS reference point of 145.17722°E and 6.17366°S and extends further to 145.17673°E and 6.16499°S during fruiting season and were assigned special and unique collection codes (KMsp001 & KMsp002). The samples were weighed to constant wet weight of 500 – 1kg of the same species for extraction.

#### **2.12 Sample Identification**

The mushroom fruit bodies were collected and identified using a guide by Neale and Syme [7] which provides general details of identification tools according to certain important features. It includes features like gill attachment to stem, spore colors under microscope, spore print color, volva and ring attachments to the stipe. This guide was used to identify the mushroom family and genera. Internet was also used to compliment the identification tools.

### **2.2 Extraction and Concentration of Secondary Metabolites**

#### **2.21 Extraction of Secondary Metabolites**

The fruiting bodies of mushrooms (about 500 -1kg) of the same species were milled into homogenous composition and pooled together in a one Liter (1L) screw capped container. The chemical constituents (secondary metabolites) were extracted using ethanol (EtOH) and dichloromethane (DCM) in the ratio of 4:1 (EtOH:DCM) and the solvents were allowed to percolate for (24 - 48 hours) before filtration.

#### **2.22 Filtration and Concentration of the Extracts**

The crude extracts were filtrated through gravity filtration by using a filter funnel. The crude extracts were filtered into a screw capped container and concentrated at 35°C using a rotary evaporator rotating at 60 revolutions per minute (rpm). 2ml of ethanol was added to the flask before the concentrated extracts were transferred into a small screw capped container for further analytical work to follow.

### **2.3 Antimicrobial Assays**

The antimicrobial activities of the crude extracts were pursued at the Australian National University (ANU) using MTT turbidity test method on *E.coli* and *S.epidermidis* from the culture collection of Research School of Chemistry at ANU.

## 2.4 MTT Assay

The freshly prepared Mueller-Hinton broth (MHB) was used as the bacterial growth medium. The inoculum was prepared in 2ml saline solution and was adjusted to 0.5 McFarland standard and diluted in the ratio 1:100. 100 $\mu$ L was used as the inoculum. Hundred micro liter of Mueller Hinton broth was dispensed into each of the sterile 96 well plates and serial two fold dilutions of each extracts (KMsp001 & KMsp002) were prepared directly on the plate by adding 100 $\mu$ L of the working solutions of each extracts to achieve the final concentration. Then 100 $\mu$ L of the inoculum was added to each well. A growth control (MHB + Bac) containing no extract and a sterile control without inoculum were also included for each extract. Kanamycin was also included to compare the activity of the extracts against the bacteria's (*E.coli* and *S. epidermidis*).

10 $\mu$ L of MTT solution (5g/L) was added into each well and the plates were covered and replaced into their original plastic bags and incubated at 37°C for 18 - 24hrs. The change in color from pale yellow to deep blue suggests no activity for the test extract as this is an indication of bacterial growth. The tetrazolium salt is reduced to formazan which results in this color. No change in color indicates some activity for the test extracts and the Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of each extract that prevented that change in colour. Minimum Inhibitory Concentration (MIC) was specified using an epoch UV Vis micro plate spectrophotometer.

## 3. Results & Discussion

### 3.1 Ethno-Mycological Details of the Mushroom Samples

This mushroom sample from the Polyporaceae family is edible and was enjoyed by the indigenous Waefo tribe for a long time. It is mainly found growing on dead logs and was traditionally cooked in wooden drums with greens. The traditional name (*Holipa lua hefola*) was related to the substrate on which it grows and the size of the fruiting body.



Figure 1: [A] The polypore in its natural environment. [B] The polypore with its unique code.

This polypore is non edible and the name (*Yomba lua muliti*) was related to the substrate on which it grows. It has no specific traditional uses and is mainly found growing on dead logs or fallen logs.

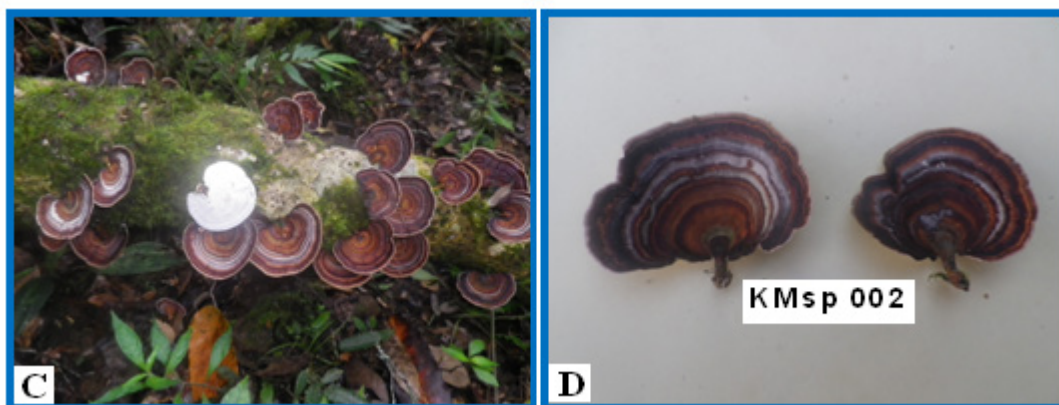


Figure 2: [C] Polypore in its natural environment. [D] The polypore with its unique code.

### 3.2 MTT Turbidity Test for *E.coli*

The positive control inhibits 100 percent of the bacterial growth at 210.53mg/mL. It would therefore be misleading to compare the activity of the mushroom extracts against kanamycin. This leads to a proposed activity range of the percentage inhibition of the mushroom extracts on bacterial growth. The proposed percentage inhibition range of the mushroom extracts was:

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1– 25%	-	(low activity)
26– 45%	-	(moderate activity)
46 – 60%	-	(high activity)
61 – 100%	-	(Very high activity)

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Both mushroom samples (KMsp001 & KMsp002) were tested against *E.coli* in duplicates for their antibacterial activity. Kanamycin was used as the positive control and a growth and sterile control were also included. KMsp002 showed activity against *E.coli*. The range of concentration tested was 15 – 0.12 mg/mL for KMsp002. KMsp001 was not active against *E.coli*.

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**Table 1** Percentage bacterial growth for KMsp002 tested after 18hrs incubation

Plate 1[A]	Kanamycin	Kanamycin (1000mg/mL)	KMsp002	KMsp002 (15mg/ml)	Growth	Sterile
Initial turbidity	0.049 0.048		0.894 1.035		0.06	0.037
Average turbidity (600nm)		210.53 0.0485 (OD)		15.00 0.9645(OD)	0.0500	0.0423
Final turbidity	0.048 0.048		1.094 1.247		0.329	0.043
Average turbidity (600nm)		210.53 0.0480 (OD)		15.00 1.1705 (OD)	0.3285	0.0399
% growth		0		74		
% inhibition		100		26		

\*tur = turbidity, \*OD = optical density

**3.21 Minimum Inhibitory Concentration (IC<sub>50</sub>) and Percentage Inhibition of the Bioactive Component**

The results of the MTT assay for the extracts on E.coli showed that KMsp002 showed activity at MIC of 15µg/mL. The percentage inhibition for KMsp002 was 26% (Table 1). This is within 26-45 percent activity range. This sample is therefore moderately active against E.coli and the IC<sub>50</sub> was calculated to be 18µg/mL.

**3.3 MTT turbidity test for S.epidermidis**

The samples (KMsp001 & KMsp002) were tested against S.epidermidis in duplicates for their antibacterial activity. Kanamycin was used as the positive control and a growth and sterile control were also included. Both samples showed activity against S.epidermidis. The range of concentrations tested were 25 - 0.20 mg/mL for KMsp001 and 15 - 0.12mg/mL for KMsp002.

**Table 2** Percentage bacterial growth for KMsp001 tested after 18hrs incubation

Plate 1[A]	Kanamycin	Kanamycin (1000mg/mL)	KMsp001	KMsp001 (15mg/ml)	Growth	Sterile
Initial turbidity	0.048 0.049		0.417 0.56		0.047	0.037
Average tur (600nm)		210.53 0.0485 (OD)		25.00 0.4885(OD)	0.0479	0.0385
Final tur	0.05 0.049		0.776 0.662		0.277	0.042
Average tur (600nm)		210.53 0.0495 (OD)		25.00 0.7190 (OD)	0.3989	0.0379
% growth		0		66		
% inhibition		100		34		

\*tur = turbidity, \*OD = optical density

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**Table 3** Percentage bacterial growth for KMsp002 tested after 18hrs incubation

Plate 1[A]	Kanamycin	Kanamycin (1000mg/mL)	KMsp002	KMsp002 (15mg/ml)	Growth	Sterile
Initial tur	0.048 0.049		1.436 1.412		0.047	0.037
Average tur (600nm)		210.53 0.0485 (OD)		15.00 1.4240(OD)	0.0479	0.0385
Final tur	0.05 0.049		1.618 1.605		0.277	0.042
Average tur (600nm)		210.53 0.0495 (OD)		15.00 1.6115 (OD)	0.3989	0.0379
% growth		0		53		
% inhibition		100		47		

\*tur = turbidity, \*OD = optical density

### 3.31 Minimum Inhibitory Concentration (IC<sub>50</sub>) and Percentage Inhibition of the Bioactive Component

The results of the MTT assay for the extracts on *S.epidermidis* showed that two extracts showed activity at MIC of 25µg/mL for KMsp001 and 15µg/mL for KMsp002. The percentage inhibition for KMsp 001 was 34% (Table 2) and KMsp002 was 47% (Table 3). The samples therefore have moderate and high activity against *S.epidermidis*. The IC<sub>50</sub> was calculated to be 28µg/mL and 15µg/mL respectively.

## 4. Conclusion

In conclusion, the forest area is estimated to host more than 80 to 100 mushroom species of which ethno-mycological details of 40 species are fully documented together with their morphological characters and the antibacterial properties of 12 samples evaluated. Three of the samples that showed activity against the test bacteria (*S.epidermidis* and *E.coli*) are reported here. Research into wild mushrooms apart from those that have traditional medicinal practices should be encouraged to isolating and identifying novel bioactive compounds as highlighted from this study. Further structural elucidative and characterization work deserves attention.

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