Antimicrobial Activity of Ageratum conyzoides against Staphylococcus aureus

P.A.D.M. Dilrukshi, S.N. Premathilake *and* K.B Wijesekara
Uva Wellassa University, Badulla, Sri Lanka.

Introduction

Ageratum conyzoides is a native annual branching herb, which grows to approximately 1 m in height and usually found open and abandoned areas . This plant is commonly used in traditional medicine, especially for wound healing (Sing et al., 2012).

Methodswhich are usedinhealing wound infections include debridement, antibiotics, tissue grafts and proteolytic enzymes. However, these methods have major drawbacks and unwanted side effects. Recently there is a tendency towards the uses of traditional medicines as it shows the better cultural acceptability and better compatibility with the human body and also fewer side effects (Parekh et al., 2005).

One of the common bacterium that causes infections in wounds is *Staphylococcus aureus*. It is a facultative, anaerobic,Gram-positivecoccibacteriumwhich isfrequently found in the human skin.Thus, themain objective of this research was to study the effect of *A.conyzoides*extracts against *S.aureus*.This study further investigated thepotentialdevelopment of a commercial herbal ointmentto heal infected wounds using*A.conyzoides*extracts.

Methodology

Fresh plant materials of *A.conyzoides* were collected and washed using tap water .Then they were separated into flowers, leaves, roots and stems and were air dried in shade for 7 days and powdered using mortar and pestle. The powdered plant materials were sieved and stored in airtight containers. Plant material extracts were obtained with 95% methanol using the soxhlet apparatus. Each extracts were concentrated and solvents were fully evaporated, by rotary evaporator (150 rpm) at 40 C. ºThe obtained concentrates were transferred to McCarthy glass vials and placed under room temperature for complete dryness. Then they were stored in airtight vials under refrigerated conditions.

*Staphylococcus aureus*pure cultures were collected from the Medical Research Institute (MRI) Colombo and from them liquid cultures were prepared using Nutrient broth. Then they were incubated at 37°C for 24 hrs.

The powdered plant materials were measured into separate McCartney bottles and appropriate volume of the extracts were added to make a stock solution of 200mg/mL.

Sterile nutrient agar plates were prepared and allowed to solidify. A 0.1 mL of liquid culture of *S. aureus*was spread equally on the solidified nutrient agar plate. After one hour five wells were dug in each plates using a sterile cork borer (5mm diameter).Concentration series of extracts (200mg/mL, 100mg/mL,50 mg/mL, 25mg/mL and 12.5 mg/mL) were prepared and from them 0.5mLof extracts were added to wells in appropriately labelled plates. As the control 95% methanol was used. The plates were left on the bench for few minutes for the extract to diffuse into the agar and later incubated at 37°C for 24hours. After the incubation the zone of clearance around each well was measured using a metric ruler by taking measurement of the inhibition zones. Minimum inhibition concentration (MIC) was determined for extracts that showed less than 7 mm(<7 mm) diameter inhibition zone.

Herbal ointment was prepared using sterilized bee wax as the base. The ointment was evaluated using physical evaluation and antibacterial test. And finally all the results were statistically analysed using MINITAB 15 statistical software.

Results and Discussion

Roots of *A.conyzoides* gave the highest amount of crude extract. However, *A. conyzoides* leaves extract showed highest inhibition zone (Figure 1) and it was noted that with the concentration of the extracts, inhibition zones showed increments.

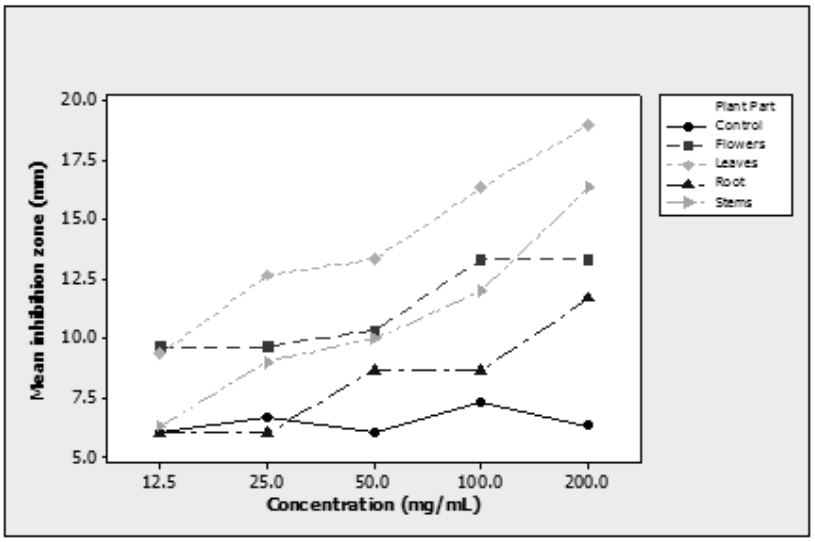

Figure 1. Mean inhibition zone of different crude types at different concentrations

According to the analysis of variance (ANOVA) both concentration and crude type significantly affected inhibition zone diameter ($P < 0.05$) (Table 1).

Table 1. Analysis of Variance for Inhibition diameter

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Plant Part	4	522.347	522.347	130.587	103.09	0.000
Con.	4	319.547	319.547	79.887	63.07	0.000
Plant Part*Con	16	124.853	124.853	7.803	6.16	0.000
Error	50	63.333	63.333	1.267		
Total	74	1030.080				

S = 1.12546 R-Sq = 93.85% R-Sq(adj) = 90.90%
Con. = Concentration of the extract

Further the best activity resulted by each crude along with the concentrations was identified through mean value comparison. Leaves extract showed highest inhibition diameter at 200mg/mL concentration. It was the best combination. According to the Dunnet Test, there were no other effective extracts compared to best selected types ($P < 0.05$) (Table 2).

Table 2: Results of Dunnett simultaneous test; Response Variable Inhibition diameter, Comparisons with Control Level

Plant level = 1 subtracted from:

Plant level	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	-2.867	0.6166	-4.65	0.0001
3	-5.933	0.6166	-9.62	0.0000
4	-3.400	0.6166	-5.51	0.0000
5	-7.667	0.6166	-12.43	0.0000

As leaves extracts gave a higher inhibition diameter at 12.5 mg/mL of Minimum Inhibition Concentration (MIC) they were selected for the preparation of herbal ointment (Figure 1).

Antibacterial test results were gained from well diffusion test for ointment compared with commercial antibacterial ointment, Betadine®. The prepared ointment showed a better antimicrobial activity against *Staphylococcus aureus* than commercial ointment (Figure 2).

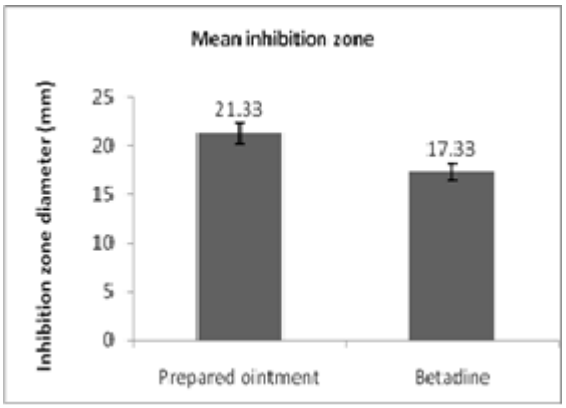

Figure 2. Mean inhibition zone resulted against prepared ointment and Betadine® after 24 hour incubation time

Conclusions

The leaf extracts of the *A.conyzoides* plant, had good antibacterial activity against *S.aureus*. Prepared herbal ointment also showed antibacterial activity against *S.aureus.*

References

Singh, S.B., Devi, W.R., Marina, A.W., Devi, I., Swapana, N., Singh, C.B., 2012. Ethnobotany, phytochemistry and pharmacology of *Ageratum conyzoides* Linn (Asteraceae). Journal of Medicinal Plants Research, 7 (8), 371-385.

Parekh, J., Jadeja, D., Chanda , S., 2005. Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. Turkish Journal of Biology, 29, 203-210.