IN-VITRO ANTIBACTERIAL SENSITIVITY OF *USNEA BARBATA* LICHEN EXTRACTED WITH METHANOL AND ETHYL- ACETATE AGAINST SELECTED *STAPHYLOCOCCUS SPECIES* FROM BOVINE MILK

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ABSTRACT

Our objective was to evaluate the antimicrobial potential of *Usnea barbata* lichen as a medicinal plant against selected *Staphylococcus* species isolated from raw milk of mastitis cows. *In-vitro* screening of the methanolic and ethyl-acetate extracts of *U. barbata* were evaluated to determine their antimicrobial activity against thirteen different *Staphylococcus* species. The selected organisms were isolated from raw bovine milk by several biochemical tests and identified with an API staph kit (bioMerieux, France). The antimicrobial activity of the extracts were evaluated using both the agar well diffusion method and the broth micro-dilution technique to determine the mean zone of inhibition and the minimum inhibitory concentration (MIC), respectively. The minimum bactericidal concentrations (MBC) of the extracts were also evaluated. Both the methanolic and ethyl-acetate extract showed variable antimicrobial activity against the *Staphylococcus* species with mean zones of inhibition ranging from 0 - 34 mm in diameter. Susceptibility by the *Staphylococcus* species tested in the methanol and the ethyl-acetate extract was 92.31% and 53.85% respectively. The MIC result for the methanol extract ranged from 0.0390 to10 mg/ml, while that of the ethyl-acetate extract ranged from 0.15625 to 5 mg/ml. The MBC's were in the range of 40 to > 160 mg/ml and 80 to > 160 mg/ml for the methanol and the ethyl-acetate extracts, respectively. Results from this study revealed the *in vitro* antimicrobial activity of *Usnea barbata* lichen and therefore validate the use of the plant in traditional medicine.

Key words: antimicrobial resistance, mastitis, microbial activity, medicinal plant

Acknowledgments: The authors wish to thank M the National Research Foundation and Govern Mbeki Research Development Centre, University of Fort Hare for funding.

INTRODUCTION

Antimicrobials are widely used for treatment of various animals. Over the years, the continuous use of antimicrobials in the dairy sector has led to the emergence of resistant strains of several pathogens that are linked to the cause of mastitis disease in animals (Pitkala *et al*., 2004). The need for cheaper and available source of active medicinal plant for mastitis therapy gave a reason for the study. The study aimed to evaluate the *in-vitro* antimicrobial activity of methanolic and ethyl-acetate extracts of *Usnea barbata* lichens on some selected *Staphylococcus* species isolated from milk of cows.

MATERIAL AND METHODS

Isolation and identification

Staphylococcus species were done using several biochemical tests including gram staining, catalase and oxidase test before they were finally identified as *Staphylococcus* species (to their species level) with API staph kit (Biomerieux Inc., Quebec).

Plant sample and extracts preparation

Ground *Usnea barbata* lichen air-dried in room temperature was serially extracted with methanol and ethyl acetate solvent, respectively. Extraction was done using a portion of 400 g of the *Usnea barbata* plant lichen in an extraction bottle with methanol and ethyl-acetate.

Testing for antimicrobial sensitivity

The agar well diffusion technique was used to test for the antimicrobial sensitivity of the plant.

Determination of minimum inhibitory concentration (MIC) and minimum bacteria concentration (MBC) of plant extracts

Minimum inhibitory concentration (MIC) of the plant extracts against the bacterial species was determined using the broth micro-dilution method in 96-well micro-titer plates (Banfi *et al*., 2003). The minimum bacteria concentration (MBC) of the plant extracts was determined from the MIC result.

RESULT AND DISCUSSION

The results obtained from the experiment showed that the zones of inhibition for methanol extract ranged from 10 to 34 mm while that of ethyl- acetate ranged from 0 to 23 mm (Table 1). Amoxicillin (0.01 µg/ml) which was used as a positive control gave a zone of inhibition in the range of 17 to 47 mm (Table 1). With reference to the break point (inhibition zone diameter $\geq$ 11), six out of the thirteen bacterial strains subjected to the plant extract were the most resistant organisms to both methanol and ethyl-acetate extract with partial zones of inhibition viz, *Staphylococcus haemolyticus*, *Staphylococcus capitis*, *Staphylococcus cohnii-urealyticus*, *Staphylococcus cohnii-cohnii*, *Staphylococcus hominis* and *Staphylococcus saprophyticus*.

Tab.1. zone of inhibition (mm) of Usnea barbata extracts and amoxicillin against the test organisms.

	Solvent extracts of *Usnea barbata* plant (mg/ml)						
	methanol			ethyl-acetate			amoxicillin
Species	5	10	20	5	10	20	0.01
S. aureus	14 ±1	ND	ND	0 ± 0	0 ± 0	15 ± 0.58	23 ± 1.5
S. sciuri	15 ±1	17 ± 2	ND	13	14 ± 1.8	15 ± 3	30 ± 0.57
S. xylosus	34 ± 1.7	ND	ND	23 ± 1.2	ND	ND	47 ± 1.5
S. chromogene	17 ± 1	18 ± 0.57	ND	13 ± 0.7	15 ± 1.7	ND	21 ± 3.1
S. lentus	14 ± 1.7	17 ± 1	19	13 ± 0.57	14 ± 1.7	ND	17 ± 0.57
S. cohnii [a]	10 ± 1.5	ND	ND	12	14 ± 0.9	19 ± 1.5	39 ± 1.5
S. haemolyticus	16 ± 0.6	ND	ND	8 ± 7.5	9 ± 6.3	14 ± 1.7	27 ± 1.5
S. capitis	16 ± 14	ND	ND	0	9 ± 6	10 ± 8	30 ± 6.5
S. epidermidis	26 ± 1.5	ND	ND	16 ± 2	19 ± 1.6	21 ± 0.6	37 ± 2.1
S. warneri	22 ± 3	ND	ND	14 ± 0.5	15 ± 0.4	16 ± 2	40 ± 0.6
S. cohnii [b]	18 ± 1.7	ND	ND	0 ± 0	7 ± 5	9 ± 7	36 ± 1.1
S. hominis	22 ± 1.7	ND	ND	0 ± 0	14 ± 1.4	17 ± 2.5	39 ± 1
S. saprophyticus	14 ± 1	ND	ND	0 ± 0	10 ± 6	12 ± 10	20 ± 2

(*S. cohnii* [a]: *Staphylococcus cohnii – cohnii*, *S. cohnii* [b]: *Staphylococcus cohnii – urealyticus*). (ND: Not Determined).
(* values are in mean ± standard deviation, n= 3).

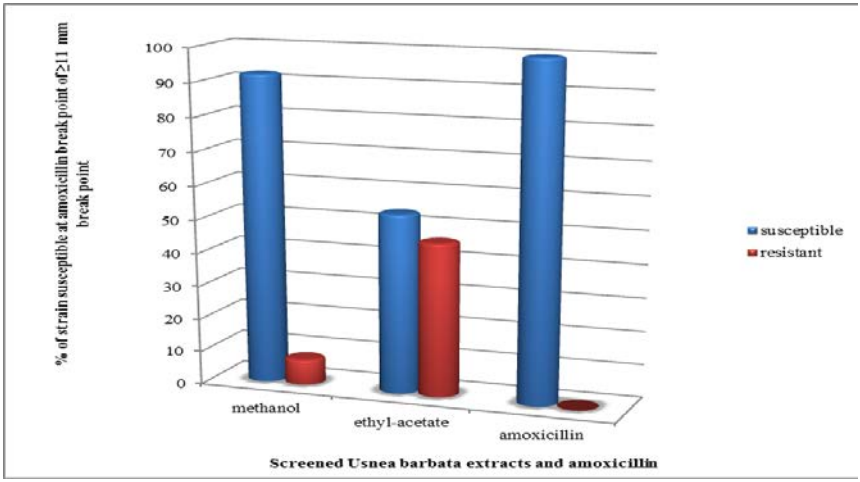

Fig. 1. Sensitivity of methanol and ethyl-acetate extracts at 5mg/ml against the test organisms.

On the other hand, four strains: *Staphylococcus xylosus*, *Staphylococcus sciuri*, *Staphylococcus lentus* and *Staphylococcus epidermidis*, were the most susceptible organisms. Out of the thirteen *Staphylococcus* species that were tested, the susceptibility of the bacterial organisms to the standard antibiotic (amoxicillin) and methanol extract was 100% and 92.31% respectively while that of the ethyl-acetate extract was 53.85% (Figure 1). The MIC results showed that methanol and ethyl-acetate had an antimicrobial activity ranging from 0.0390 to 10mg/ml and 0.15625 to 5mg/ml respectively while the MIC for amoxicillin on the other hand had a ranged of 0.625 to10 µg/ml (Table 1). The minimum bactericidal concentration (MBC) of methanol extract ranged from 40 to > 160 mg/ml while that of ethyl-acetate ranged from 80 to > 160 mg/ml. The overall activity of both extracts for all the tested organisms vary between 0.039 - 10 mg/ml. This result suggests that *Usnea barbata* lichen extracted with methanol and ethyl-acetate solvents possess some potential antimicrobial compound that inhibited the tested organisms and may be broad spectrum. Further investigation on the activity of this plant against the tested microbial organisms (using other solvents) may be required in the present search for new antimicrobial drugs.

Tab. 2. Antibacterial activity of Usnea barbata extracts and amoxicillin against the test organisms.

	MIC[a] (mg/ml)		
Staphylococcus species	methanol	ethyl-acetate	amoxicillin
S. aureus	1.25	2.5	$6.25x10^{-4}$
S. sciuri	$3.125x10^{-1}$	$1.5625x10^{-1}$	$3.125x10^{-4}$
S. xylosus	na[b]	na	$7.8125x10^{-5}$
S. chromogene	10[c]	1.25	$6.25x10^{-4}$
S. lentus	10	$6.25x10^{-1}$	$5x10^{-3}$
S. cohnii- cohnii	$3.125x10^{-1}$	$3.125x10^{-1}$	$2.5x10^{-3}$
S. haemolyticus	$6.25x10^{-1}$	$6.25x10^{-1}$	$1.25x10^{-3}$
S. capitis	$6.25x10^{-1}$	$1.5625x10^{-1}$	1.562^{-4}
S. epidermidis	$1.5625x10^{-1}$	$3.125x10^{-1}$	1.562^{-4}
S. warneri	$3.90x10^{-2}$	$3.125x10^{-1}$	$2.5x10^{-3}$
S. cohnii-urealyticus	$3.125x10^{-1}$	$3.125x10^{-1}$	$6.25x10^{-4}$
S. hominis	$6.25x10^{-1}$	2.5	$1.0x10^{-2}$
S. saprophyticus	10	5	$5x10^{-3}$

[a]: minimum inhibitory concentration, [b]:Not active, [c] : highest concentration of extract tested.

CONCLUSIONS

The experiment showed that methanol and ethyl-acetate extracts of *Usnea barbata* exhibited *in-vitro* antimicrobial activities with the methanol extracts being more active and bactericidal. It is therefore proposed that further investigation should be carried out on the plant lichen to determine the natural bioactive compounds present in the plant.

REFERENCES

Banfi, E., Scialino, G. and Monti-Bragadin, C. 2003. Development of a microdilution method to evaluate *Mycobacterium tuberculosis* drug susceptibility. *Journal of Antimicrobial Chemotherapy*, 52: 796-800.

Pitkala, A., Haveri, M., Pyorala, S., Myllys, V. and Honkanen-Buzalski, T. 2004. Bovine Mastitis in Finland 2001- Prevalence, Distribution of Bacteria, and Antimicrobial Resistance. *Journal of Dairy Science*, 87: 2433-2441.