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Susceptibility of tetracycline- and ampicillin-resistant strains of *Staphylococcus aureus* and *Salmonella typhi* to bioactive column fractions from the lichen *Ramalina farinacea* (L.) Ach

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Cases of infections caused by strains of *Staphylococcus aureus* and *Salmonella typhi* resistant to cheap and commonly available antibiotics such as ampicillin and tetracycline abound in Nigeria. However, several species of lichens with antibiotic properties are also scattered in many parts of Nigeria and are used locally in the Eastern region for the treatment of certain infections. The antibiotic properties of lichen substances have been known for long (Bustinza, 1951; Marshak, 1947; Ingólfsdóttir et al, 1985; Bérdy, 1982). Usnic acid, a very popular lichen substance has found clinical applicability in the treatment of infections in several European countries (Bérdy, 1982).

The antimicrobial properties of two column chromatographic fractions from the lichen *Ramalina farinacea* (L.) Ach. were evaluated against a total of 25 and 20 clinical isolates of *Staphylococcus aureus* and *Salmonella typhi* respectively resistant to both tetracycline and ampicillin (MIC > 1000 μg/ml). These fractions (B and C) were previously demonstrated to be biologically active by the brine shrimp lethality assay technique (Mclaughlin et al, 1991). These column fractions were obtained thus: A chloroformic extract of the lichen was fractionated in a glass column (internal diameter 20 mm and length 19 cm) using n-hexane : ethylacetate (4 : 1) as mobile phase and silica gel S (particles size 0.063 - 0.1 mm) as stationary phase. Fraction B is green in colour while fraction C is golden yellow. Both fractions, monitored by TLC consist of two components, with R_f values of 0.03/0.18 for fraction B and 0.19/0.93 for fraction C. The minimum inhibitory concentrations (MICS) of these fractions against the isolates were determined by a modification of the cup-agar diffusion plate method as described by Ebi and Ofoefule (1997). This involves making two-fold serial dilutions of the fraction in Dimethylsulphoxide (DMSO). Two drops (0.02 ml per drop) of these different dilutions are introduced into wells bored in nutrient agar plates already seeded with a standardized (about 10^5 CFU/ml) inoculum of the test microorganism. After incubation, the zones of inhibition are measured and the MIC obtained from the intercepts on the Y-axis of the graph of logarithm of concentration (Log Conc.) against the squares of the inhibition zone diameter (IZD^2). The results obtained (Table 1) show that fraction B is more effective against *Salmonella typhi* while C is more effective against *Staphylococcus aureus*. The parameter used for assessing the efficacy of the fractions is their MIC_{90} (the MIC of 90% of the isolates) and MIC_{50} (the MIC of 50% of the isolates). The MIC range of the fractions against all the clinical isolates generally reveals that the fractions have a commendable antimicrobial activity against these tetracycline and ampicillin resistant bacteria. That these fractions exhibit this degree of antimicrobial activity even without being purified is equally commendable. Currently, purification of the fractions and chemical/structural identification of the antibiotic component is in progress. It is possible that further purification of the fraction, could reduce their MIC against these microorganisms and hence improve their antimicrobial efficacy.

Table 1: MICs of fractions B and C against the test microorganisms

Fraction	MIC_{90} (μg/ml)		MIC_{50} (μg/ml)		MIC range (μg/ml)	
	Staph. aureus	*S. typhi*	*Staph. aureus*	*S. typhi*	*Staph. aureus*	*S. typhi*
B	302.5	95.5	194.5	64.5	70.8-504.2	11.2-158.5
C	230.5	254.5	158.5	161.0	10.3-398.6	114.8-389.7

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