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Antagonistic potential of *Coriolopsis* species extracts on *Escherichia coli* isolated from well water in Akure, Nigeria

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ABSTRACT

The current study evaluates the inhibitory effect of extracts of *Coriolopsis* sp., a wild macro fungus, on *E. coli* obtained from well water. A total of fifty well water samples were collected from different parts of Akure metropolis in South West, Nigeria. Fifteen of the samples were positive for *Escherichia coli*. Ethanolic extract obtained from *Coriolopsis* sp (CET) displayed a significant inhibitory effect on *E. coli* from well water at concentration of 100mg/ml. The zones of inhibition range between 1.5 to 13mm. However, ethyl acetate and petroleum extracts (CEA and CPE) do no show any inhibitory effect at concentrations of 12.5mg/ml to 100mg/ml. Fractions obtained from CET were able to inhibit the growth of *E. coli* with zones of inhibition in the range of 12 to 27mm. The study revealed that extracts of *Coriolopsis* species contains bioactive compounds that can serve as alternative in combating multi-drug resistant *E.coli*.

KEYWORDS

Inhibition;
Extracts;
Coriolopsis;
Well;
Water.

INTRODUCTION

In the last three decades, there has been a renewed search for new sources of biopharmaceutical to serve as alternative to synthetic antibiotics because of the increasing phenomenon of antibiotic resistance. Cases of gastroenteritis as a result of bacterial etiological agents are usually treated with antibiotics. The emergence of multi-resistant bacteria has become a problem for the treatment of infectious diseases[1]. This is because microorganisms have developed versatile resistance mechanisms to toxic effects of antibiotics as well as other drugs through mutation[2,3]. Several reports worldwide show that bacterial isolates are resistant to antibiotics, particularly in developing countries[4,5]. The major mechanism by which bacteria become resistant to antibiotics is through acquisition of resistant plasmid (R-plasmid). Microbial infection can be acquired mainly through food and water.

Water supply in Akure, Nigeria is mainly from well. Previous report shows that well water in Akure metropolis contains *E. coli* which is above WHO recommended level of microorganisms in water[6]. The organisms commonly used as indicators of microbial pollution are *Escherichia coli* and the coliform groups as a whole[7]. Most strains of *E. coli* live as commensals; many are opportunistic pathogens of humans and animals. *E. coli* and several related species of bacteria found in faeces can be the cause of serious infections of the gastrointestinal tract[8]. Non-intestinal *E. coli* such

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as enterotoxigenic, may cause traveler's diarrhea; enteroinvasive, resembling shigellosis, or enteropathogenic, which cause infant diarrhea[8]. A previous report by Oyetayo *et al*.[6] revealed that *E. coli* isolated from well water in Akure metropolis displayed multiple resistances to synthetic antibiotics. This therefore necessitates a search for alternative source of antibiotics to treat waterborne infections as a result of bacterial etiological agents.

Macrofungi had received attention because they need antibacterial and antifungal compounds to survive in their natural environment. Hence, they may be rich sources of natural antibiotics. Both fruiting body and the mycelium contain compounds with wide-ranging antimicrobial activity. Preliminary screening of some polypores had revealed significant antimicrobial effect. Suay *et al*.[9] reported that extracts of more than 75% polypores mushroom species surveyed showed antimicrobial activity and 45% of 204 mushroom species inhibited wide variety of microorganisms. Moreover, the inhibition of the growth of methicillin-resistant *Staphylococcus aureus* (super bug) and other bacteria by new sesquiterpenoid hydroquinones produced by European *Ganoderma* species *Ganoderma pfeifferi* Bres. had been reported[10].

It has been estimated that the number of mushrooms on earth is about 140,000 yet only 14,000 (10%) are known[11]. A large number of the unknown species of mushrooms may be in Africa and probably species whose antimicrobial property had not been reported are more abundant in Nigeria. The present study therefore aimed at assessing the antimicrobial effect of extracts of *Coriolopsis* species, a common polypore found in Nigeria, on *E. coli* isolated from well water.

MATERIALS AND METHODS

Fungal material

Fruit bodies of *Coriolopsis* species were collected in the forest around Federal University of Technology Akure, Nigeria (Lat. 07º 14¹N Long. 05º 11¹E). The fruit bodies were morphologically identified macroscopically. The identity of the macrofungus, *Coriolopsis* species was further confirmed by molecular methods at The Key Laboratory of Systematic Mycology and Lichenology, Institute of Microbiology Chinese Academy of Sciences, Beijing 100101, People's Republic of China. The Internal Transcribed Spacer (ITS4 and ITS5) of nuclear ribosomal DNA (nrDNA) was amplified and sequenced. The sequence of the DNA fragment was compared with data obtained from NCBI GenBank. Its closest relative was *Coriolopsis polymyza* (EJ627247.1). The percentage level of relationship was 98%.

Preparation of *Coriolopsis* species extracts

Dried samples of *Coriolopsis* species was ground into a fine powder with an electric mill. The bioactive components were extracted sequentially from non-polar to polar solvents by using petroleum ether, ethyl acetate and ethanol. The extraction by all solvents was performed in Erlenmeyer flasks shaken on an orbital shaker at 300 rpm and at room temperature for 48 h. The extracts obtained from the extracting medium were dried to constant weight in a SUN LAB proctector laboratory hood overnight to a constant weight. The extracts were designated CPE (Petroleum ether extract), CEA (ethyl acetate extract) and CET (Ethanol extract). Fractions of CET were obtained by eluting it through chromatographic column using different solvents.

Isolation and Characterization of *Escherichia coli* Sourced from Well Water

A total of 50 water samples were randomly collected from wells in Akure metropolis. Total microbial load was assessed by plating dilutions of well water on Nutrient agar (LAB M) while *E. coli* count was assessed by plating aliquot of water on Eosin methylene blue (EMB) agar (LAB M). Positive colonies with golden green metallic sheen were further characterized using standard morphological and biochemical characteristics.

Antimicrobial activity

Antimicrobial activity of extracts was determined by the agar well diffusion method [12]. *E. coli* isolated from well water were cultivated on nutrient agar medium at 36 ± 1ºC for 24 hour. Aliquot of culture (100 ìl) was evenly spread on the surface of the solidified agar. Wells of 7 mm were bored in the agar with sterile cork borers. Concentrations (100mg/ml) of extracts were prepared respectively for CPE, CEA and CET using the extraction solvents. The extracts were filter sterilized through 0.22 ìm membrane and was introduced into the wells already seeded with *E. coli* isolated from well water. The plates were incubated at 36 ± 1ºC for

24 hour. Tetracycline and ampicillin were used as standard antibacterial agents while the extracting solvents serve as negative control.

Minimum inhibitory concentration

Dilutions of extracts (CEA, CPE and CET) ranging from 12.5 mg/mL to 100 mg/mL were prepared. The agar diffusion method described above was used to screen the antimicrobial effect of the different concentrations of extracts. Agar well in which the extracting solvent was added served as negative control. The tests were performed in duplicates.

RESULTS AND DISCUSSION

Natural antibiotics in clinical use were developed from fungal and actinomycetes metabolites. However, in the last 2 to 3 decades several pathogenic microorganisms had developed resistance to available antibiotics. This then necessitate the search for new antibiotic with novel mechanism of action. Attention of researchers had been turned to macrofungi. This is because they are perceived to produce antibacterial and antifungal compounds which help them to survive in their natural environment[13]. Hence, they are rich sources of natural antibiotics. Both fruiting body and the mycelium contain compounds with wide-ranging antimicrobial activity. Pharmacological potentials of wild macrofungi have remained untapped[14].

In the present study, inhibitory potential of extracts obtained from a macrofungus, *Coriolopsis* species on

TABLE 1: Bacteria count in water samples

Well water samples	Total microbial count (cfu/ml)*	Total *E. coli* count (cfu/ml)*	Well water samples	Total microbial count (cfu/ml)*	Total *E. coli* count (cfu/ml)*
Ajipowo	120	21	Ayedun	42	-
Ajegboro	68	15	Aba	181	-
Danjuma junction	142	19	Oke ogba	143	-
Peace avenue	135	26	Irowo	98	-
Ayetutu	85	30	Alaba avenue	72	-
Arakale	225	80	Ijemikin	114	-
Ondo road	157	-	Odopetu	68	13
Odi olowo	152	-	Adesida road	155	-
Orita obele	195	-	Ijomu	92	-
Igboyegun	98	-	Apatapiti	130	-
Oyemekun	165	45	Aratusin	265	-
Isinkan	74	12	Iyange	132	-
Obe street	112	-	Unity quarters	23	-
Olotu avenue	135	18	Adebayo avenue	252	46
Oke aro	95	29	Oluwafemi quarters	65	-
Adebobajo avenue	72	-	Odokoyi	182	34
Ilesa garage	56	-	Leo junction	187	-
Adegbola junction	103	-	Osokoti	130	-
Old garage	28	-	Kajola	88	-
Odo ile	42	-	Okejebu	71	-
Fanibi avenue	238	-	Okuta elerinla	60	-
Gbogi	51	-	Oja oba	136	16
Aule	47	-	Ajebamidele	24	-
FUTA junction	163	-	Alakunre	114	-
Ijoka	196	-	Ijare road	107	21

***Values are means of replicates (n=2)**

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TABLE 2 : Zone of inhibition (mm)* of *Coriolopsis* species extracts (100mg/ml) against E .*coli* isolated from well water

Location of well where *E. coli* was isolated	CET	CEA	CPE	Tetracycline (30µg/ml)	Ampicillin (40µg/ml)
Ayetutu	3.0	-	-	-	3.5
Danjuma	2.0	-	-	-	-
Ajipowo	6.0	-	-	4.3	-
Okearo	2.5	-	-	-	-
Oyemekun	1.5	-	-	3.0	3.0
Adebayo avenue	2.0	-	-	-	-
Ijare road	6.5	-	-	5.3	-
Ojaoba	4.0	-	-	6.7	-
Odokoyi	2.0	-	-	-	-
Odopetu	3.0	-	-	-	-
Isinkan	13.0	-	-	-	-
Olotu	3.5	-	-	6.0	-
Arakale	10.0	-	-	1.0	-
Ajegboro	1.5	-	-	-	-
Peace avenue	-	-	-	6.0	-

***Values are means of replicates (n=2) : No inhibition**
CET: Ethanolic extract of *Coriolopsis species*; CPE: Petroleum extract of *Coriolopsis species* and CEA: Ethyl acetate extract of of *Coriolopsis species*.

TABLE 3 : Minimum inhibitory concentration of ethanolic extract of *Coriolopsis* species against E .*coli* from well water

E. coli from locations of well	MIC (mg/ml)
Ayetutu	50
Danjuma	12.5
Ajipowo	50
Okearo	100
Oyemekun	100
Adebayo avenue	50
Ijare road	12.5
Ojaoba	12.5
Odokoyi	12.5
Odopetu	12.5
Isinkan	50
Olotu	25
Arakale	100
Ajegboro	12.5
Peace avenue	-

***Values are means of replicates (n=2)**

E. coli isolated from well water was investigated. The results of the total microbial and *E. coli* counts of Well water showed that not all the water samples collected from Akure contain *E. coli* (TABLE 1). The antibiotic sensitivity test (TABLE 2) revealed that *E. coli* isolated from different Wells vary in their sensitivity pattern to the various antibiotics. In TABLE 2, almost all the *E. coli* isolates exhibited resistance to Ampicillin (AMP) while few of the isolates show resistance to Tetracycline (TRA). Ethanol extract (CET) was the only extract that inhibited *E. coli* isolated from Well water while Ethyl acetate extract (CEA) and Petroleum ether extract (CPE) had no effect at 100mg/ml (TABLE 2). In a previous study, ethanol was observed as the best solvent for extracting antimicrobial substances from two wild edible mushrooms: *Lycoperdon pusilum* and *L. giganteum*[15]. It was also observed that the *E. coli* obtained from Ayetutu which was resistant to all the antibiotics used was inhibited by CET (TABLE 2). The minimum inhibitory concentration (MIC) of CET on *E. coli* isolated from Well water is also presented in TABLE 3. Most of the *E. coli* isolates were inhibited at concentration of 12.5mg/ml except *E. coli* from Peace Avenue that showed resistance at 100mg/ml concentration. TABLE 4 revealed that the different fractions of *Coriolopsis* species were more effective against *E. coli* and the fraction E4:1 eluted by combination of Ethyl acetate and ethanol in ratio 4:1 had the highest zone of inhibition (27mm).

TABLE 4 : Inhibitory effect of fractions of CET extract against *E. coli*.

Solvent used	Fractions	Zones of inhibition (mm)
Petroleum ether	P	12
Petroleum ether and ethyl acetate	P4: 1	16
Ethyl acetate	E	13
Ethyl acetate and ethanol	E 1:1	19
Ethyl acetate and ethanol	E 4:1	27
Ethanol	E	14

***Values are means of replicates (n=2); P- Petroleum ether fractions; P4:1- Petroleum ether and ether acetate in ratio 4:1 fractions; E – Ethyl acetate fractions; E1:1 - Ethyl acetate and ethanol in ratio 1: 1fractions; E4:1 - Ethyl acetate and ethanol in ratio 4:1 fractions; E- Ethanolic fractions**

This study shows that bioactive compounds that can be used in treating multi-drug resistant strain of *E. coli* isolated from well is present in *Coriolopsis* species. Extracts of this macrofungus will be a good alternative where well known antibiotics have become inef-

fective. Further work aimed at isolation and identification of specific antimicrobial agents in extracts is the next focus of this research.

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