GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL 16, NO. 2, 2010: 205-208

COPYRIGHT© BACHUDO SCIENCE CO. LTD PRINTED IN NIGERIA. ISSN 1118-0579
www.globaljournalseries.com; Email: info@globaljournalseries.com

ANTIMICROBIAL ACTIVITY OF "DUTSEN DAN LIBYA"

E. D. PAUL, M. S. ABDULLAHI, E. E. OGABIELA, F. G. OKIBE AND C. E. GIMBA

(Received 8, August 2009; Revision Accepted 26, October 2009)

ABSTRACT

Dutsen dan Libya is a rock material used in traditional medicine for the treatment of a myriad of ailments like cold, fever, stomach disorder, diarrhea and Jaundice. To evaluate the scientific basis for the use of the rock, the antimicrobial activities of its water extract was studied against some common gram positive and gram negative bacteria and fungi. The antimicrobial activity of the aqueous extract was evaluated by determination of the diameter of zone of inhibition using the paper disk diffusion method. Results showed sensitivity to *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Corynebacterium ulcerans*, *Klebsiella pneumoniae* and *Candida albicans*. *Bacillus subtilis*, *Salmonella typhi*, *Escherichia Coli* and *Neisseria gonorhoea* were resistant. Studies on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the rock-extract solution on the test organisms showed the lowest MIC at the dose level of 1.25mg/ml and MBC at the dose level of 2.50mg/ml respectively against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. The highest MIC (2.5 mg/ml) and MBC (5.00mg/ml) were exhibited against *staphylococcus aureus*, *corynebacterium ulcerans*, *klebsiella pneumoniae* and *Candida albicans*.

KEYWORDS: Antimicrobial, Infectious disease, Pathogenic, Bacteria, Fungi

INTRODUCTION

Infectious disease is one of the causes of death worldwide. It accounts for approximately 50% of death in tropical countries (Iwu et al., 1999). This may be due to poverty and increasing incidence of multiple drug resistance. Bacterial resistance to almost all antimicrobial agents has been reported (Truiti et al., 2003). This resistance is due to indiscriminate use of antimicrobial drugs commonly used in the treatment of infectious diseases (Afolayan and Aliero, 2006). Furthermore, some antibiotics have serious undesirable side effects which limit their application. There is, therefore, a serious need to develop new antimicrobial agents that are effective with minimal side effects.

Dutsen dan libya is a mineral/ rock formation commonly found in North Africa. It is reported to be very common in Libya where the rock derived its name. Preliminary examination places the rock in the pyroxene group. A survey of literature on Dutsen dan libya revealed that there is no report of any antimicrobial study on this rock extract. In this study, the antimicrobial activities of the aqueous extract of the rock against some pathogenic gram positive, gram negative bacteria and a fungus were investigated to ascertain the scientific basis of the use of this rock for the treatment of some diseases.

MATERIALS AND METHODS

Antimicrobial Screening

The antimicrobial activities of the water extract from the rock sample were determined using some pathogenic microorganisms. The microbes were obtained from the department of medical microbiology, Ahmadu Bello University Teaching Hospital, Zaria. All the isolates were checked and maintained in slants of nutrient agar while the fungus [*Candida albicans*] was maintained in a slant of S. D. A.

The paper discs method (Bauer-Kirby), was used for checking the antimicrobial activities of the extracts of the rock sample against the micro-organisms. 0.1 g of the powdered rock was dissolved in 10mls of distilled water to obtain a concentration of 10.0 mg/ml.

Blood agar base was prepared by dissolving 40.0g of the agar in a litre of distilled water in a flask caped with cotton wool and boiled to dissolve on a Bunsen burner .The solution of blood agar was sterilized at 121 ^{o}C for 15mins. The medium was allowed to cool. 20.0 mls of the sterilized medium was poured into each sterilized Petri dish. The plates were covered and allowed to solidify. The plates containing the medium were then seeded with the test micro-organisms by spread plates techniques and left to dry for half an hour. Filter paper disks were cut and sterilized at 160 ^{o}C for 30mins. The sterilized paper discs were soaked in the solution of the rock extract. The paper discs were allowed to dry at 45 ^{O}C for one hour, the dried paper discs were then planted on the medium previously seeded with the test microbes. The plates were then incubated for 24 hours at 37 ^{O}C and 25 ^{O}C for the bacteria and the fungus respectively, after which they were inspected for the zone of inhibition of growth. All tests were carried out in duplicates. The zones were measured with a transparent ruler and the diameters recorded in millimeters.

E. D. Paul, Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria
M. S. Abdullahi, National Research Institute for Chemical Technology, Zaria, Nigeria
E. E. Ogabiela, National Research Institute for Chemical Technology, Zaria, Nigeria
F. G. Okibe, Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria
C. E. Gimba, Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria

MIC

Minimum Inhibition Concentration was carried out on the extract that showed growth inhibitory effects on the microbes and was done using the broth dilution method. Nutrient broth was prepared according to the manufacturer's method, boiled and 10mls was dispensed into a set of 5 screw cap test tubes. This was sterilized at 121^{O}C for 15 minutes. The medium was allowed to cool.

Mc – Farland's turbidity standard scale number 0.5 was prepared to give a turbid solution. 10.0 mls of normal saline was used to make a turbid suspension of the microbes. The dilution of the microbes suspension was done continuously using the normal saline until the turbidity marched that of the Mc – Farland's scale by visual comparison, at this point the microbes have the concentration of 3.0 X 10^{-8} mg/ml. 0.1 g of the powdered rock was dissolved in 10.0 mls of the sterile nutrient broth.

Two fold serial dilution of the rock in the broth was done continuously to give a concentration of 10.0 mg/ml, 5.0 mg/ml, 2.5 mg/ml, 1.25 mg/ml and 0.625 mg/ml respectively. All these were obtained by withdrawal of 2.0 ml from a proceeding test tube into the next and from the last tube 2.0 mls of the solution was withdrawn and discarded using a sterile pipette. 1.0 ml of the microbes suspension in the saline was transferred into each of the test tubes. The test tubes were then incubated at 37 ^{O}C for 24 hours after which they were observed for turbidity. The tubes with the lowest concentration of the extract showing a clear solution represent the MIC.

MBC

Minimum bactericidal concentration determination was carried out in order to determine the microbes sensitivity.

Blood agar plates were prepared according to the manufacturer's instruction. The contents of the MIC tubes and proceeding tubes in the serial dilution were sub cultured onto the labeled plates by dipping a sterile loop into each of the test tubes and streaking the plates. The plates were inoculated at 37 ^{O}C for 24 hours after which they were observed for microbial growth. The MBC was the plate with the lowest concentration of the rock extract without growth.

RESULTS

The results of the antimicrobial activities, the zone of growth inhibition in millimeters, MIC and MBC tests carried out on the various microorganisms are presented in the following Tables 1, 2, 3, and 4 respectively.

Test organism	DDL
Staphylococcus aureus	S
Streptococcus pyogenes	S
Bacillus subtilis	R
Corynebacterium ulcerans	S
Salmonella typhi	R
Escherichia Coli	R
Klebsiella pneumoniae	S
Pseudomonas aeruginosa	S
Neisseria gonorhoea	R
Candida albicans	S

Table 1: Antimicrobial Activities of DDL
Key=S=Sensitive R= Resistance

Test organism	Zone of growth inhibition
Staphylococcus aureus	27
Streptococcus pyogenes	35
Bacillus subtilis	0
Corynebacterium ulcerans	28
Salmonella typhi	0
Escherichia Coli	0
Klebsiella pneumoniae	29
Pseudomonas aeruginosa	31
Neisseria gonorhoea	0
Candida albicans	20

Table 2: Zone of Growth Inhibition of DDL Extract against the Micro – Organism (mm)

Table 3: Minimum Inhibition Concentration (MIC) of DDL against the Microbes

Test Organism	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	0.625mg/ml
Staphylococcus aureus	-	-	*+	+	++
Streptococcus pyogenes	-	-	-	*+	+
Bacillus subtilis					
Corynebacterium ulcerans	-	-	*+	+	++
Salmonella typhi					
Escherichia Coli					
Klebsiella pneumoniae	-	-	*+	+	++
Pseudomonas aeruginosa	-	-	-	*+	+
Neisseria gonorhoea					
Candida albicans	-	-	*+	+	++

Key: - = No growth (Clear Solution) *+ = MIC
+ = Light growth (Turbid) ++ = Dense growth

Table 4: Minimum Bactericidal Concentration of DDL against the Microbes

Test Organism	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	0.625mg/ml
Staphylococcus aureus	-	*+	+	++	+++
Streptococcus pyogenes	-	-	*+	+	++
Corynebacterium ulcerans	-	*+	+	++	+++
Bacillus subtilis					
Salmonella typhi					
Escherichia Coli					
Pseudomonas aeruginosa	-	-	*+	+	++
Klebsiella pneumonia	-	*+	+	++	+++
Neisseria gonorhoea					
Candida albicans	-	*+	+	++	+++

Key: - = No Growth *+ = MBC + = Growth ++ = Moderate Growth
+++ = Heavy Growth

DISCUSSION

The results of this study show that water extract of this rock has demonstrated significant antimicrobial activities against the tested microbes. With reference to table 1, the rock extract was discovered to have antimicrobial activities on both gram positive and negative bacteria. The sensitive microbes include bacteria such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium ulcerans*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. It also inhibited a fungus; *Candida albicans*.

The rock extract has high zone of inhibition, an indication of a strong antimicrobial activity, on *Pseudomonas aeruginosa*, a bacteria associated with burn infections. These infections are usually difficult to combat due to the organism's multidrug resistance (Afolayan and Aliero 2006). Thus the rock is a potential source of a drug compound that could be used against infections caused by this microorganism. DDL also inhibited *Streptococcus pyogenes*, an organism associated with sore throat, causing pus in infected wounds.

Another significant observation from the results is the strong activity of the rock extract against *Staphylococcus aureus* (zone of inhibition = 27 mm). This is the microorganism known to play significant role in skin diseases (Srinivasan et al., 2001). This indicates that the rock can be a good source of a compound that can be effective against skin infections. *Staphylococcus aureus* has demonstrated a resistance to known drugs in recent times. This drug resistance phenomenon is already causing major problems in hospitals where staphylococcus infections that do not respond to antibiotic treatments are becoming more common (Greif, 2008). It also inhibited *Klebsiella pneumoniae*, an encapsulated gram – negative, none motile, pathogenic bacterium that causes severe pneumonitis in humans. DDL was also found to inhibit *Corynebacterium ulcerans*, a pathogenic micro organism responsible for zoonotic infections similar to diphtheria (Takahashi, 2003). *Candida albicans* a microorganism that causes a fungal infection of the mucus membrane known as thrush, mycosis or candidiasis, was also inhibited by DDL. These microorganisms are associated with a lot of infectious diseases.

The inhibitory activities of DDL extract gives promise to its potential application in the treatment of microbially induced ailments or diseased conditions. This explains the basis for its use in traditional medicine for the treatment of infections.

REFERENCES

Afolayan, A. J. and Aliero, A. A., 2006. Antimicrobial Activity of Solanum Tomentosun. Afr. J. Biotech. 5 (4): 369 - 372

Barry, A. L., Thumsberry C., 1985. Susceptibility tests. Diffusion test procedure J. Chem. Pathol. 19: 492 – 500.

Bauer, A. W.; Kirby, W. M. M.; Sherris, T. C. and Turck M., 1966. Antibiotic Susceptibility Testing by Standardized Single Disc Method. Am. J. Clinical Pathology 45: 493 – 496

Greif, J., 2008. Fighting the Future: Mason Scientists Address Bacteria's Resistance to Modern Antibiotics. The Mason Gazette, http://gazette.gmu.edu/articles/11862

Iwu, M. M.; Duncan, A. R. and Okunji, C. O., 1999. New Antimicrobials of Plant Origin. In: Perspective on Crops and Uses, Janick, J. (Ed.) Ashs Press, Alexandria V. A., Pp 457 - 462

Kimura T., But P. P. H., Guo J-X, Sung C. K., Eds: 1996. International Collation of Traditional and Folk Medicine. NG Publishing Co. Ptc. Ltd

King A., Brown D. F., 2001. Quality Assurance of Antimicrobial Suceptibility testing by disc diffusion. J. Antimicrob. Chemother. 48 : 71 – 76. Pubmed Abstract.

National Committee for Clinical Laboratory Standards, 2000. Method for Dilution Antimicrobial Susceptibility tests for Bacteria that grow Aerobically: Approved Standards. 5 ed. Wayne: NCCLS, (M100 – 510/ M7).

Selwyn, S.; Lacey, R. W. and Bakhtiar M., 1980. Resistance Strains in the lactam Antibiotics penicillins and cephalosporin in perspective. Hodder and Stouughton. London 1980 224 – 228.

Takahashi, M., 2003. Corinebacterium Ulcerans Diphtheria in Japan, *Emerging Infectious Diseases* http://www.thefreelibrary.com/Corynebacterium+ulcerans+diphtheria+in+Japan.+(Letters).-a0103563817

Truiti, M. C. T.; Sarragiotto, M. H.; Filho, B. A. A.; Nakamura, C. V. and Filho, B. P. D., 2003. In Vitro Antibacterial Activities of a 7 –Q – β – D – glucopyronosyl – nutanocoumarin from Chaptalia nutans (Asteraceae) Mem. Inst. Oswaldo. Cruz.; 98 (2): 283 - 286

USP., 2006. (51) Antimicrobial effectiveness testing United States Pharmacopeia 29:2499 – 2500