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Antibacterial activities of *Daldina concentrica*, an ascomycetous fungus from Nigeria

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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 ($P<0.05$) against the test microorganisms except Bacilllus cereus. Staphylococcus aureus is the most sensitive organism to the extract of this fungus. Chloroform extract of Daldinia concentrica possessed higher anti -bacterial activity against the five tested microrganisms. The effect of fresh macro-fungus on test organisms was also studied. Fresh Daldina concentrica was more active against Proteus mirabilis in comparison witn other pathonenic microoganssms.

KEYWORDS

Anti-bacterial;
extracts;
Nigerian mushroom;
human infection.

INTRODUCTION

Daldina concentrica is an ascomycetous fungus that is mostly found in tropical and temperate counties of the world[17,27]. It belongs to the division of Ascomycota,class Ascomycets,order xylariales and family xylariacea[3,4]. This fungus is an interesting genus in that it forms large hemispherical stroma with a zoonate inner stroma tissues[17,27]. The fruit bodies appear as a hard hemispherical cushion up to 4cm in diameter on dead trunks and decaying logs[17]. the surface of the sporophores is black and glossy with minute spores formed by the ostioles of perithecia[27]. This higher fungus with other medicinal ingredients has been used by traditional doctors in Yoraba land,South Western Nigeria in the treatment of pneumonia and other bacteria infections[24,25].

Mushrooms have been employed for several useful purposes[2,5,6]. 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[13]. Mushrooms can also be canned for consumption and exported to foreign countries[5,18,20]. Higher fungi especially, mushrooms have been utilized for environmental and medicinal purposes[17,25]. Antibiotics, therapeutic agents have been produced for medicinal use from some fungi such as Penicillium notatum, Aspergillus, Pleurotus species, Lycoperdom species, Polyporus species[21-23].

They have been observed by Nigerian herbalists of possessing some curative effects against some bacterial infections and intestinal disorders[5,6,24]. Jonathan et al[20]. also reported the antagonistic effect of extracts of

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some three Ganoderma species against selected pathogenic microorganisms. Likewise, Gbolagade and Fasidi[15]. also reported the inhibitory potentials of some 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 disease of medical importance[9-12]. Many green plants and mushrooms have been implicated of possessing various degree of anti microbial activities against some disease causing microorganisms[6,7,8,11,20]. 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. Collected samples were cut into bits, dried at 40^{0}C 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 macrofungus using the procedures of Jonathan et al[19].

Isolation and Identification procedures: 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^{o}C for 24hrs. Well isolated colonies obtained from agar medium and different broth cultures of Gram-negative and Gram-positive bacteria were constantly sub-cultured into agar slants from time to time, incubated at 37^{0}C for 24hrs and stored at 4^{0}C[26].

Preliminary screening for anti-bacterial activity using hole diffusion method: The aim of this experiment was to compare the anti-microbial activity of the fungus and to know which of the solvents would extract its active component. Well diffusion method was used for the test. Glass Petri dishes were sterilized in an oven at 160^{0}C for 3 hours. Nutrient agar was poured into sterilized plates . 6mm cork borer was used to make wells on the solidified medium[22]. 1ml of each of chloroform, ethanolic, and distilled water extracts of Daldina concentrica were dropped in holes 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^{0}C. 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-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 0.1g of the fungus on the plates. The plates were incubated at 37^{0}C. 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 0.6mm using sterile cork borer and sterile blade[17]. These filter paper disks were sterilized in an oven at 100^{0}C 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^{0}C for 18hours so as to allow proper diffusion of the extract into the media before incubating at 37^{0}C for 24hours. Inhibitory zones were also measured and recorded[20].

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 extract. distilled water, ethanolic and chloroform extracts were kept at 25^{0}C, 37^{0}C and 45^{0}C temperatures for 60minutes[17]. After storage, the extract was tested on the test organisms using hole diffusion method. Plates were incubated at 37^{0}C for 24hours. The sizes of the inhibitory zones observed were recorded[15].

RESULTS

TABLE 1 shows that the chloroform extract of Daldina concentrica possessed anti-bacterial activities against all the tested bacteria. The highest inhibitory zones (17mm) 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 in Bacillus cereus and Escherichia coli. These values were closely followed by 12.5 mm inhibitory zones in Pseudomonas aeruginosa. The least zone of inhibition 9mm 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 show 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 solvent s are essentially required to obtained 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 extract did not show any anti-bacterial activity against the microorganisms. Ethanolic extract inhibited the organisms tested except Bacillus cereus, while Staphylococcus aureus only was not inhibited when chloroform extract was used. Chloroform extract has the greatest activity(30.0mm) against Bacillus cereus

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^{0}C.

At storage temperature of extracts at 25^{o}C (TABLE 5), Distilled water showed no anti-bacterial action against all the test organisms while chloroform and ethanol did 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 did not.

DISCUSSIONS

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[20] on Ganoderma species Therefore, the non-effectiveness of the fresh macro-fungus on the isolates except Proteus mirabilis may be due the importance of extraction to obtained ingredients from this fungus. Chloroform extract of Daldina concentrica could be useful in preventing the infestation of of Bacillus cereus.

Very good inhibitory activities were observed using ethanolic and chloroform extracts for Daldina concentrica. Similar results were reported by Jonathan[17] on some selected Nigeria higher fungui. At 25^{o}C and 45^{o}C storage temperatures, distilled water extract of Daldina concentrica was not active against the test organisms. Similar result was observed by Ajayi et al[6], fossential oil of some medicinal plants. The high anti-microbial activities of Daldina concentrica is similar to the observation of[1] for chewing sticksin the prevention of Streptococcus mitis causing dental caries.

Therefore, these observations show that distilled water is not a good extracts to remove bioactive components from the fungal tissues, while the chloroform and Ethanol possess a bacteriocidal or bacteriostactic properties against the test fungus. Similar observations were made by Olorundare et al.[23], on anti-bacterial activities of Cassia alata leaves. Hence, there is need to employ broad range of extracting solvents. Jonathan[17] also reported that Distilled water extract was not active against the growth of bacteria. This may be due to the fact that active component of Daldina concentrica is not soluble in water[22, 23].

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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 Urinogenital tract infection (Escherichia coli, Proteus mirabilis, Bacillus cereus)was a sure 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.

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

Extracts	Bacterialisolates				
	S. Aureus	*B. cereus*	*E. coli*	*Ps. aeruginosa*	*P. mirabilis*
Distilled Water	-	-	-	-	-
Ethanol	17mm	-	10mm	13mm	10mm
Chlorform	9mm	16mm	16mm	12.5mm	15mm

When the effect of fresh macro-fungus was carried out on test organisms (TABLE 2). Daldina concentrica possessed anti-microbial activity against Proteus mirabilis only.

TABLE 2 : Effect of fresh macrofungi on test organisms Fungi

Species	Bacterial isolates				
	S. Aureus	*B. cereus*	*E. coli*	*Ps. aeruginosa*	*P. mirabilis*
Daldina Concentric	-	-	-	-	5mm

When distilled water ethanol and chloroform extracts were assayed against test organisms using filter paper disk method (TABLE 3), 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.

Similarly, from (TABLE 4), ethanolic and Chloroform inhibited 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°C.

TABLE 3 : Screening for anti-bacterial substance of Daldina concentric using filter paper disc method

Extracts	Bacterial isolates				
	S. Aureus	*B. cereus*	*E. coli*	*Ps. aeruginosa*	*P. mirabilis*
Distilled Water	-	-	-	-	-
Ethanol	2mm	-	4mm	4mm	5mm
Chlorform	-	30mm	17mm	25mm	10mm

TABLE 4 : Effect of storage temperature of Daldina concentrica extracts on test organisms at 37°C

Extracts	Bacterial isolates				
	S. aureus	*B. cereus*	*E. coli*	*Ps. aeruginosa*	*P. mirabilis*
Distilled Water	-	-	-	-	-
Ethanol1	9mm	15mm	16mm	15mm	-
Chlorform	20mm	18mm	18mm	7mm	4mm

At storage temperature of extracts at 25°C (TABLE 5), distilled water showed no –anti-bacterial action against test organisms while chloroform and Ethanol did with the exception of Bacillus cereus for ethanolic extract.

TABLE 5 : Effect of storage temperature of Daldina concentrica extracts on test organisms at 25°C

Extracts	Bacterial isolates				
	S. aureus	*B. cereus*	*E. coli*	*Ps. aeruginosa*	*P. mirabilis*
Distilled Water	-	-	-	-	-
Ethanol	17mm	-	10mm	13mm	10mm
Chlorform	9mm	16mm	16mm	12.5mm	15mm

TABLE 6 shows that ethanolic and chloroform extracts possessed anti-microbial activities

against the micro-organisms tested while Distilled water extract did not.

TABLE 6 : Effect of storage temperature of Daldina concentrica extracts on test organism at 45°C.

Extracts	Bacterial isolates				
	S. aureus	*B. cereus*	*E. coli*	*Ps. aeruginosa*	*P. mirabilis*
Distilled Water	-	-	-	-	-
Ethanol	2mm	3mm	2mm	2mm	mm
Chlorform	3mm	4mm	7mm	4mm	3mm

REFERENCES

[1] S.K.Adesina, B.J.Oguntimehin; Journal of Crude Drug Research **18**, 45-48 **(1980)**.
[2] F.R.Alofe, E.A Odu, H.C Illoh; The Nigerian Fields **63**, 3-18 **(1978)**.
[3] Ainsworth, G.C.; A general purpose classification for Fungi Systematic Mycology **11**, 1-4 **(1966)**.
[4] C.J.Alexopolous, C.W.Mimis, C.W., M.Blackweel; Introductory mycology. 4th Edition. USA, John Wiley and Sons Inc., **(1996)**.
[5] O.D.Adejoye, O.D.,I.O.Fasidi, I.O.; Agricultural and Food Chemistry (EJEAFche), **8(11)**, 1186-1193 **(2009)**.
[6] I.A.Ajayi, S.G.Jonathan, A.Adewuyi, R.A.Oderinde; World Applied Sciences Journal **3(1)**, 79-81 **(2008)**.
[7] T.V.Benjami, T.C.Anucha, P.G Hugbo, Journal. of Plant Anatomy, **34**, 7-11 **(1986)**.
[8] Cridland, A.A.; Mycology **54**, 230-234 **(1962)**.
[9] R.Duguid, J.P., Marmion, R.H.; Journal of Applied Bacteriology **(4)2**, 299-309 **(1978)**.
[10] K.S.Diker, M.Akan, M.Gulsenttascelik, M.Yurdakok; M.Letters in Applied Microbiol. **(12)**, 34-35 **(1991)**.
[11] H.Dold, H., Knapp; Journal of Hygiene, Infektionshr **127**, 251-253 **(1984)**.
[12] F.Elsaid, S.O.Fadulu, J.O., Kever, B.A.Sofowora; Lioyodia **34(1)**, 171-173 **(1971)**.
[13] I.O.Fasidi, U.U.Ekuere; Food Chemistry, **48**, 255-258 **(1993)**.
[14] B.AFox, A.G.; Cameron Food Science Nutrition and Health, **15**, 80-90 **(2000)**.
[15] J.Gbolagade, I.Fasidi; African Journal of Biomedical Sciences **8(2)**, 83-87 **(2005)**.
[16] K.Hiller; Pharmazie **19**, 167-185 **(1984)**.
[17] S.G.Jonathan'Vegetative growth requirements and antimicrobial activities of some higher fungi in Nigeria. Ph.D Dissertation. University of Ibadan, Nigeria **(2002)**.
[18] S.G.Jonathan, I.O.Fasidi, I.O.; African Journal of Biomedical Research **6**, 85-90 **(2003)**.
[19] S.G.Jonathan, L.T.Kigigha, E.Ohimain; African Journal of Biomedical Research **11**, 192-202 **(2008)**.
[20] S.G Jonathan, F.EAwotona; African Journal of Biomedical Research **13(2)**, 119-125 **(2010)**.
[21] C.K.Kokake, K.C.Varma, K.C.; Journal of Chemical Science **81**, 651-652 **(1972)**.
[22] S.A.Malcom,E.A.Sofowora; Lloyodia **32**, 512 **(1989)**.
[23] E.O.Olorundare, T.S.Emudiarughe, G.S.Khasar, S.A.Kuteyi, O.N.Irobi; Bulletin of Science Association of Nigeria **17**, 106-107 **(1991)**.
[24] B.AOso; Economic Botany Bot., **31**, 367-371 **(1977)**.
[25] B.A.Oso; Inaugural Lecture, 1-50 **(1981)**.
[26] M.J.Pelczar, E.C.N.Chan, N.R.Krieg; Microbiology, Concepts and Application, MacGraw-Hill Inc. New York, **(1983)**.
[27] H.M.Zoberi, Tropical Macrofungi, Macmillan Press, London, **(1972)**.