Shigellocidal Properties of Three Nigerian Medicinal Plants: *Ocimum gratissimum*, *Terminalia avicennoides*, and *Momordica balsamina*

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**ABSTRACT**

The prevalence of multidrug-resistant shigellae is an important concern in the treatment of shigellosis in many developing countries, and other therapies, including herbal agents, may provide an important alternative to antimicrobial agents. In this study, three Nigerian medicinal plants: *Ocimum gratissimum*, *Terminalia avicennoides*, and *Momordica balsamina* were investigated for their activities against multidrug-resistant *Shigella* species isolated from patients with bacillary dysentery in Lagos. Decoctions of *O. gratissimum* and concoctions of *O. gratissimum* and *T. avicennoides* at crude concentration of 3,000 µg/mL markedly inhibited the growth of all isolates tested. Zones of inhibition indicating susceptibilities of the organisms varied from 18.3 to 21.5 mm for *Shigella dysenteriae*, 15.3 to 16.3 mm for *S. flexneri*, 18.8 to 19.3 mm for *S. sonnei*, and 16.5 mm for *S. boydii*. Except *S. flexneri*, minimum inhibitory concentration and minimum bactericidal concentration revealed a higher shigellocidal property of *O. gratissimum/T. avicennoides* concoction than other extracts in *S. dysenteriae* (300–515.6 vs 337.5–1,312.5 µg/mL), *S. sonnei* (309.4–543.8 vs 403.1–1,312.5 µg/mL), and *S. boydii* (243.8–337.5 vs 253–1,312.5 µg/mL). *O. gratissimum* showed a greater shigellocidal effect against the *S. flexneri* isolates, while extracts of *M. balsamina* possessed low shigellocidal potential. The results suggest that aqueous extracts of *O. gratissimum* and *T. avicennoides* as decoctions and concoctions could be useful in the treatment of shigellosis and should be clinically evaluated specially in Nigerian region.

**Key words:** Plants, Medicinal; *Ocimum gratissimum*; *Terminalia avicennoides*; *Momordica balsamina*; Anti-infective agents; *Shigella*; Dysentery, Bacillary; Nigeria

**INTRODUCTION**

Diarrhoeal diseases still remain a public-health problem in developing countries (1,2). In Nigeria, shigellosis is endemic in most communities. Severe infections initially seen in children aged less than five years are now frequently occurring among adolescents and young adults (3,4). This observation emphasized the need of better treatment, preferably using simple but useful local ingredients and herbs.

The medicinal plants, including *Ocimum gratissimum* (Lamiaceae), *Momordica balsamina* (Curcurbitaceae), and *Terminalia avicennoides* (Combretaceae) are abundantly grown in Nigeria and are used in folk medicine for treating ailments, including various digestive disorders, and asthma (5). The essential oil obtained from the seeds of *O. gratissimum* contains thymol and eugenol in amounts ranging from 32% to 65%. These substances
are antiseptic, antitussive, and antispasmodic in functions (6). Ilori et al., Akinsinde and Olukoya, and Akinyemi et al. have shown that the extracts of leaf and bark of these plants possess antidiarrhoeal properties (7-9). However, the effects of these compounds on specific pathogens, such as shigellae, are not known. It is, thus, important to determine the antibacterial effects of these compounds against shigellae, specifically multidrug-resistant *Shigella* infections that have been so common in the country during the last 10 years (10).

In the present study, we have examined the antibacterial activities of decoctional and concoctional preparations of three plants: *O. gratissimum* (Lamiaceae), *M. balsamina* (Cucurbitaceae), and *T. avicennoides* (Combretaceae) against *Shigella* species isolated from stools of patients with bacillary dysentery.

**MATERIALS AND METHODS**

**Medicinal plants**

Fresh leaves of *O. gratissimum* and *M. balsamina* detached from their stems and the stem-bark of *T. avicennoides* purchased from a local market in Lagos were used in this study. The plants were vouchered as Morb-002, Ogr-010, and Tav-005 after authentication at the Botany Department of Lagos State University, Nigeria.

**Preparation of extracts**

Aqueous extracts of the plants were prepared according to the method of Olukoya et al. (11). Seventy gram each of dried, ground leaves and 50 g of powdered stem-bark were soaked separately in 200 mL of autoclaved water for seven days at 30–32 °C under sterile conditions. The extracts were filtered through a millipore filter (0.25 µm). The resulting filtrate was concentrated under reduced pressure at 50 °C and then transferred to a labelled sterile bottle.

**Shigella isolates**

Four isolates each of *Shigella dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei* recovered from the stools of patients with shigellosis in Lagos, Nigeria, were screened. All the isolates were sensitive to ofloxacin but showed ≥75% resistance to tetracycline, co-trimoxazole, streptomycin, colistin sulphate, ampicillin, and streptomycin.

**Antimicrobial activity**

An inoculum of size 10^8cfu/mL of each of the isolates was prepared according to the method of Bauer et al. (12). A loopful (0.002 mL) of each inoculum corresponding to 1x10^7cfu per plate was streaked evenly on Mueller-Hinton agar (Difco, USA). The plates were dried in the air and then mounted with ofloxacin disc (5 µg), and discs were pre-soaked with crude extracts of the medicinal plants (3,000 µg/mL). Another inoculum (similar size) of *Escherichia coli* ATCC25922 was also used on Mueller-Hinton agar to serve as a negative control. The inoculated plates containing discs pre-soaked with sterile water served as positive control. All the plates were incubated aerobically at 37 °C for 24 hours. Diameters of zones of inhibition were measured in mm and recorded.

**Minimum inhibitory concentrations**

Decoctions and concoctions of the extracts were tested for shigellocidal activity using the macrobroth dilution method. Each decoction was diluted with Mueller-Hinton broth to yield concentrations of 1,500, 750, 375, 300, 187.5, and 150 µg/mL. The two- and three-extract concoctions were prepared in 1:1 and 1:1:1 ratio by volume respectively. Minimum inhibitory concentration (MIC) assays were set up to contain 1 mL of extract preparation, 1 mL of Mueller-Hinton broth, and 5x10^4cfu/mL of bacterial cells per tube. A 2-mL Mueller-Hinton broth containing 5x10^4cfu/mL of bacterial cells of each *Shigella* species served as control.

**Minimum bactericidal concentrations**

Minimum bactericidal concentrations (MBCs) of extract preparations were determined by the agar diffusion method (13). Ten microlitre suspension from each negative tube in the MIC assays and from the positive growth control tubes were inoculated into Mueller-Hinton agar. The plates were incubated aerobically at 37 °C for 24 hours. MBCs were defined as the lowest concentrations of extracts that produced negative subcultures or only one colony.

**RESULTS**

The results on the investigation of shigellocidal properties of aqueous extracts of the stem-bark of *T. avicennoides* and leaves of *O. gratissimum*, and *M. balsamina* are presented in Table 1–3.

At a concentration of 3,000 µg/mL, decoctions of *O. gratissimum* and concoctions of *T. avicennoides*/*O. gratissimum* displayed a relatively higher growth inhibition against all *Shigella* isolates tested and *E. coli*.
controls. In terms of diameter of the zone of inhibition, these preparations inhibited bacterial growth on Mueller-Hinton agar ranging from 18.3±2.6 to 21.5±3.4 mm in S. dysenteriae, 15.3±1.3 to 16.3±3.5 mm in S. flexneri, 18.8±1.7 to 19.3±1.5 mm in S. sonnei, 16.5±2.1 to 16.5±2.9 mm in S. boydii, and 18.3±1.5 to 20.3±4.8 mm in E. coli.

In all the organisms tested, decoctions and concoctions involving M. balsamina produced smaller inhibition zones ranging from 4.0±1.6 to 9.5±0.5 mm (Table 1).

### DISCUSSION

The aqueous extracts of O. gratissimum and T. avicennoides, when used singly or in combination, showed marked shigellocidal activity that is comparable to the antibacterial effects of ofloxacin. This observation indicates that the two plants have shigellocidal properties and provide a scientific basis of their therapeutic use, especially in rural areas where these herbal preparations are commonly used. This study has further indicated the wide range of Gram-negative coverage of T. avicennoides. The vibriocidal property of the plant was reported by Akinsinde and Olukoya (8), while Malcolm and Sofowora (14) demonstrated its antibiotic action against Gram-positive organisms, such as Sarcina lutea, Staphylococcus aureus, and Mycobacterium phlei.

The 3,000-µg/mL dose, at which we compared the efficacy of O. gratissimum with ofloxacin, 24.3.8-431.3 µg/mL at which O. gratissimum and concoction with

### Table 1. Antibacterial effects of decoctions and concoctions of plant extracts by the disc-diffusion technique

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates</th>
<th>Decoctions (3,000 µg/mL)</th>
<th>Concoctions (µg/mL)</th>
<th>Ofloxacin (5 µg)</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>a+b</td>
<td>a+c</td>
</tr>
<tr>
<td>S. dysenteriae</td>
<td>4</td>
<td>18.3±2.6</td>
<td>16.3±2.8</td>
<td>9.5±5.0</td>
<td>21.5±3.4</td>
</tr>
<tr>
<td>S. flexneri</td>
<td>4</td>
<td>16.3±3.5</td>
<td>12.5±1.3</td>
<td>4.0±1.6</td>
<td>15.3±1.3</td>
</tr>
<tr>
<td>S. sonnei</td>
<td>4</td>
<td>18.8±1.7</td>
<td>15.0±2.2</td>
<td>7.8±1.7</td>
<td>19.3±1.5</td>
</tr>
<tr>
<td>S. boydii</td>
<td>4</td>
<td>16.5±2.9</td>
<td>12.3±2.8</td>
<td>6.8±1.0</td>
<td>16.5±2.1</td>
</tr>
<tr>
<td>E. coli</td>
<td>1</td>
<td>18.3±1.5</td>
<td>15.8±2.2</td>
<td>5.8±1.3</td>
<td>20.3±4.8</td>
</tr>
</tbody>
</table>

Figures represent diameters of growth inhibition zones expressed in mm as mean±standard deviation of four determinations

### Table 2. Minimum inhibitory concentrations of decoctions and concoctions of medicinal plants

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates</th>
<th>Decoctions (µg/mL)</th>
<th>Concoctions (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>S. dysenteriae</td>
<td>4</td>
<td>337.5</td>
<td>468.8</td>
</tr>
<tr>
<td>S. flexneri</td>
<td>4</td>
<td>393.8</td>
<td>431.3</td>
</tr>
<tr>
<td>S. sonnei</td>
<td>4</td>
<td>403.1</td>
<td>431.3</td>
</tr>
<tr>
<td>S. boydii</td>
<td>4</td>
<td>253</td>
<td>337.5</td>
</tr>
<tr>
<td>E. coli</td>
<td>1</td>
<td>206.3</td>
<td>309.4</td>
</tr>
</tbody>
</table>

Figures represent MIC values in µg/mL as means of four determinations

The shigellocidal properties of extract preparations, as demonstrated by diffusion on Mueller-Hinton agar, using negative cultures from MIC assays, further confirmed the greater shigellocidal effect of concoctions of O. gratissimum/T. avicennoides when compared with other extract preparations, except in S. flexneri in which O. gratissimum was more effective. Analysis of MBC profiles revealed 515.6 vs 562.5–1,312 µg/mL in S. dysenteriae, 750 vs 703.1–1,125 µg/mL in S. flexneri, 543.8 vs 731.3–1,312.5 µg/mL in S. sonnei, and 337.5 vs 403.1–1,312.5 µg/mL in S. boydii (Table 3).
Seed-oils of *O. gratissimum* have also been employed to provide MIC and MBC values far lower than what we obtained (13,15). These differences could be linked to species and strain differences, susceptibility-testing conditions, and the fact that changing chemical composition is a property found among strains of *O. gratissimum* and within the Basil family (16). Except *S. flexneri* in which *O. gratissimum* showed more effectiveness, we observed that concoctions of *O. gratissimum* and *T. avicennoides* showed more shigellocidal effect in terms of growth inhibition, MIC, and MBC than other extract preparations, and this suggests synergistic action of active principles in both the plants against shigellae. In their study on the antibacterial properties of Vietnamese cajeput oil and *Ocimum* oil combination, Jedlikova et al. made a similar suggestion (17). Interestingly, *S. flexneri* was found not to succumb to the synergy between *T. avicennoides* and *O. gratissimum*. This further reveals the relevance of speciation in the management of shigellosis and supports the ongoing efforts to develop and set clinical trials on different vaccines for infections due to *S. dysenteriae* and *S. flexneri* (18,19). Our results on decoctions and concoctions involving *M. balsamina* tend to suggest that the plant has low shigellocidal potentials. This is not consistent with what was reported by Akinyemi *et al.* on the efficacy of this plant against local isolates of *Salmonella typhi* and *S. paratyphi* (9).

**Table 3.** Minimum bactericidal concentrations of decoctions and concoctions of medicinal plants by the agar diffusion method

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates</th>
<th>Decoction (µg/mL)</th>
<th>Concoction (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td><em>S. dysenteriae</em></td>
<td>4</td>
<td>562.5</td>
<td>937.5</td>
</tr>
<tr>
<td><em>S. flexneri</em></td>
<td>4</td>
<td>703.1</td>
<td>750</td>
</tr>
<tr>
<td><em>S. sonnei</em></td>
<td>4</td>
<td>731.3</td>
<td>750</td>
</tr>
<tr>
<td><em>S. boydii</em></td>
<td>4</td>
<td>403.1</td>
<td>562.5</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1</td>
<td>337.5</td>
<td>543.8</td>
</tr>
</tbody>
</table>

Figures represent MBC values in µg/mL as means of four determinations
a=Aqueous leaf extract of *O. gratissimum*
b=Aqueous bark extract of *T. avicennoides*
c=Aqueous leaf extract of *M. balsamina*
NT=Not tested

It can be concluded that *O. gratissimum* and *T. avicennoides* are effective in inhibiting the growth of multidrug-resistant shigellae. These preparations need to be further characterized as potential therapeutic agents in shigellosis.

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REFERENCES


*T. avicennoides* elicited MIC and 290.6-750 µg/mL at which minimum bactericidal action was elicited, was far greater than 4 mg/mL and 8.0 mg/mL reported by Ilori *et al.* (7) as MIC and MBC of aqueous extract of *O. gratissimum* against a local strain of *S. dysenteriae*.


