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ANTIBACTERIAL SCREENING OF FOUR LOCAL PLANTS USING AN INDICATOR-BASED MICRODILUTION TECHNIQUE

[1]Umeh, E.U., [1]Oluma, H.O.A., and [2]Igoli, J.O.
[1]Department of Biological Sciences, University of Agriculture, Makurdi, Benue State, Nigeria, .[2]Department of Chemistry, University of Agriculture, Makurdi, Benue State, Nigeria. E-mail: jceu1@yahoo.com

Abstract

The antibacterial activities of the plants, *Mitragyna inermis, Morinda lucida, Khaya senegalensis* and *Nauclea latifolia* were investigated using an indicator-based microdilution technique. The extracts of the plants in water, methanol, chloroform and petroleum ether inhibited growth of bacteria in broth cultures. *M. inermis* with the lowest minimal inhibitory concentration (MIC) of 0.03g/ml was the bacteriologically most active plant. The chi-square value (10.25) and F-statistic value (4.36) were significant at less than 0.05 level and implied that inhibition of bacterial growth was significantly associated with the type of plant investigated. The R value obtained on regressing bacterial inhibition on the independent variables – type of plant, plant part (leaf or stem bark) and extraction solvent used - was 0.57 ($p < 0.01$ level), and means that the independent variables put together influenced inhibition of bacterial growth . The findings of this study suggest the effectiveness of the microdilution technique in the phytochemical screening of plants for antibacterial activities.

Keywords: indicator-based, microdilution technique, antibacterial, *Mitragyna inermis, Morinda lucida, Khaya senegalensis, Nauclea latifolia.*

Introduction

Infectious diseases have continued to ravage most developing nations of the world. Nearly half of all deaths in developing nations are attributed to microbial infections (Lamikanra, 1989). Coupled with the scourge of infectious diseases is the recent emergence of drug-resistant microorganisms that have reduced the effectiveness of antimicrobial agents (Montefiore et al. 1989). Bioactive compounds with antibacterial effects need to be explored from local natural resources. Being new, such compounds may

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not have the problem of microbial resistance, and with some structural modification their activity could be diversified.

Seeking remedies for human ailments from the environment has formed the basis for therapeutics (Potier et al. 1990). According to Bringmann and Pokorny (1995), African plants constitute a rich untapped pool of natural products. The plants *Mitragyna inermis, Morinda lucida, Khaya senegalensis* and *Nauclea latifolia* are used in many African countries by traditional medical practitioners for the treatment of various ailments including bacterial diseases. Extracts of different parts of these plants (e.g. fruits, leaves, stem-bark and roots) in hot water or alcohol are used in form of infusions, decoctions or concoctions (Irvine, 1961 and Agoha, 1974). The purpose of this study was to screen these four plants for antibacterial activities using a simple and accurate indicator-based microdilution technique. The relative bioactivities of the four plants were also investigated. Such a study may be useful in rapid identification of cheaper, more readily available and culturally acceptable treatment for bacterial diseases.

Materials and Methods

Test plants

The leaves or stem bark of four plant species, *Morinda lucida*, *Mitragyna inermis, Khaya senegalensis* and *Nauclea latifolia* were investigated for antibacterial activity. The plants were purchased from a herbal shop in Makurdi metropolis and identified by Dr. H.O.A. Oluma, Department of Biological Sciences, University of Agriculture, Makurdi. The voucher specimens were deposited in the herbarium of the Department of Biological Sciences.

Test bacteria

Escherichia coli, Staphylococcus aureus and *Streptococcus spp.* isolated from clinical specimens (urine, wound and throat swabs) and identified at the TOSEMA Diagnostic Laboratories, Makurdi were used for the bacterial sensitivity tests.

Extraction procedure

Thirty milliliters (30 ml) of solvent (chloroform, methanol, water or petroleum ether) were added to dry pulverized leaf or stem bark (20 g) of each plant. The resulting suspension was allowed to stand in a tightly covered bottle for 48 h at room temperature after which it was filtered using Whatman's filter paper. The filtrate was collected as plant extract in sterile test tube and was concentrated by evaporation.

Antibacterial activity assay

The antibacterial activity of the various plant extracts was assayed using a slight modification of the microdilution techniques described by Drummond and Waigh (2000). An indicator solution was prepared by dissolving one tablet of resazurin dye in 40 ml of

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sterile water. An overnight broth culture of a test bacterium in nutrient broth was diluted serially in 0.1% peptone water to obtain 10^6 colony forming units/ml of broth culture. Extract solution was serially diluted two-folds in an appropriate solvent (acetone or dimethylsulphoxide) and placed in microtitre wells so that each well contained one hundred microliters (0.1 ml) of a dilution. The wells were labeled 1 – 10. An equal amount of broth culture (0.1 ml) and indicator solution (0.1 ml) were placed one after the other in each labeled well. Growth control solution comprised indicator solution and broth culture, while the sterile control consisted of indicator solution and sterile broth. The microtitre tray was incubated at 37^{o}C for 6 hours. Blue coloured solution meant growth inhibition in test wells, while pink coloured solution indicated growth or absence of inhibition. The highest dilution showing growth inhibition was taken as the minimal inhibitory concentration (MIC).

Results and Discussion

Extracts of the plants, *M. inermis, Mo. lucida, K. senegalensis* and *N. latifolia*, prevented growth of bacteria in broth cultures. Antibacterial activities of these plants, although on agar diffusion plates, have been reported (Abreu et al., 1999; Omer et al., 1998; Laurens et al., 1985; and Makinde et al., 1994). The results of the present study demonstrate the effectiveness of the microdilution technique in phytochemical screening of plants for antibacterial activities.

Antibacterial activity varied according to type of plant, part of plant use, extraction solvent and the test organism. For example, as shown in Table 1, *M. inermis* had the lowest MIC (0.03g/ml) and the highest percentage growth inhibition (100%) of the plants used in this study. These results show that *M. inermis* was the most bacteriologically active plant. The chi-square value (10.25), and the F-statistic (4.36) were significant at less than 0.05 level, and indicate that antibacterial activity was significantly associated with the type of plant used in treatment. Thus the differences in means or percentages of inhibition among the four types of plants were not due to chance. Leaf extracts showed a higher percentage of growth inhibition (77.8%) than stem bark extracts (53.3%). This suggests that the antibacterial constituents of the plants are preferentially concentrated in the leaves.

Although the inhibitory effects of aqueous and methanolic extracts of medicinal plants are usually reported (Ogunlana and Ramstad, 1975; Tignokpa *et al.,* 1986; Omer *et al.,* 1998 and Olayinka et al., 1992), our findings showed that petroleum ether and chloroform extracts of the plants studies have more inhibitory effect than methanolic and aqueous extracts. Among the three test bacteria, *E. coli* appeared to be the most sensitive to the inhibitory effects of the plant extracts. *E. coli*, though a Gram-negative bacterium, has been reported to be sensitive to extracts of *N. latifolia* (Omer *et al.*, 1998 and Abreu *et al.*, 1999) and *K. senegalensis* (Kudi *et al.*, 1999). Similarly, the studies of Ebana *et al.* (1993) showed that *E. coli* and some other Gram-negative bacteria were sensitive to extracts of *Strophantus lipidus* and *Secamone afzeli*.

Table 2 shows the regression result in which sensitivity (growth inhibition) was regressed on the independent variables – type of plant, plant part and extraction solvent. The R^2 (coefficient of determination) was 0.32 and the corresponding t-statistic was 4.59

Table 1: Mean and percentage values of inhibitory activity of four herbal plants used in ethnomedical practice.

Varia-bles		Coding numb-ers	Sensi-tive	Percent (%) sensi-tive	Mean	Non-sensi-tive	Total	Chi-square	MIC** (g/ml)	F-ratio
Plant type	*Morinda lucida*	1	5	55.6	1.6	4	9	10.25 (0.17)*	0.12	4.355 (0.01)
	Mitragyna inermis	2	9	100.0	2.0	0	9		0.03	
	Khaya senegalensis	3	3	33.3	1.3	6	9		0.25	
	Nauclea latifolia	4	5	83.3	1.8	1	6		0.06	
	Total		**22**			**11**	**33**			
Plant part	Leaf	1	14	77.8	1.8	4	18	2.20 (0.13)		2.21 (0.15)
	Stem bark	2	8	53.3	1.5	7	15			
	Total		**22**			**11**	**33**			
Extra-ction solvent	Chloroform	1	10	83.3	1.8	2	12	5.25 (0.15)		1.83 (0.16)
	Methanol	2	6	50.0	1.5	6	12			
	Water	3	3	50.0	1.5	3	6			
	Petroleum ether	4	3	100.0	2.0	0	3			
	Total		**22**			**11**	**33**			
Test bact-erium	*Escherichia coli*	1	9	81.8	1.8	2	11	3.55 (0.17)		1.81 (0.18)
	Staphylo-coccus aureus	2	5	54.5	1.4	6	11			
	Streptoco-ccus spp.	3	8	72.7	1.7	3	11			
	Total		**22**			**11**	**33**			

*Figures in parenthesis show level of statistical significance; **MIC = Minimal inhibitory concentration (g/ml).

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($p < 0.01$ level). Thus the regression line fits the data well. This shows that the independent variables put together influenced the *in vitro* inhibition of bacterial growth. The negative coefficient indicate that plant parts are inversely related to antibacterial activity, meaning that leaf extracts are more likely to be inhibitory to bacterial growth than stem bark.

The findings of this study demonstrate the usefulness of the indicator-based microdilution technique in phytochemical screening.

Table 2: Estimated multiple linear regression equation (coefficients) for growth inhibition of four plant extracts.

Explanatory variables	Variable identity	Estimated unstandardized coefficients (B)
Types of plants	V_1	0.47 (3.21)*
Plant parts	V_2	-1.15 (-3.68)*
Extraction solvent	V_3	-5.5×10^{-2} (-0.12)
Constant term	A	2.35 (7.80)*
R value	R	0.57
R square	R^2	0.33
F-statistic	F	4.59*
Sample size	N	33

Figures in parenthesis represent t-ratios; *Significance at 0.01 level.

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