IN-VITRO
SUSCEPTIBILITY OF
MYCOBACTERIUM
ULCERANS TO HERBAL
PREPARATIONS

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INTRODUCTION

- Surgery is the main treatment option for Buruli ulcer because antimicrobials have not been that effective.
- The World Health Organization has provisionally recommended the use of selected antimycobacterials in combination with surgery, while the search for an effective antimycobacterial continues.
- There are unconfirmed reports of successful treatment of BU in Ghana with herbal preparations
- Therefore this study sort to:
  1) Identify herbal preparations with anti-\textit{M. ulcerans} activity
  2) Determine their minimum inhibitory concentrations (MICs).
METHODOLOGY

Identification of herbal Preparations

Since the effective herbal preparations are trade secrets of the herbal practitioners, this study sort to identify the anti-BU herbs by following their ethnomedical uses:

i. Treatment of sores, new/old wounds, ulcers
ii. Treatment of skin disorders (rashes, measles, herpes, cancer)
iii. Have antimicrobial property

On this basis, and the cooperation of a vendor of herbal medicines, trust of 4 (BU) herbal practitioners and literature, 69 herbal preparations were obtained

Criteria for inclusion in study

(i) Limited resources and (ii) long incubation period of *M. ulcerans* necessitated sifting of the herbs obtained
Rapid verification of antimicrobial property in 24-48 hours

- Herbs prepared into infusions (20%), juices (20%), decoctions (10%) and lyophilised powders. Liquids used at 1:5 dilution; Powders used at 50mg/ml [Reference drugs: Pefloxacin, Miconazol]

- Agar dilution method (incubation for 24-48 hours)

- Battery of 11 bacteria and 2 fungi (some with predilection for the skin. Used at 0.5 McFarland )
  a) 5 Gram-positive bacteria: Staphylococcus aureus, Staphylococcus spp., Bacillus cereus, Bacillus spp and Streptococcus spp.
  b) 6 Gram-negative bacteria: Pseudomonas aeruginosa, Escherichia coli, Haemolytic E. coli, Salmonella typhimurium, Salmonella spp and Klebsiella spp
  c) 2 fungi: Candida albicans and Aspergillus spp.
RESULT OF RAPID SCREENING

Fifty-two (75.40%) of the 69 herbal preparations demonstrated antimicrobial activity by inhibiting the growth of at least 1 of the 13 microbes tested.

Screening of the 52 herbal preparations for anti-\textit{M. ulcerans} activity

- Agar dilution method (incubation for 8 weeks)
- Battery of 8 (phenotype/PCR authenticated) \textit{M. ulcerans} isolates
  - 2 reference isolates (Ghana, Benin – Kindly provided by Prof. Francoise Portaels)
  - 6 clinical isolates (Ghana: Amasaman, Nsawam)

Concentration of incorporation: 1:5 dilution
Concentration of \textit{M. ulcerans} inoculum: McFarland 1(10^{-1})
RESULT OF ANTI-M. *ULCERANS* SCREENING

- Twenty-eight (53.85%) of the 52 herbal preparations demonstrated *in-vitro* anti-*M. ulcerans* activity.
- Twenty (71.43%) of the 28 herbal preparations with anti-*M. ulcerans* activity are purportedly used for the treatment of sores, wounds, ulcers, BU, TB and leprosy.

**Determination of the Minimum Inhibitory Concentrations of the 28 herbal preparations**

- Agar proportion method/Two fold serial dilution
- Concentration of inoculum McFarland 1($10^{-1}$, $10^{-3}$)
- Concentration of herbs: Liquids 1:5, Powders 50mg/ml
**RESULTS**

b. 1. Extended Antimicrobial Profile of herbal preparations with anti-\textit{M. ulcerans} activi

<table>
<thead>
<tr>
<th>Scientific &amp; Common Names</th>
<th>Other Microbes Inhibited \textit{In-Vitro}</th>
</tr>
</thead>
</table>
| 1. \textit{Hydrastis canadensis}  
Goldenseal                    | St, Pa, Ca, Sa, Asp, Bsp, Strep, Staph, Bc [9/13] |
| 2. \textit{Allium sativum}  
[White garlic]                | St, Ca, Sa, Asp, Bsp, Strep, Sal, Staph, Ek, Hek, Kp Bc [12/13] |
| 3. \textit{Allium sativum}  
[Purple garlic]                | St, Ca, Sa, Asp, Bsp, Strep, Sal, Staph, Ek, Hek, Kp, Pa, Bc [13/13] |
| 4. \textit{Syzygium aromaticum}  
[Clove]                        | St, Ca, Sa, Asp, Bsp, Strep, Sal, Staph, Ek, Hek, Kp Pa, Bc [13/13] |
| 5. Tonic 1                  | St, Pa, Sa, Asp, Bc, Bsp, Strep, Sal, Staph, Kp [10/13] |
| 6. Swedish bitters           | St, Ca, Sa, Asp, Bsp, Strep, Sal, Staph, Ek, Hek, Kp Pa, Bc [13/13] |
| 7. Tonic 2                  | St, Sa, Bc, Bsp, Strep, Sal, Staph, Kp [8/13] |
| 8. Tonic 3                  | Sa, Asp, Bc, Bsp, Strep, Sal, Staph [7/13] |

\textit{St} (\textit{Salmonella typhimurium}), \textit{Ca} (\textit{Candida albicans}), \textit{Sa} (\textit{Staphylococcus aureus}), \textit{Asp} (\textit{Aspergillus} spp.), \textit{Bsp} (\textit{Bacillus} spp.), \textit{Strep} (\textit{Streptococcus} spp.), \textit{Sal} (\textit{Salmonella} spp), \textit{Staph} (\textit{Staphylococcus} spp.), \textit{Ek} (\textit{Escherichia coli}), \textit{Hek} (\textit{Haemolytic E. coli}), \textit{Kp} (Klebsiella spp), \textit{Pa} (\textit{Pseudomonas aeruginosa}), \textit{Bc} (\textit{Bacillus cereus})
RESULTS (cont 1)

Fig. 1. Extended Inhibitory Activity of Herbal Preparations with Anti-M. ulcerans activity
### Table 2a. Minimum Inhibitory Concentration (MIC) of Liquid Herbal Preparations

<table>
<thead>
<tr>
<th>HERBS</th>
<th>Reference Isolates</th>
<th>Clinical Isolates</th>
<th>Mean MIC of Herb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ghana Ref. ATCC 970321</td>
<td>Benin Ref. ATCC 990826</td>
<td></td>
</tr>
<tr>
<td>2. Tonic 2 (from herbal practitioner)</td>
<td>6.25 12.50</td>
<td>12.50 6.25 12.50 6.25 6.25 6.25</td>
<td>8.59 (3.23)</td>
</tr>
<tr>
<td>4. Syzygium aromaticum [Clove]</td>
<td>12.50 12.50</td>
<td>12.50 12.50 12.50 12.5 12.50 12.50</td>
<td>12.50 (.00)</td>
</tr>
<tr>
<td>5. Allium sativum, white variety [White Garlic]</td>
<td>1.56 3.13</td>
<td>1.56 3.13 6.25 0.39 0.78 0.39</td>
<td>2.15 (1.98)</td>
</tr>
<tr>
<td>6. Allium sativum, purple variety [Purple Garlic]</td>
<td>3.13 3.13</td>
<td>3.13 1.56 3.13 3.13 3.13 1.56</td>
<td>2.74 (0.73)</td>
</tr>
<tr>
<td>7. Swedish bitters</td>
<td>3.13 1.56</td>
<td>1.56 0.20 1.56 0.78 1.56 1.56</td>
<td>1.49 (0.84)</td>
</tr>
<tr>
<td>8. Hydrastis canadensis [Goldenseal]</td>
<td>0.78 3.13</td>
<td>3.13 1.56 3.13 0.20 0.20 0.20</td>
<td>1.54 (1.39)</td>
</tr>
<tr>
<td>Mean Susceptibility of Individual Isolates</td>
<td>6.54 (5.18) 6.06 (4.29)</td>
<td>6.25 (5.22) 3.93 (3.89) 5.67 (4.41) 3.69 (4.08) 3.84 (3.97) 3.59 (4.09)</td>
<td></td>
</tr>
<tr>
<td>Mean Susceptibility of Isolates by Community</td>
<td>5.28 (4.45)</td>
<td>3.70 (3.87)</td>
<td></td>
</tr>
</tbody>
</table>
## RESULTS (cont 4)

Table 2b. Minimum Inhibitory Concentration (MIC) of Liquid Herbal Preparations, presented as cumulative number

<table>
<thead>
<tr>
<th>Concentration of Liquid Herbal Preparations (%vol/vol), [Titre])</th>
<th>HERB</th>
<th>0.20</th>
<th>0.39</th>
<th>0.78</th>
<th>1.56</th>
<th>3.13</th>
<th>6.25</th>
<th>12.50</th>
<th>25.00</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[1:640 dil]</td>
<td>[1:320 dil]</td>
<td>[1:160 dil]</td>
<td>[1:80 dil]</td>
<td>[1:40 dil]</td>
<td>[1:20 dil]</td>
<td>[1:10 dil]</td>
<td>[1:5 dil]</td>
<td></td>
</tr>
<tr>
<td>1. Tonic 1</td>
<td></td>
<td>6</td>
<td>1(7)</td>
<td>1(8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Tonic 2</td>
<td></td>
<td>5</td>
<td>3(8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Tonic 3</td>
<td></td>
<td>5</td>
<td>1(6)</td>
<td>2(8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Syzygium aromaticum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8(8)</td>
<td></td>
</tr>
<tr>
<td>[Clove]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Allium sativum, white variety</td>
<td></td>
<td>2</td>
<td>1(3)</td>
<td>2(5)</td>
<td>2(7)</td>
<td>1(8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[White garlic]</td>
<td></td>
<td></td>
<td>1(3)</td>
<td>2(5)</td>
<td>2(7)</td>
<td>1(8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Allium sativum, purple variety</td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Red garlic]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Swedish bitters</td>
<td></td>
<td>1</td>
<td>1(2)</td>
<td>5(7)</td>
<td>1(8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Hydrastis canadensis</td>
<td></td>
<td>3</td>
<td>1(4)</td>
<td>1(5)</td>
<td>3(8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goldenseal</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Number of M. ulcerans isolates (n=8)
Cumulative number of M. ulcerans isolates inhibited at each dilution (n=8)
RESULTS (Cont 5)

Fig. 2. Inhibitory activities of herbal preparations against M. ulcerans isolates

Inhibitory concentrations (% vol/vol) vs. M. ulcerans isolates

- Tonic 1
- Tonic 2
- Tonic 3
- Syzygium aromaticum
- Allium sativum (white)
- Allium sativum (purple)
- Swedish bitters
- Hydrastis canadensis
Discussions, Conclusions, Recommendations

- Extended antimicrobial activity exhibited by 75.40% of the herbal preparations,
- Confirms the veracity of their ethnomedical uses
- The assertion by several other ‘phytoscienists’, that the surest way to identify higher plants for drug discovery is by follow-up of their ethnomedical uses (Spieler, 1981; Ogura et al., 1982; Oubre et al., 1997; Fabricant and Farnsworth, 2001).

- The anti-\textit{M. ulcerans} findings have:
  - Confirmed the anti-BU claims of 2 traditional herbal practitioners who provided Tonics 1, 2, 3; while negating that of the other two.
  - Indirectly confirmed claims that a number of BU patients in the rural areas are being successfully treated with herbs since 71.43% of the herbal preparations that demonstrated anti-\textit{M. ulcerans} activity are traditionally used for the treatment of sores, wounds and ulcers.
Several herbal preparations demonstrated anti-\textit{M. ulcerans} activity, suggesting that herbal therapy may hold promise as a BU treatment option.

The activities (MICs) of the 8 herbal preparations were largely determined by the herbal preparations themselves (P < 0.01; \( \eta^2 = 72.5\% \))

Very minimally influenced by the isolates (P > 0.05, \( \eta^2 = 7.9\% \))

Very minimally influenced by the source of the isolates (P > 0.05, \( \eta^2 = 3.6\% \))

THEREFORE, strongly suggesting that the herbal preparations investigated may be effective against other Ghanaian \textit{M. ulcerans} isolates.
Discussions, Conclusions, Recommendations

- At some stage of BU infection a large number of bacilli are present, suggesting that a favourable condition for the selection of resistant mutants could be created. (combination therapy)

- Unfortunately though, the use of the TB drugs could lead to an increase in drug resistance, especially in undiagnosed TB patients, which could eventually result in an increase in TB cases, especially in TB/BU endemic communities.

- In view of this, herbal therapy would be worth considering and actively pursued as a BU treatment option, since:
  - A single herb is never a single compound but a group of compounds, which potentiate each other or create synergy (Stermitz et al., 2000).
  - The use of an herb or herbal cocktail would simulate combination therapy, which may prevent, or at worse, delay the development of antimicrobial resistance.
Observations in BU patients and *M. ulcerans* infected animals demonstrate that *M. ulcerans* disseminates into regional lymph nodes, visceral organs and bone marrows, with resultant osteomyelitis, (Portaels *et al.*, 1998; Addo *et al.*, 2005).

These complication are often secondarily infected by other microorganisms.

The use of a single herbal preparation with activity against both *M. ulcerans* and other microbes will simultaneously treat the *M. ulcerans* infection and some of the accompanying complications.

**THEREFORE HERBAL THERAPY SHOULD BE STUDIED FURTHER, SINCE IT MAY BE AN EFFECTIVE MEANS OF TREATMENT**
The team members are grateful to the World Health Organization for funding the study, Professor Françoise Portaels for providing the reference isolates and Drs. Mensah Quianoo (Amasaman Hospital) and Aninakwa (Nsawam) for providing the biopsies.

The team members also acknowledge with gratitude the invaluable assistance of traditional herbal practitioners, vendors and users of traditional herbal medicines and staff of the Botany Department of the University of Ghana.

Research Team (Noguchi Mem. Institute)


THANK YOU