Antibacterial Activity of Medicinal Plants Used to Treat Infectious Disease in Northern Peru

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ABSTRACT

OBJECTIVE: In order to compare the treatment used by the curanderos (healers) with Western medical practice, the antibacterial activity of plant extracts from various plants such as Uncaria tomentosa (Ecuador’s Chornus), Borrago officinalis (Echinacea), Desmodium indicum (African Mollicum), Physelinus nitens (Pinecones), Picthops tomentosus (Uncaria tomentosa), and Libertia flava was measured.

HYPOTHESIS: The plant extracts will have antibacterial activity.

METHODS: Plants were purchased, dried, ground. Alcoholic extracts were prepared, concentrated, dried, and suspended in boiling water, and sterilized. Bacterial growth inhibition against S. aureus and E.coli was measured spectrophotometrically at various concentrations. RESULTS: Dose was normalized to percent growth and IC50 values were calculated. Plant extracts were more efficacious against S. aureus than E.coli. The IC50 of plant extracts used for infections against S. aureus was 250 ng/mL. Two of these had IC50 values against S. aureus <1000 ng/mL.

CONCLUSION: Antibacterial activity was observed in all plants. Further study of plants with high antibacterial activity could identify new antibacterial compounds.

BACKGROUND

Northern Peru is thought to be the center of the "Central Andean Health Axis," an area with a rich supply of potential pharmaceuticals. This region includes the "Central Andean Biodiversity Hotspot." With its diverse climates (Fig. 1) Peru has a high degree of biodiversity (Figure 2) among the flora native to Northern Peru. Native healers of Northern Peru have a long history of using plants to combat infectious disease. Traditional medicine is still practiced in Northern Peru and is a critical component of daily life.

Since the late 1900s, hundreds of plants used by the curanderos have been taxonomically classified. In addition, many of these plants have been identified for use in medicine to treat a variety of conditions including bacterial infections.

Today, modern technology has allowed scientists, to explore the chemical composition of medicinal plants. This project was designed to study plants for the treatment of diseases believed by allopathic medicine to be caused by bacterial infections. Through the evaluation of antibacterial activity of selected plant extracts and plant mixture extracts, we were able to observe antimicrobial activity of plants used by curanderos to treat infectious disease.

RESULTS

Antimicrobial Activity of Plant Extracts:

Concentration-response curves were used to show the effects of various plant extract concentrations on the bacterial growth of E.coli and S.aureus. In all 12 experiments of Figure 7, bacterial growth was seen at lower extract concentrations, and in all experiments, bacterial inhibition was seen at higher extract concentrations. Bacterial growth at lower extract concentrations is thought to be due to the natural chemical nutrients in plants which stimulate the growth of bacteria rather than inhibit their growth. Achicoria (TB and TG), Mentha (TD and TJ) and Alamos (TE and TJ) are said to be noninhibitory (NI) since their concentration-response curve did not show any inhibition of bacterial growth. In all 10 experiments, plant extracts screened against S.aureus showed a greater inhibition of bacterial growth at lower extract concentrations then their corresponding experiments in E.coli.

CONCLUSIONS

Antibacterial activity was seen in all of the plant extracts, used by the curanderos to treat infectious disease.

Antibacterial activity for plants used to treat infectious disease by the curanderos show similarity to allopathic medicine.

REFERENCES

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METHODS

Plant Collection:

Voucher specimens used in this study were purchased at a local market (Fig. 4), identified by botanists, and stored at the Universidad Nacional de Trujillo, Peru and the Missouri Botanical Garden, MO, USA.

Extract Preparation:

Plants purchased from the market were dried, ground, and incubated in 1L ethanol for one week to extract chemical components. Extracts made from single plants contained 50g of plant material in 1L of ethanol. Extracts were then filtered to remove plant debris, dried by rotary evaporation, transferred to a glass vial, and stored at 0°C. As needed, frozen extracts were thawed, resuspended in boiling water, and filtered sterilized. Extract dry weights were used to determine concentration.

Bacterial Growth Assay (BGA):

Bacterial strains Escherichia coli ATCC 29242 and a clinical isolate of Staphylococcus aureus were used. A single colony was inoculated into 2 mL of Mueller-Hinton Media and incubated for 24 hours at 37°C. Fresh overnight bacteria were prepared for each experiment from this master stock which was made fresh each week.

Plant extracts were diluted 1:2 in media, and the diluted extracts were serially diluted (1:4). Each dilution was subsequently aliquoted into four tubes to give an n=4. The bacteria were added to each tube. Controls are prepared for media alone, for extract alone, for total growth with no extract, and with an antibiotic for growth inhibition. Next 4 steps are visualized below.

Data Analysis:

Data were normalized to percent survival or percent growth, in comparison to negative controls (without extract). Data were then plotted as concentration-response curves and the section of the curves showing maximum change was fit to a line (y=mx+b). The concentration which caused 50% inhibition of bacteria (IC50) were calculated where y=0.

RESULTS and DISCUSSION

Figure 8: (A) Uninhibited screening curves for the inhibition of S. aureus growth by Borraga. (B) IC50 values were calculated by modifying the initial curve to contain a negative slope. The line of best fit for the modified graph is shown. (C) IC50 values were calculated by interpolating the concentration that results in 50% growth, using the line of best fit. The IC50 values for each test were then averaged and the standard deviation was calculated.

Analysis of IC50:

Table 1 shows the IC50 values of the two plant sets. The same volume of resuspended plant extract was used for each set to eliminate potential confounding variables. For Manayapa, it is important to note that only 3 replicates were used due to the absence of a S. aureus pellet post-centrifugation. The concentration-response curve for Chancapiedra against S.aureus had to be modified twice to determine the corresponding IC50 values. According to Table 1, all plant extracts tested had at least one bacterial strain that it could inhibit. For all of the plant extracts there was a much lower concentration required to inhibit the growth of S.aureus than required to inhibit the growth of E.coli (lower IC50). This observation shows less bacterial strains that are incubated with plant extracts of varying concentrations.

TABLE 1: Summary of Extract IC50 values against S.aureus and E.coli

<table>
<thead>
<tr>
<th>Latin Botanical Name</th>
<th>Common Name</th>
<th>IC50 (mg/mL)</th>
<th>IC50 (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthospermum spinosum</td>
<td>Alamos</td>
<td>18.3 ± 0.3 (4)</td>
<td>0.0376-9.438 (4)</td>
</tr>
<tr>
<td>Borrago officinalis</td>
<td>Borraga</td>
<td>7.0 ± 2.0 (5)</td>
<td>20.7 ± 0.2 (4)</td>
</tr>
<tr>
<td>Deodorum mollis</td>
<td>Manayapa</td>
<td>0.38 ± 0.05 (7)</td>
<td>29 ± 4 (5)</td>
</tr>
<tr>
<td>Physilcanus nitens</td>
<td>Chancapiedra</td>
<td>0.15 ± 0.02 (8)</td>
<td>18 ± 3 (6)</td>
</tr>
<tr>
<td>Picthops tomentosus</td>
<td>Anticoccra</td>
<td>13 ± 1 (12)</td>
<td>0.0655-16.25 (4)</td>
</tr>
<tr>
<td>Uña de Gato</td>
<td>Uña de Gato</td>
<td>0.5 ± 0.1 (18)</td>
<td>9.6 ± 0.1 (4)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are round to show n=4, 7, 8 or 12. Plant extracts that did not inhibit growth (non-inhibitory) at the indicated concentrations are marked as "NI." The plant extracts that were determined to be NI have IC50 values that are too high to be therapeutically relevant (IC50 ≥ 10 mg/mL). Ideally, IC50 values are minimized (i.e., a lower concentration is needed to kill bacteria).

The plant extracts that determined to be NI were the plants Chornus, Chornus, Chornus, and Chornus. The IC50 values were calculated for these plants. The plant extracts that were determined to be NI have IC50 values that are too high to be therapeutically relevant (IC50 ≥ 10 mg/mL). Ideally, IC50 values are minimized (i.e., a lower concentration is needed to kill bacteria).