THE EFFECT OF SOME NIGERIAN LOCAL HERBS ON HELICOBACTER PYLORI

1Smith, S. I., 2Oyedoji, K.S., 3Opere, E., 4Iwalokun, B. A., 5Omonigbehin, E. A.
1Department of Microbiology, 2Department of Botany and Microbiology, 3Department of Genetics and
Blood Disorders, Nigerian Institute of Medical Research. P.M.B 2013, Yaba, Lagos, Nigeria.
4Department of Biochemistry, Lagos State University, Ojo, P.M.B. 1087, Apapa, Lagos, Nigeria.

Correspondence to: Dr. S.I. Smith (E-mail: stellaismith@hotmail.com)

Four Nigerian medicinal plants commonly used in the treatment of bacterial infections were tested for
antimicrobial activity against twenty local strains of Helicobacter pylori recovered from patients with
gastro-duodenal ulcers and gastritis. In vitro agar diffusion assay revealed anti-Helicobacter pylori
activity of ethanolic extracts of C. papaya and M. lucida to 80% (16/20) of the isolates tested. While the
ethanolic extracts of O. gratissimum and P. amarus inhibited the colonial growth of 35% (7/20) of
these strains. The zones of inhibition ranged from 5 - 20 mm in diameter. Contrastingly, the aqueous
extracts of these plants appeared to lack anti-Helicobacter pylori activity except in M. lucida and O.
gratissimum where inhibition of a total of three isolates was observed. The present results suggest
the presence of anti-Helicobacter pylori principles in ethanolic extracts of C. papaya and M. lucida
and support their future use in the treatment of ulcers and gastritis in Nigeria.

INTRODUCTION

It is over a decade that Helicobacter pylori infections were
known as a major cause of gastro-
duodenal ulcers, gastritis and
stomach cancer with greater
burden of cases documented in
developing countries (1, 2). Most
effective therapies employ a
synergic action between a gastric
acid release inhibitor and one or
more antibiotics to eradicate
Helicobacter pylori, its urease and
associated diseases (3, 4). However, the implementation of
these therapies in communities
where the poor bears the greater
brunt of the disease is cost
ineffective as concerned drugs are
poorly patronized (5).

In Nigeria, Helicobacter pylori
is fastly replacing non steroidal
anti-inflammatory drugs (NSAIDs)
as causal agent of gastro-duodenal
ulcers and duodenal perforation
cases are also of significant
increase (6). More worrisome is the
increased resistance trend of
Helicobacter pylori isolates to
metronidazole, amoxycillin and
tetracycline in vitro (7) in a manner
that discourage their future clinical
use against Helicobacter pylori
infection in the country. In Nigeria,
a triple therapy involving
omeperazole, metronidazole and
amoxycillin are widely used in
severe cases and treatment failures
with this combination have been
reported (8). Alternative triple
therapies that could be used are expensive, have undesirable side effects (9) and a long list of contraindications (10). The discoveries that Allium vegetables habit anti-Helicobacter pylori substances (11,12) have further heightened the global search for similar compounds in other medicinal plants and this to some extent has yielded encouraging results that have the therapeutic applications in areas of discovery as a barrier (13). Morinda lucida (Rubiaceae), Ocimum gratissimum (Lamiaceae), Carica papaya (Caricaceae) and Phyllanthus amarus (Euphorbiaceae) extracts are among the folkloric remedies that have been confirmed scientifically to possess clinical values against protozoal and bacterial infections in Nigeria (14, 15, 16, 17). These plants grow abundantly in Nigerian soils and are not ethnically or age group biased in use. This further explains why their potentials in the future treatment of gastritis and ulcers in Nigeria are investigated.

**MATERIALS AND METHOD**

**Plant materials**

The plants, were collected from various local markets in Lagos, identified and confirmed by Mrs. B. Opare of the Department of Botany, Lagos State University. Voucher samples of these plants were subsequently deposited in the Department. The plants used were listed in Table 1.

**Extraction**

A simple extraction procedure of Olukoya et al (18) was adopted to prepare aqueous and organic extracts of the plants tested. To prepare aqueous extracts, 1.1 g of plants' leaves (previously dried at 50°C and ground into fine powders) were steeped in 10 ml of sterile-distilled water at 30 – 32°C for five days. The organic extracts were prepared by steeping 1.2 g of plant materials in 5 ml of 40% ethanol. Extracts were then passed through Hemmings filters (BTI UK) and the resulting sterile filtrates were aseptically transferred to sterile bottles and labeled as crude extracts of individual plants. The organic extracts were subsequently reconstituted with phosphate buffered saline solution (pH 7.2) to nullify the effect of ethanol on the tested organisms. A mixture of 0.1 ml of sterile water and 5 ml of 40% ethanol was prepared as a control.

**Microbial cultures**

Twenty strains of Helicobacter pylori recovered from the biopsy samples of patients with gastritis and gastro-duodenal ulcers from Western Nigeria were used as test organisms. Helicobacter pylori ATCC 49503 was used as control. All organisms were cultured on Columbia agar base (Oxoid, CM331) containing 7% sheep blood.
Sensitivity Testing

Antimicrobial susceptibility testing was carried out using the agar diffusion technique. In brief, Isosensitest agar (Oxoid, UK) plates were holed (6 mm in diameter) with the aid of a sterile cork-borer and seeded with 10 μL of H. pylori suspension (McFarland 3). The plates were dried in the air and 100 μL of plant extract was introduced into the wells. The plates were incubated microaerophilically (5% O₂, 10% CO₂) at 37°C for 4 days. Holes containing bacterial suspension (10 μL of 9 × 10⁸ CFU/ml) and sterile water or ethanol (100 μL) were used as controls. Diameters of zones of inhibition of both the tested organisms and standard strain (H. pylori ATCC 45903) were measured in millimeters (mm) and recorded.

RESULTS.

Table 2 gave the summary of the antimicrobial activity of aqueous and ethanolic extracts of the plants against the twenty H. pylori isolates and the standard strain. The ethanolic extracts of C. papaya, M. lucida, O. gratissimum and P. amarus provided evidence of anti-Helicobacter pylori activity in 80% (16/20) and 30% (7/20) of the isolates tested. Helicobacter pylori ATCC 49503 was observed to be susceptible to the organic extracts of C. papaya and M. lucida only. The zones of growth inhibition were 5 – 20 mm in diameter. Apart from the water extracts of M. lucida and O. gratissimum, which inhibited the growth of 10% (2/20, 5 – 15 mm) and 5% (1/20, 10 – 15 mm) of the isolates tested, aqueous extracts of other plants were found susceptible to these isolates. The standard strain was resistant to all aqueous preparations. Unlike sterile water wells, no colonial growth was found in 40% ethanol control wells.

<table>
<thead>
<tr>
<th>Table 1: The local herbs selected for testing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Botanical name</strong></td>
</tr>
<tr>
<td>C. papaya</td>
</tr>
<tr>
<td>M. lucida</td>
</tr>
<tr>
<td>O. gratissimum</td>
</tr>
<tr>
<td>P. amarus</td>
</tr>
</tbody>
</table>

*Nigerian (Yoruba) names.*
Table 2: Antimicrobial activity of ethanol and water extracts of the four local herbs

<table>
<thead>
<tr>
<th>Strain code no</th>
<th>C. papaya</th>
<th>M. lucida</th>
<th>O. gratissimum</th>
<th>P. amarus</th>
<th>Sterile water 40% Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>W</td>
<td>E</td>
<td>W</td>
<td>E</td>
</tr>
<tr>
<td>Hp 1</td>
<td>0</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hp 2</td>
<td>2+</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>Hp 3</td>
<td>1+</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hp 4</td>
<td>2+</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>2+</td>
</tr>
<tr>
<td>Hp 5</td>
<td>1+</td>
<td>0</td>
<td>3+</td>
<td>0</td>
<td>2+</td>
</tr>
<tr>
<td>Hp 6</td>
<td>3+</td>
<td>0</td>
<td>1+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hp 7</td>
<td>2+</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>2+</td>
</tr>
<tr>
<td>Hp 8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hp 9</td>
<td>2+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hp 10</td>
<td>2+</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hp 11</td>
<td>2+</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>2+</td>
</tr>
<tr>
<td>Hp 12</td>
<td>1+</td>
<td>0</td>
<td>3+</td>
<td>0</td>
<td>2+</td>
</tr>
<tr>
<td>Hp 13</td>
<td>2+</td>
<td>0</td>
<td>1+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hp 14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hp 15</td>
<td>2+</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>2+</td>
</tr>
<tr>
<td>Hp 16</td>
<td>1+</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>Hp 17</td>
<td>2+</td>
<td>0</td>
<td>3+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hp 18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hp 19</td>
<td>2+</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>Hp 20</td>
<td>3+</td>
<td>0</td>
<td>3+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hp 49305</td>
<td>2+</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Keys: W = water extract; E = Ethanollic extract; 0 = No inhibition; 1+ = 5 - 9 mm diameter zone of inhibition; 2+ = 10 - 15 mm; 3+ = 16 - 20 mm; Hp = Helicobacter pylori strains.

**DISCUSSION**

In this study, ethanolic extracts of *C. papaya* and *M. lucida* were observed to prevent the growth of 80% of Helicobacter pylori strains tested in vitro. Similar extracts of *O. gratissimum* and *P. amarus* also demonstrated anti-Helicobacter pylori activity but in only 35% (7/20) of the isolates. The lack of inhibition observed in water wells confirmed the viability of all the isolates tested. While the inhibitory effect of 40% ethanol attested to the appropriateness of the reconstitution procedure, the poor antibacterial activity of the water extracts of these plants implies that water has inadequate power to extract anti-Helicobacter pylori principles from these plants. However, With respect to organic extraction, this finding has provided scientific evidence for antibacterial activity of Morinda lucida in vitro as most scientific findings described the plant as an anti-malaria herb (14). The study of Agomo et al (19), which demonstrated a complex array of cellular responses to *M. lucida*.
administered to mice infected with *Plasmodium yoelii nigeriensis* seemed to provide an indication that numerous biological properties are inherent in this plant. *Ocimum gratissimum* has been extensively demonstrated to inhibit aetologic agents of diarrhoeal *in vitro* and *in vivo* (15, 20). However, the result obtained from this study should be interpreted with caution as strains of *O. gratissimum* are characterized by varying chemical composition (21). The present study has also extended the biological functions of *Carica papaya* whose seeds have demonstratable evidence of having antifertility effects in rats (22). Although, antimalaria activity of *P. amarus* in mice and rats has been observed in the laboratory (*Personnal communication*), this is first time anti-*Helicobacter pylori* activity will be ascribed to this plant in Nigeria. Based on our findings, there is no doubt that these plants hold tremendous clinical promise especially in rural communities, which provide the greater number of patients and severe cases of gastro-duodenal ulcer. The present study is still in its infancy and therefore invite more research studies to elucidate the active anti-*Helicobacter pylori* substances in these plants, investigate synergy associated with extract combination leading to ultimate suggestion of whether these plants can be combined with orthodox drugs to met the criteria of gastric acid suppression, *H. pylori* eradication and stomach protection in the treatment of ulcers and gastritis. In conclusion, the present study has revealed, the tremendous potentials inherent in ethanolic preparations of *C. papaya* and *M. lucada* if adopted for future treatment of ulcers and gastritis in Nigeria.

REFERENCES


