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Studies on antibacterial activities of Nigerian selected fungi: *Fomes lignosus* and *Daldina concentrica.*

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ABSTRACT: The anti-bacterial activities of the distilled water, ethanolic and chloroform extracts of two Nigerian Macrofungi; *Fomes lignosus* and *Daldina concentrica* were investigated on three Gram – negative bacteria (*Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa* and two Gram -positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) causing food poisoning, wound, urinary tract and respiratory infections in man, using hole diffusion and filter paper disc methods. The most sensitive was hole diffusion method. The anti – bacterial activities of the tested macro – fungi vary and are target microbes specific. Of the tested macro fungi, Ethanolic extract of *Daldina concentrica* showed a significantly anti-bacterial activity ($p<0.05$) against test organisms except *Bacilllus cereus*. *Staphylococcus aureus* was the most sensitive organism. Chloroform extract of *Daldina concentrica* possessed significantly higher anti -bacterial activity ($p<0.05$) against the five tested organisms. The activity of *Fomes lignosus* extract of chloroform and ethanol against *Staphylococcus aureus* was significantly higher ($p<0.05$, 5mm-11mm) than that exhibited by *Daldina concentrica.* The effect of fresh macro-fungi on test organisms was also studied. Gram-negative bacteria are more sensitive to fresh *Fomes lignosus* than Gram-positive bacteria, while the anti- bacterial activity of *Daldina concentrica* was more active against *Proteus mirabilis* compared to other test organisms. Therefore, the study of anti-bacterial substances gives room for improvement of our technological knowledge in the determination of anti-bacterial substances from Nigerian selected higher fungi which will be useful in production of some anti-biotic drugs in Nigeria pharmaceutical industries.

Key words: Anti-bacterial,extracts,Nigerian selected fungi,Human infections.

Introduction

Fomes lignosus and *Daldina concentrica* are higher macrofungi that are mostly found in humid and sufficient hot areas natural habitat where the soil is rich in nitrogen pack forest near manure pit in green house (Elsik, 1983). *Fomes lignosus* belongs to *Eumycota* sub division of *Basidiomycotina* class of Hymenomyces group of fungi while *Daldina concentrica* is a member of *Ascomycotina* class of *Eumycotina* sub-division. The strain of *Daldina concentrica* that commercially evolved are blackish with some red patches in colour while *Fomes lignosus* are amber (pale –yellow) in colour (Ainsworth, 1966).

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Although some edible fungi belonging to Basidiomycetes are useful for human's consumption when they are lightly cooked and prepared into dish as they serve as vegetable source. Grinded mushroom into powder are additive to all kinds of fodder as it is suitable for fish meal, as fresh food and feeding of livestock. Mushrooms can also be canned for consumption and exported to foreign countries (Sac, 1975; Angelo, 1985; Fasidi and Ekuere, 1993; Jonathan and Fasidi, 2003).

Fungi have been extensively studied by Mycologists in educational research fields. Antibiotics, therapeutic agents have been produced for medicinal use from some fungi such as *Penicillium notatum*, *Aspergillus*, *Pleurotus species*, *Lycoperdom species*, *Polyporus species* (Oso, 1977a; Angelo, 1985; Jonathan,2002). They have been observed by Nigerian herbalists of possessing some curative effects against some bacterial infections and intestinal disorders (Jonathan, 2002 and Oso ,1977a, 1977b). Jonathan *et al*. (2007) also reported the antagonistic effect of extracts of some Nigerian higher fungi against selected pathogenic microorganisms.

Likewise Jonathan *et al*. (2008) also evaluated the inhibitory potentials of some higher Nigerian fungi against some pathogenic microorganisms.

Both cellular components and secondary metabolites of a large number of mushroom have been shown to effect the immune system of the host and therefore could be used to treat a variety of diseased states (Wasser and Weis, 1999).

Materials and Methods

Sources of materials and extract preparations: *Fomes lignosus* and *Daldina concentrica* were two different isolates of macro fungi samples used in this study. *Fomes lignosus* and *Daldina concentrica* were collected from the rubber tree and *Fagana leprieurii* tree respectively at the Botanical garden of the University of Ibadan, Ibadan, Nigeria. Collected samples were cut into bits, dried at 40^0C and grinded aseptically into powder using milling machine.

Distilled water, Ethanol and Chloroform were solvents used for carrying out the extraction of powdered samples of the macro-fungi. The mixtures were filtered using Whatsman filter paper No 1. The filtrates obtained were concentrated using rotary evaporator at 40^0C until the volumes were reduced to 20ml under vacuum pump and stored at 4^0C in refrigerator prior to testing.

Isolation and Identification procedures: Isolates of test organisms were obtained from the stored stock culture of *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus* collected from Department of Pharmaceutical Microbiology, University of Ibadan,Nigeria, using prepared nutrient agar, nutrient broth, MacConkey agar (MCA) and Blood Agar. The plates were incubated at 37^oC for 24hrs. Well isolated colonies obtained from agar medium and different broth cultures of Gram-negative and Gram-positive bacteria were constantly subcultured into agar slants from time to time, incubated at 37^0C for 24hrs and stored at 4^0C (Harrigan and McCance, 1976). Identification was based mainly on the followings; (i) Indole production (ii) Presence of catalase (iii) Haemolysis on blood agar (iv) Coagulase test (v) Urease test (vi) Oxidase test (vii) Citrate test (viii) Spore test (ix)Acid and gas production (x) Microscopic and macroscopic examination of morphology (xi) Gram stain. Identification of the different strains were carried out using Bergey's manual (Sneath, 1986).

Preliminary screening for anti-bacterial activity using hole diffusion method: The aim of this experiment was to compare the anti-microbial activity of the fungi and to know which of the solvents would extract the active component of the fungi. Well diffusion method was used for the test. Glass Petri dishes inside the canister were sterilized in an oven at 160^0C temperatures for 3 hours. Prepared sterilized nutrient agar was melted and poured into sterilized plates which have been labeled properly. 6mm cork borers, wrapped in Aluminum foil were also sterilized in an oven at 160^0C temperature for 3 hours. 30mls was poured per plate so that holes bored on the surface of agar plates could take large amount of the extracts. Holes were bored into the solidified medium using different sterilized cork borers (Toba *et al*., 1989). 1ml of each of Chloroform, Ethanol, and Distilled water extract of *Fomes lignosus* and *Daldina concentrica* were dropped in hole of different plates using calibrated Pasteur pipette. The plates were previously streaked with 24 hrs old of cultured organisms of *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus cereus*. A hole was left as control in each of the

plates without an extract. The plates were incubated at 37^0C . After 24 hours incubation , the plates were examined for inhibitory zones. Inhibitory zones present were measured and recorded. Presence of zones of inhibition around each of the wells signified the presence of anti-microbial action while absence indicates absence of anti-microbial action.

Effect of fresh macrofungi on test organisms: The aim of this experiment was to know whether the solvent used for extraction could extract the active component from fungi compared to an unextracted freshly cut macrofungi. The fresh macrofungi were tested on the bacteria directly. Sterilized nutrient agar was poured into different sterilized Petri- dishes. Test organisms were streaked on the solidifying medium before placing 0.1g of fungi on the plates. The plates were incubated at 37^0C. After 24 hours incubation, the plates were examined for inhibition. Zones of inhibition were measured and recorded.

Screening for anti-bacterial substances using filter paper disk method: Whatsman filter paper No 1 were cut into disks of 0.6mm using sterile cork borer and sterile blade (Cruickshank *et al*., 1975). These filter paper disks were then steriled in an oven before use at the temperature of 140^0C for 60 minutes. Dried sterile filter paper disks were dipped into various extracts. Sterile pour plate of nutrient agar were spread over using sterile glass spreader with a loop full of 24hours nutrient broth culture of test organisms. The filter paper disks containing the extracts were placed on the seeded plates. Plates were kept in refrigerator at 4^0C for 18hours so as to allow proper diffusion of extracts into the media before incubating at 37^0C for 24hours. Inhibitory zones were also measured and recorded.

Effect of storage temperature of extracts on test organisms: The aim of this experiment was to show the effect of various storage_temperatures on the anti- microbial activities of the extracts. Distilled water, Ethanol and Chloroform extracts were kept at 25^0C , 37^0C and 45^0C temperatures for 60minutes (Diker *et al*., 1991) . After storage, the extracts were tested on the test organisms using hole diffusion method (Toba ,1989). Plates were incubated at 37^0C for 24hours. The size of the inhibitory zones observed were recorded.

Results

Table 1 shows that chloroform extract of *Daldina concentrica* possessed an anti-microbial activities against all the micro-organisms tested, while distilled water extracts did not. *Bacillus cereus* was the only test organism that was not inhibited when Ethanolic extract was used.

From Table 2, Distilled water, Ethanolic and Chloroform extracts of *Fomes lignosus* did not produce any measurable activity against *Escherichia coli* organism used for the study, while *Staphylococcus aureus* isolates were all inhibited when Distilled water, Ethanolic and Chloroform extracts were used. However, Ethanolic extract of *Fomes lignosus* possessed anti-microbial activity against *Bacillus cereus, Pseudomonas aeruginosa,Proteus mirabilis* and *Staphylococcus aureus,* while Distilled water, and chloroform did not show any inhibitory activity against *Bacillus cereus*.

When the effect of fresh macrofungi was carried out on test organisms (Table 3), *Daldina concentrica* possessed anti-microbial activity against *proteus mirabilis* while *Fomes lignosus* did not possess any measurable activity against *Staphylococcus aureus* and *Bacillus cereus*.

When Distilled water, Ethanolic and Chloroform extracts were assayed against test organisms using filter paper disk method (Table 4), Distilled water extract did not show any anti-microbial activity against the micro- organisms. Ethanolic extract inhibited the organisms tested except *Bacillus cereus*, while *Staphylococcus aureus* only was not inhibited when Chloroform extract was used.

Table 5 shows that Distilled water did not show any measurable activity against test organisms while Ethanolic and Chloroform extracts are active against *Bacillus cereus* and *Proteus mirabilis*.

Similarly, from (Table 6), Ethanolic and Chloroform extracts inhibited all the test organisms except *Proteus mirabilis* for Ethanolic extract, while Distilled water showed no inhibitory action when the extracts were stored at the temperature of 37^0C.

Observation from Table 7 shows that Chloroform extract of *Fomes lignosus* clearly inhibited the growth of all the test organisms. *Pseudomonas aeruginosa* and *proteus mirabilis* were not acted upon by Distillled water extract while ethanol extract did not produce measurable activity against *Escherichia coli*.

At storage temperature of extracts at 25°C, (Table 8), Distilled water showed no anti-microbial action against all the test organisms while Chloroform and Ethanol did with the exception of *Bacillus cereus* for Ethanol extract. The extract of *Fomes lignosus* (Table 9) using distilled water, Ethanol and Chloroform was active against *Staphylococcus aureus* while *Escherichia coli* did not show inhibitory zones by the extracts. Table 10 and 11 show that Ethanol and Chloroform extracts possessed anti-microbial activities against all the micro-organisms tested, while distilled water extract did not.

Table 1: Preliminary Screening for anti-bacterial activity of *Daldina concentrica* using hole diffusion method.

Extracts	Bacterial isolates				
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Distilled water	-	-	-	-	-
Ethanol	17 mm	-	10 mm	13 mm	10 mm
Chloroform	9 mm	16 mm	16 mm	12.5 mm	15 mm

Table 2: Preliminary Screening for anti-bacterial activity of *Fomes lignosus* using hole diffusion method.

Extracts	Bacterial isolates				
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Distlled water	5mm	-	-	-	-
Ethanol	10mm	16mm	-	16mm	12mm
Chloroform	11mm	-	-	11mm	15mm

When the effect of fresh macrofungi was carried out on test organism (Table 3). *Daldina concentrica* possessed anti-microbial activity against *Proteus mirabilis* while *Fomes lignosus* did not possess any measurable activities against *Staphylococcus aureus* and *Bacillus cereus*.

Table 3: Effect of fresh macrofungi on test organisms

Fungi Species	Bacterial isolates				
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Daldina concentrica	-	-	-	-	5mm
Fomes lignosus	-	-	7mm	3mm	5mm

When distilled water ethanol and Chloroform extracts were assayed against test organisms using filter paper disk method (Table 4), Distilled water extracts did not show anti-microbial activity against the micro-organisms. Ethanolic extract inhibited the micro-organisms tested except *Bacillus cereus* while *Staphylococcus aureus* was not inhibited when Chloroform extract was used.

Table 5 shows that distilled water did not show any measurable activity against test organisms, while Ethanolic and Chloroform extracts are active against *Bacillus cereus* and *Proteus mirabilis.*

Table 4: Screening for anti-bacterial substance of *Daldina concentrica* using filter paper disc method

Extracts	Bacterial isolates				
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Distilled water	-	-	-	-	-
Ethanol	2mm	-	4mm	4mm	5mm
Chloroform	-	30mm	17mm	25mm	10mm

Table 5: Screening for anti-bacterial substance of *Fomes lignosus* using filter paper disc method

Extracts	Bacterial isolates				
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Distilled water	-	-	-	-	-
Ethanol	-	5mm	-	-	2mm
Chloroform	-	3mm	-	-	6mm

Similarly, from Table 6, Ethanolic and Chloroform inhibited test organisms except *Proteus mirabilis* for Ethanolic extract, while Distilled water showed inhibitory action when the extracts were stored at a the temperature of 37^{o}C.

Observation from Table 7 shows that Chloroform extract of *Fomes lignosus* clearly inhibited the growth of some test organisms. *Pseudomonas aeruginosa* and *Proteus mirabilis* were not acted upon by Distilled water extract while Ethanolic extract did not produce measurable activity against *Escherichia coli*.

Table 6: Effect of storage temperature of *Daldina concentrica* extracts on test organisms at 37^{o}C

Extracts	Bacterial isolates				
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Distilled water	_	_	_	_	_
Ethanol	19mm	15mm	16mm	15mm	_
Chloroform	20mm	18mm	18mm	7mm	4mm

Table 7: Effect of storage temperature of *Fomes lignosus* extracts on test organisms at 37°C

Extracts	Bacterial isolates				
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Distilled water	11mm	12mm	10mm	_	_
Ethanol	16mm	17mm	_	9mm	18mm
Chloroform	20mm	19mm	19mm	14mm	17mm

At storage temperature of extracts at 25°C (Table 8) ,Distilled water showed no –anti-microbial action against test organisms while Chloroform and Ethanol did with the exception of *Bacillus cereus* for Ethanolic extract.

The extract of *Fomes lignosus* (Table 9) using Distilled water, Ethanol and Chloroform was active against *Staphylococcus aureus* while *Escherichia coli* did not show inhibitory zones by the extracts.

Table 8: Effect of storage temperature of *Daldina concentrica* extracts on test organisms at 25°C

Extracts	Bacterial isolates				
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Distilled water	-	-	-	-	-
Ethanol	17mm	-	10mm	13mm	10mm
Chloroform	9mm	16mm	16mm	12.5mm	15mm

Tables 10 and 11 show that ethanol and chloroform extract possessed anti – microbial activities against the micro – organism tested while distilled water extract did not.

Table 9: Effect of storage temperature of *Fomes lignosus* extracts on test organisms at 25°C

Extracts	Bacterial isolates				
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Distilled water	5mm	-	-	-	-
Ethanol	10mm	16mm	-	16mm	12mm
Chloroform	11mm	-	-	11mm	15mm

Table 10: Effect of storage temperature of *Daldina concentrica* extracts on test organism at 45^{o}C

Extracts	Bacterial isolates				
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Distilled water	-	-	-	-	-
Ethanol	2mm	3mm	2mm	2mm	3mm
Chloroform	3mm	4mm	7mm	4mm	3mm

Table 11: Effect of storage temperature of *Fomes lignosus* extracts on test organisms at 45^{o}C

Extracts	Bacterial isolates				
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Distilled water	-	-	-	-	-
Ethanol	5mm	2mm	5mm	1mm	4mm
Chloroform	5mm	6mm	7mm	3mm	5mm

Discussion

Tables 1 and 2 show that Ethanolic and Chloroform extracts of *Fomes lignosus* and *Daldina concentrica* possess measurable anti-microbial activities against *Staphylococcus aureus* causing some human infections such as skin boils, whit low of finger, breast abscesses, broncho-pneumonia and surgical wounds. Similar observation was reported by (Duguid, 1978), when aqueous Ethanol leaf extracts of *Acalypha wilkesiana* possessed measurable in vitro anti-microbial activities of fresh macro-fungi (Table 3) were effective against only *Proteus mirabilis* may be due to solvent that was not used to extract the active component of fresh macro-fungi.

Result from Table 5 shows that Ethanol and Chloroform extract of *Fomes lignosus* can be useful in preventing the growth of *Proteus mirabilis* on soil sewage and manure so as to reduced its pathogenic effect on man's Urinary tract as well as to avoid septicemia. Similarly, the formation of heat resistant spores of *Bacillus cereus* can be inhibited due to the presence of anti-microbial activities in *Fomes lignosus* extract when Chloroform and Ethanol solvents were used.

Tables 6 and 7 show how inhibitory activities were observe using Ethanolic and Chloroform extracts for *Daldina concentrica* and *Fomes lignosus*, while distilled water extract for *Fomes lignosus* inhibited the growth of *Stapylococcus aureus*, *Bacillus cereus* and *Escherichia coli*. At 25^{o}C and 45^{o}C storage temperatures of extracts, distilled water extracts of *Fomes lignosus*, and *Daldina concentrica* were not active against the test organisms as observed by Agu (1980), in phytochemical studies on the volatile product of some Nigerian Medicinal plants in treatment of diseases. *Fomes lignosus* is related to its local use for gastrointestinal disorder and skin diseases. The high anti-microbial activities of *Daldina concentrica* and *Fomes lignosus* confirms the work of Elsaid (1971), when the aqueous extracts of some African chewing sticks inhibited the activities of *Streptococcus mitis* implicating dental caries.

Therefore, these observations show that distilled water extract must be considered to be static in action, while the Chloroform and Ethanol possess a cidal property. Similar observations were made by Olorundare *et al*. (1991), while studying the anti-bacterial activities of *Cassia alata* leaves. Hence there is need to employ broad range of extracting solvents as reported by Kadara(1985). It shows distilled water extract was not active against the growth of bacteria. This may be due to the fact that active component of *Fomes lignosus*, and *Daldina concentrica* are not soluble in water as reported by Fox and Cameron (1989).

The fact that the Chloroform and Ethanolic extracts of *Fomes lignosus*, and *Daldina concentrica* produced inhibitory activities against some of the micro-organisms implicated in the pathogenesis of skin infections, (*Staphylococcus aureus, Escherichia coli, Proteus mirabilis),* food poisoning (*Staphylococcus aureus, bacillus cereus*), Gastro-intestinal tract and Urino-genital tract infection (*Escherichia coli, Proteus mirabilis, Bacillus cereus).* This provides some scientific basis for the utilization of *Fomes lignosus, and Daldina concentrica* in folk medicine.

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