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Research article

Antibacterial Activities of *Daldina concentrica*

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ABSTRACT: Activities of the distilled water, ethanolic and chloroform extracts of *Daldina concentrica* an ascomycetous fungus was investigated on *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Bacillus cereus* and *Staphylococcus aureus*) using agar well diffusion method. Ethanolic extract of *Daldina concentrica* showed significantly antibacterial activity against all the test microorganisms except *Bacilllus cereus* ($P \leq 0.05$). *Staphylococcus aureus* was the most sensitive organism to the extracts of this fungus with 17.0mm zone of inhibition with ethanol extract. It was also shown that chloroform extract of *Daldinia concentrica* possessed higher anti-bacterial activity against the five tested microorganisms. The effect of fresh tissues of the fungus on test bacteria indicated that *Proteus mirabilis* was the only sensitive organism with 5.0mm zone of inhibition while other bacteria were resistant. The implications of these findings were discussed.

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Keywords: bacteria, extracts, mushroom, human infection

INTRODUCTION

Daldina concentrica is an ascomycetous fungus that is mostly found in tropical and temperate countries of the world (Zoberi, 1972; Jonathan, 2002).It belongs to the division of Ascomycota,class Ascomycets,order xylariales and family xylariacea (Alofe *et al*,1998). This fungus is an interesting genus in that, it forms large stroma with a zoonate inner fibrous tissues(Zoberi,1972; Jonathan, 2002).The fruit bodies appear as a hard hemispherical cushion up to 4cm in diameter ,on dead trunks and decaying logs (Jonathan,2002). The surface of the sporophores is black and glossy with minute spores formed by the ostioles of perithecia (Zoberi,1972).This higher fungus with other medicinal ingredients has been used by traditional doctors in Yorubaland, South Western Nigeria in the treatment of pneumonia and other bacterial infections (Oso, 1977; Oso, 1981; Jonathan,2002).

Mushrooms have been employed for several useful purposes. They have been employed in pharmaceutical, food and agro allied industries (Alofe *et al*, 1998; Fasidi and Ekuere, 1993; Adejoye and Fasidi, 2009; Akinfemi *et al*,2009) . They could be milled into powder and added as additives to all kinds of fodder as it is suitable for fish meal, as fresh food and feeding livestock Akinfemi *et al*, 2009,2010). Mushrooms can also be canned for consumption and exported to foreign countries (Jonathan and Fasidi,2003;Adejoye and Fasidi,2009; Jonathan and Awotona,2010) .Higher fungi especially ,mushrooms have been utilized for environmental and medicinal purposes(Oso,1981;Jonathan *et al*,2008). Antibiotics, therapeutic agents have been produced for medicinal use from some fungi such as *Penicillium notatum*, *Aspergillus*, *Pleurotus species*, *Lycoperdom species*, *Polyporus species* (Fox and Cameron,1989;Olorundare *et al*,1991). They have been observed by Nigerian herbalists of possessing some curative effects against some bacterial infections and intestinal disorders (Ajayi *et al*, 2008). Jonathan *et al* (2010) also reported the antagonistic effect of extracts of some three *Ganoderma* species against selected pathogenic microorganisms. Likewise, Gbolagade and Fasidi (2005), also reported the inhibitory potentials of some

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higher Nigerian fungi against some disease causing microorganisms.

Both cellular components and secondary metabolites of a large number of mushrooms and other green plants have been shown to affect the immune system of the host and therefore could be used to treat a variety of diseases (Prashanth *et al*,2001; Erdogrul, 2002; Kloucek *et al*,2005; Parekh *et al*,2005; Buwa and Staden,2006)**.** Many green plants and mushrooms have been implicated of possessing various degree of anti-microbial activities against some disease causing microorganisms(Benjamin *et al*,1986; Jonathan and Awotona,2010).It was therefore the aim of this present investigation to scientifically prove the claim of the local people from South Western Nigeria that *Daldinia concentrica* could be used to treat some bacterial infections

MATERIALS AND METHODS

Sources of materials and extract preparations: *Daldina concentrica* samples used in this study were collected from the decaying log of *Fagana leprieurii* tree at the Botanical Gardens of the University of Ibadan, Ibadan, Nigeria. 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 the extraction of powdered samples of the macrofungus using the procedures of Jonathan *et al* (2008)

Test bacteria: Isolates of test organisms were obtained from the stored stock culture of *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Bacillus cereus* and *Staphylococcus aureus* collected from Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria, using prepared nutrient agar and Blood agar. The plates were incubated at 37^oC for 24hrs. The organisms were constantly sub-cultured into plates of nutrient agar slants from time to time. Incubation was done at 37^0C for 24hrs (Pelczar *et al*, 1983).

Screening for antibacterial activity using hole diffusion method: Nutrient agar was poured into sterilized petri dishes. Seven(7.0)mm cork borer was used to make wells on the solidified mediu· 1ml of each of chloroform, ethanolic, and distilled water extracts of *Daldina concentrica* were dropped in holes of different plates using calibrated Pasteur pipettes. 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 and plates incubated at 37^0C . After 24 hours incubation, the plates were examined for inhibitory zones which were measured and recorded. Presence of zones of inhibition around each of the wells signified the presence of anti-bacterial action while absence indicates absence of anti-bacterial action.

Effect of fresh macro-fungus on test organisms: The aim of this experiment was to know whether the solvent used for extraction could extract the active component from the fungus compared to an unextracted freshly cut macro-fungus. The fresh macro-fungus was 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 0.25g of the fungus 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: Whathman filter papers No 1 were cut into disks of 7.0mm using sterile cork borer and sterile blade(Buwa and Staden,2006)[l]. These filter paper disks were sterilized in an oven at 100^0C for 60 minutes. Dried sterile filter paper disks were dipped into various extracts. Sterile nutrient agar were poured on petri dishes. A loop full of 24hours nutrient broth culture of test organisms were used to streak the plates . 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 the extract into the media before incubating at 37^0C for 24hours.Inhibitory zones were also measured and recorded(Jonathan *et al*,2008)·

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-bacterial activities of the extracts. Distilled water, ethanolic and chloroform extracts were kept at 25^0C , 37^0C and 45^0C for 24hours(Adesina *et al*,1980)· After storage, the extract was tested on the test organisms using hole diffusion method. Plates were incubated at 37^0C for 24hours. The sizes of the inhibitory zones observed were recorded (Gbolagade and Fasidi,2005).

RESULTS

Table 1 shows that the chloroform extract of *Daldina concentrica* possessed anti-bacterial activities against all the tested bacteria .The highest inhibitory zones (17.0mm) were noticed with *Staphylococcus aureus* using ethanol as an extractive solvent .When

chloroform was used as extractive solvent,16.0mm zones of inhibition were produced with *Bacillus cereus* and *Escherichia coli* .These values were closely followed by 12.5 mm inhibitory zones in *Pseudomonas aeruginosa* .The least zone of inhibition 9.0mm was seen in *Staphylococcus aureus.*Ethanol extract was second best extractive solvent.But the extract did not show any effect on *Bacillus cereus.* Distilled water extracts showed very poor action on the test microorganisms.

When fresh macro fungus was plated directly on the agar plates, all the tested bacterial species were not sensitive except *Proteus mirabilis* (Table 2).This shows that extractive solvents are essentially required to obtained bio-active ingredients from this ascomycetous fungus.When distilled water, ethanolic and chloroform extracts were assayed against test organisms using filter paper disk method (Table 3), distilled water extracts did not show any activity against the microorganisms. Ethanolic extract inhibited all the organisms tested except *Bacillus cereus*, while *Staphylococcus aureus* was not inhibited with chloroform extracts. The greatest activity(30.0mm)was seen against *Bacillus cereus* with chloroform extract.Similarly, from (Table 4), 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.

At 25^oC (Table 5), distilled water showed no anti-bacterial action against all the test organisms while chloroform and ethanol exhibited antibacterial action in allthe test bacteria with the exception of *Bacillus cereus* for ethanolic extract.Table 6 shows that ethanol and chloroform extracts possessed anti-bacterial activities against all the micro-organisms tested while distilled water extract possessed no activity.

Table 1:
Activities of *D. concentrica* using hole diffusion method.

Extracts	**Bacterial isolates /Zone of inhibition(mm)**				
	S. aureus	*B. cereus*	*E. coli*	*Ps. aeruginosa*	*P. mirabilis*
Distilled Water	-	-	-	-	-
Ethanol	17.0a	-	10.0 b	13.0 a	10.0b
Chlorform	9.0a	16.0a	16.0 a	12.5 a	15.0a

Values followed by the same letters are not significantly different by Duncan's multiple range test (P≤0.05)

Table 2:
Effect of fresh *D. concentrica* tissue on test organisms

Extracts	**Bacterial isolates /Zone of inhibition(mm)**				
	S. aureus	*B. cereus*	*E. coli*	*Ps. aeruginosa*	*P. mirabilis*
D. concentrica	-	-	-	-	-

Table 3:
Activities of *D. concentrica* extracts using filter paper disc method

Extracts	**Bacterial isolates /Zone of inhibition(mm)**				
	S. aureus	*B. cereus*	*E. coli*	*Ps. aeruginosa*	*P. mirabilis*
Distilled Water	-	-	-	-	-
Ethanol	2.0 a	-	4.0 b	4.0b	5.0b
Chlorform	-	30.0a	17.0 a	25.0a	10.0a

Table 4:
Effect of temperature on activities of *D.concentrica* extracts at 37^oC

Extracts	**Bacterial isolates /Zone of inhibition(mm)**				
	S. aureus	*B. cereus*	*E. coli*	*Ps. aeruginosa*	*P. mirabilis*
Distilled Water	-	-	-	-	-
Ethanol	19.0 a	15.0 b	16.0b	15.0	-
Chlorform	20.0 a	18.0 a	18.0 a	7.0	4.0

Table 5:
Effect of temperature on activities of *D.concentrica* extracts at 25°C

Extracts	Bacterial isolates /Zone of inhibition(mm)				
	S. aureus	*B. cereus*	*E. coli*	*Ps. aeruginosa*	*P. mirabilis*
Distilled Water	-	-	-	-	-
Ethanol	17.0 a	-	10.0 b	13.0 a	10.0b
Chlorform	9.0 b	16.0a	16.0 a	12.5 a	15.0a

Table 6:
Effect of temperature on activities of *D. concentrica* extracts at 45°C

Extracts	Bacterial isolates /Zone of inhibition(mm)				
	S. aureus	*B. cereus*	*E. coli*	*Ps. aeruginosa*	*P. mirabilis*
Distilled Water	-	-	-	-	-
Ethanol	2.0b	3.0 b	2.0a	2.0 b	3.0a
Chlorform	3.0a	4.0 a	7.0 b	4.0 a	3.0a

DISCUSSION

Daldina concentrica possess measurable anti-bacterial activities against *Staphylococcus aureus* causing some human infections such as skin boils, whitlow of finger, abscesses, broncho-pneumonia and surgical wounds. Similar observations was reported by Jonathan and Awotona(2010) on *Ganoderma* species .Few other Nigerian mushrooms have been reported of containing anti-microbial activities (Gbolagade and Fasidi,2005;Jonathan *et al*,2008). The non-effectiveness of the fresh tissues of *D.concentrica* on the bacterial isolates may be linked with the importance of extractive solvents in the removal of bio active compounds from this fungus.

Very good inhibitory activities were observed using ethanolic and chloroform extracts for *Daldina concentrica*. Similar results were reported by Jonathan (2002), on some selected Nigeria higher fungi. At 25°C and 45°C , distilled water extract of *Daldina concentrica* was not active against the test organisms. Similar result was observed by Ajayi *et al* (2008), for essential oil of some medicinal plants. The demonstration of good anti-microbial activities by *Daldina concentrica* is similar to the observation of Olawuyi *et al* (2010) for *Fomes lignosus* Likewise, Adesina *et al* (1980)reported that some chewing sticks could be used in the prevention of *Streptococcus mitis* ,the causative agent dental caries. The results also showed that distilled water was not a good extracts to remove bioactive components from the fungal tissues, while the chloroform and ethanol possessed good extractive tendencies. This may be due to the fact that active component of *Daldina concentrica* were not soluble in water .Similar observations were made by Olorundare *et al* (1991),' on anti-bacterial activities of *Cassia alata* leaves. Hence, there is need to employ broad range of extracting solvents. Jonathan(2002), also reported that distilled water extract was not active against the tested bacteria. (Erdogrul, 2002; Olorundare *et al*,1991).

The fact that the chloroform and ethanolic extracts of *Daldina concentrica* produced inhibitory activities against some of the microorganisms implicated in the pathogenesis of skin infections, (*Staphylococcus aureus, Escherichia coli* and *Proteus mirabilis)*, food poisoning (*Staphylococcus aureus, Bacillus cereus*), gastro-intestinal tract and urino-genital tract infection (*Escherichia coli, Proteus mirabilis, Bacillus cereus)* was an evidence that this fungus could be used in the control of some human pathogens. This provides some scientific basis for the utilization of *Daldina concentrica* by traditional doctors among Yoruba people of south Western Nigeria.

REFERENCES

Adesina ,S. K., Oguntimehin, B.J. and Akinwasi, D.D. (1980). Phytochemical and Microbiological examination of the leaves of *Acalypha wilkesiana*. Quarterly *Journal of Crude Drug Research* 18: 45-48.

Akinfemi A,Babayemi,O.J and Jonathan,SG(2009). Bioconversion of maize husks by white rot fungi.Revista Cietifica Agricola 9(4):972-978.

Akinfemi A.,Jonathan SG and Adu O.A (2010). Improving the Nutritive Value of Millet Stover Using Biological Treatment. Libyan Agriculture Research Center Journal International 1 (3): 195-201, 2010

Alofe,F.R.,Odu,E.A.and IIloh,H.C.(1998). Mushrooms,man and nature,edible wild mushrooms on decaying wood in Obafemi

Awolowo University Campus.The Nigerian Fields.63:3-18.

Alexopolous C.J.,Mims,C.W. and Blackwell,M.(1996) .Introduction to mycology .(4th Edt.).New York John Wiley.

Adejoye ,O.D.and Fasidi I.O.(2009). Effect of cultural conditions on biomass and laccase production in submerged medium by *Schizophyllum commune* (Fr.), a Nigerian edible mushroom. Electron J Environ Agric Food Chem (EJEAFChe) 2009;8:1186-93.

Ajayi, I.A.,Jonathan,S.G., Adewuyi,A.A. and Oderinde,R.A. (2008). Antimicrobial screening of the essential oil of some herbal plants from western Nigeria. World Applied Sciences Journal 3(1):79-81.

Benjamin, T. V., Anucha, T. C. and Hugbo, P. G. (1986). Approach in the study of medicinal plants with anti – microbial activities by reference to *Mitracarpusscrabra.* Workshop of Medicinal plant:In Journal. of Plant Anatomy. 7 – 11; Ife Nigeria Pp 34.

Buwa, L.V.and Staden JV(2006). Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa**.** *Journal of Ethnopharmacology* 103**:**139-142.

Erdogrul, O.T(2002). Antibacterial activities of some plant extracts used in folk medicine. Pharmaceutical Biology , 40**:**269-273.

Fasidi, I. O. and Ekuere,U.U.(1993).. Studies on *Pleurotus tuber- regium*(Fries).Singer: Cultivation ,proximate compositions and mineral contents of the*Sclerotia*. Food Chemistry.48: 255 – 258.

Fox ,B.A. and Cameron, A.G (1989). Tea, Food Science Nutrition and Health.pp 80-90.

Gbolagade,J. and Fasidi,I. (2005). Antimicrobial activities of some selected Nigerian mushrooms .African Journal of Biomedical Sciences.8 (2):83-87.

Jonathan, S. G.(2002).. Vegetative growth requirements and antimicrobial activities of some higher fungi in Nigeria.Ph.D thesis. University of Ibadan ,Nigeria.

Jonathan S. G. and Fasidi,I.O. (2003). Antimicrobial activities of two Nigerian edible macrofungi-*Lycoperdonpusilum* (Bat. Ex) and *Lycoperdongigantum*(Pers) African Journal of Biomedical Research 6:85 – 90.

Jonathan ,S. G. , Kigigha, L. T. and Ohimain, E. (2008). .Evaluation of the inhibitory potentials of Eight higher Nigerian Fungi against pathogenic Microorganisms. African journal of Biomedical Research 11: 192 – 202.

Jonathan, S.G. and Awotona F.E (2010). Studies on Antimicrobial Potentials of three G*anoderma species*. African Journal of Biomedical Research 13(2):119-125.

Kloucek, P., Polesny ,Z,. Svobodova, B., Vlkova ,E. and Kokoska L(2005). Antibacterial screening of some Peruvian medicinal plants used in Callería District.Journal of Ethno pharmacology 99:309-312.

Olawuyi, O.J.,Jonathan, S.G,.Aina, D.A. and Baysah GI(2010). Studies on antibacterial activities of *Fomes lignosus*-A Nigerian mushroom. Nigerian Journal of Microbiology 24(1):2153-2159

Olorundare ,E.O., Emudiarughe T.S., Khasar, G.S., Kuteyhi, S.A. and Irobi, O.N (1991). Antibacterial activity of leaf extract of *Cassia alata.* Bulletin of Science Association of Nigeria 17 :106-107.

Oso, B. A. (1977). Mushrooms in Yoruba mythology and medicinal practices.Eco. Bot. 31: 367 – 371.

Oso, B. A. (1981). Fungi and mankind.Universty of Ibadan,Nigeria.Inaugural Lecture.**1-50.**

Parekh, J., Jadeja ,S. andChanda S(2005) . Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity**.** Turkish Journal of Biology, 29:203-210.

Prashanth ,D., Asha ,M.K., and Amit A(2001). Antibacterial activity of *Punica granatum.* Fitoterapia 72:171-173.

Pelczar, M.J., Chan, E.C.N. and N.R. Krieg, N.R. (1983). Microbiology: Concepts and Application. MacGraw-Hill Inc. New York.

Zoberi,H.M.(1972). Tropical Macrofungi. Macmillan Press, London.