Antitrichomonal and antioxidant activities of *Dorstenia barteri* and *Dorstenia convexa*

**Abstract**

*Dorstenia barteri* and *D. convexa* extracts and some isolated components of the former were investigated for effectiveness against *Trichomonas gallinarum* and compared with quercetin and quercitrin. The antioxidant activity of the extracts/compounds was also determined. The minimum lethal concentrations (MLCs) for the extract of *D. barteri* leaves and twigs at 24 h were found to be 15.625 and 15.625 µg/ml, respectively. However, the MLCs of the leaf and twig extract of *D. convexa* were 125 and 437.5 µg/ml, respectively. The prenylated and geranylated chalcones were as active as the prenylated flavones, 6-prenylapigenin and the diprenylated derivative 6,8-diprenyleridictyol. The order of the antitrichomonal activity of the compounds at 24 h was: quercetin (0.121 µg/ml) > quercitrin (0.244 µg/ml) ≥ bartericin B (0.244 µg/ml) > bartericin A (0.73 µg/ml) > stigmasterol (0.98 µg/ml) > 6,8-diprenyleridictyol = isobavachalcone = dorsmanin F (31.25 µg/ml). *D. barteri* extracts, quercitrin, and bartericin A, and the prenylated flavonoids had potent antioxidant properties. The twig extract of *D. barteri* was more potent than the leaf extract. Moderate (EC₅₀ >50 µg/ml) and high (EC₅₀ <50 µg/ml) antioxidant activities were detected in the leaf and twig extracts of *D. barteri* and the prenylated flavonoids. Prenylated flavonoids and the isolated compounds with antioxidant properties described here may account for the anti-inflammatory action of these extracts. The antitrichomonal and antioxidant activities shown by the extracts and compounds in this study are consistent with the ethnomedicinal and local use of the *Dorstenia* species studied.

**Introduction**

There are about 170 species of the genus *Dorstenia* (Moraceae) worldwide (1). Decoctions of the leaves of some of these species are used for cough, headache and stomach pain (2). Other uses include gout and various skin diseases (3).

Trichomoniasis affects men and animals causing untold economic loses in poultry and livestock and sometimes high morbidity in man. The prevalence of trichomoniasis is
significantly higher in communities with high HIV prevalence (29.3% in Kisumu and 34.3% in Ndola) than in Cotonou (3.2%) and Yaoundé (17.6%) (4). In Nigeria, prevalence ranges from 6 to 46% depending on the age, profession and location of the subjects (5-9). *Trichomonas gallinarum* affects birds including poultry, causing high morbidity and mortality especially in young birds. There is no information available in the literature concerning the antitrichomonas activity of *Dorstenia* species. Antioxidant polyphenols are common in plants (10). Many defense mechanisms within the organism have evolved to limit the levels of reactive oxidants and the damage they inflict (11). It is estimated that 5% of all *T. vaginalis* patients’ isolates display some level of resistance to metronidazole (12). In addition, patients also have adverse reactions to high doses of metronidazole or are allergic to this agent (13). Therefore, the search for a new antitrichomonal agent is certainly justified. The present study was carried out to examine and identify an agent from the array of compounds and extracts of *Dorstenia* species that possess antitrichomonal and antioxidant activities, to complement the use of this plant in the treatment and/or management of human disorders including arthritis, rheumatism, gout, stomach disorders, cough, headache, and skin diseases (1-3).

**Material and Methods**

**Plants and compounds**

Plant samples were collected from the Central Province of Cameroon. Specimens of the plants are deposited at the National Herbarium, Yaounde, Cameroon. Combined CH$_2$Cl$_2$/MeOH (1:1) extracts of *Dorstenia convexa* and *D. barteri* were obtained as previously described (14). Four hundred and fifty grams of the twigs and 210 g of the leaves were extracted with a mixture of methylene chloride and methanol (1:1) for 24 h. Dorsmanin F and 6,8-diprenyleridictyol (Table 2) were isolated from *D. manni* as previously described (15-17). Quercetin (18), quercitrin (19), amethoflavone (20), and gedunine were obtained from *Carapa grandifolia* (21). Ascorbic acid was obtained from Hach Company, Loveland, CO, USA, while 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma, St. Louis, MO, USA.

**Antitrichomonal assay**

*Trichomonas gallinarum* was cultured in Ringer’s-egg-serum medium according to the method of Boeck and Drbohlav (22) as modified by Levine (23). According to Meingasser and Thurner (24), the minimal lethal concentration (MLC) is the lowest concentration of the test extract or compound at which no motile organism is observed. Samples of the compounds (4-10 mg) were dissolved in 1 ml dimethylsulfoxide and further diluted to appropriate final concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1, 5, 10, 100, 250, 500, 1000 µg/ml) on 96-well flat bottom microtiter plates held at 37°C in an incubator. At least three different concentrations were tested for each compound/extract in triplicate analyses. MLCs were determined by the microplate method (13). End points (defined as lack of motility) were assessed at 24 and 48 h.

**Free radical scavenging activity**

The free radical scavenging activity of each extract and/or compound was analyzed by the DPPH assay (25) as described by Sanchez-Moreno et al. (26). The test compounds, at concentrations ranging from 10 to 100 µg/ml, were mixed with 3 ml 0.1 mmol DPPH/l (in ethanol) in a cuvette. The time-course of the change in absorbance at 517 nm was monitored for 20 min. The antioxi-
Antitrichomonal activities of the extracts/compounds were evaluated by measuring the value of the absorbance at 517 nm when the reaction plateau step was reached. A minimum of three different concentrations for each compound/extract were tested in triplicate analyses. The percentage of remaining DPPH was calculated according to the equation:

$$%\text{DP HH}_{\text{REM}} = \frac{[\text{DPPH}]_0}{[\text{DPPH}]_0} \times 100$$

where $[\text{DPPH}]_0$ is the remaining concentration of the stable radical without the antioxidant and $[\text{DPPH}]_0$ is the remaining concentration at the reaction plateau step. For each compound/extract tested, a simple regression analysis was used to relate the response variable (percentage of remaining DPPH) to the independent variable (antioxidant concentration). The EC$_{50}$ was interpolated or extrapolated from each related model. The EC$_{50}$ values are expressed in terms of µg antioxidant per mg of DPPH.

**Results**

**Compounds isolated**

The twigs and the leaves yielded 60 g of extract each. Chromatographic separation of these extracts yielded 40 mg isobavachalcone (0.067%) from the twigs and 36 mg (0.06%) from the leaves; 29 mg 4-hydroxylonchocarpin (0.048%) from the twigs and 18 mg (0.03%) from the leaves; 35 mg bartericin A (0.058%) from the twigs and 46 mg (0.077%) from the leaves, while 30 mg bartericin B (0.05%) was obtained from the twigs but was not detected in the leaves.

**Antitrichomonal assays**

The MLCs for the extract of *D. barteri* leaves and twigs were found to be 15.625 and 15.625 µg/ml, respectively. *D. convexa* leaf extract with an MLC of 125 µg/ml was found to be less potent than *D. barteri* extract (Table 1). The activities of the compounds isolated from *D. barteri*, such as isobavachalcone, 4-hydroxylonchocarpin, bartericins A and B, and stigmasterol, were compared with quercetin and quercitrin isolated from *Mallotus oppositifolium*, 6-prenyl-apigenin isolated from *D. kameruniana* and 6,8-diprenylidictyol, dorsmanin F from *D. manni*; amenthoflavone from *Cannarium shwuenfurthi*, and gedunine from *Carapa grandifolia* (Table 2). The order of antitrichomonas activity of the compounds is: quercetin (0.121 µg/ml) > quercitrin (0.244 µg/ml) ≥ bartericin B (0.244 µg/ml) > bartericin A (0.73 µg/ml) > stigmasterol (0.98 µg/ml) > 6,8-diprenylidictyol = isobavachalcone = dorsmanin F (31.25 µg/ml). Some of these compounds were more effective than metronidazole (0.625 µg/ml). Quercetin, with an MLC of 0.121 µg/ml at 24 h, is the most active compound.

**Antioxidant assays**

Figure 1 shows the relationship between the concentration of DPPH radicals and the time which elapsed since mixing the DPPH solution with the extracts and compounds examined. The lower the percent remaining DPPH, the higher the antioxidant activity. The twig extract of *D. barteri* was more effective than the leaf extract. Four of the compounds and the twig extract displayed high antioxidant activities (EC$_{50}$ <50 µg/ml).

Table 1. Antitrichomonas activities of *Dorstenia* extracts.

<table>
<thead>
<tr>
<th>Product</th>
<th>Plant name</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td><em>D. convexa</em></td>
<td>125 ± 0</td>
<td>125 ± 0</td>
</tr>
<tr>
<td>Twigs</td>
<td><em>D. convexa</em></td>
<td>437.5 ± 125</td>
<td>416 ± 144</td>
</tr>
<tr>
<td>Leaves + twigs</td>
<td><em>D. barteri</em></td>
<td>125 ± 0</td>
<td>125 ± 0</td>
</tr>
<tr>
<td>Leaves</td>
<td><em>D. barteri</em></td>
<td>15.625 ± 0</td>
<td>15.625 ± 0</td>
</tr>
<tr>
<td>Twigs</td>
<td><em>D. barteri</em></td>
<td>15.625 ± 0</td>
<td>15.625 ± 0</td>
</tr>
</tbody>
</table>

Data are reported as means ± SD for assays in triplicate. MLC = minimum lethal concentration.
Table 2. Comparison of antitrichomonas activity of *Dorstenia* compounds with other active compounds.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>MLC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td><em>Isobavachalcone</em></td>
<td>31.25</td>
</tr>
<tr>
<td><em>4-Hydroxylonchocarpin</em></td>
<td>800 ± 0</td>
</tr>
<tr>
<td><em>6-Prenylapigenin</em></td>
<td>93.6 ± 0</td>
</tr>
<tr>
<td><em>6,8-Diprenylidictyol</em></td>
<td>31.25 ± 0</td>
</tr>
<tr>
<td><em>Dorsmanin F</em></td>
<td>31.25 ± 0</td>
</tr>
<tr>
<td>Amemothoflavone</td>
<td>500 ± 0</td>
</tr>
</tbody>
</table>

Continued on next page
Table 2  continued.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>MLC (µg/ml)</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gedunine</td>
<td>500 ± 0</td>
<td>500 ± 0</td>
<td></td>
</tr>
<tr>
<td>*Bartericin B</td>
<td>0.244 ± 0.2</td>
<td>0.121 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>*Bartericin A</td>
<td>0.73 ± 1.0</td>
<td>0.121 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.121 ± 0.2</td>
<td>0.121 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Quercitrin</td>
<td>0.244 ± 0.2</td>
<td>0.121 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>*Stigmasterol</td>
<td>0.98 ± 0.85</td>
<td>0.121 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

Data are reported as means ± SD for assays in triplicate. MLC = minimum lethal concentration. *Compounds present in Dorstenia species.
These results are clearly shown in Table 3. The effective median concentrations show that ascorbic acid has the highest activity. The order of potency of the compounds tested was: ascorbic acid > quercitrin > 6,8-diprenyleridictyol > bartericin A > dorsmanin F > stigmasterol > isobavachalcone > 6-prenylapigenin.

**Discussion**

The observations that *T. vaginalis* is becoming resistant to metronidazole in about 5% of the population (12) coupled with the fact that metronidazole has unpleasant adverse effects (13) have led to search for phytochemicals in African medicinal plants with potential antitrichomonal activities. The results of the present study have shown that the extracts of *D. barteri* and *D. convexa* possess antitrichomonal activity. The active components (bartericins A and B and isobavachalcone) isolated from *D. barteri* were very active (0.121-31.25 µg/ml) against *T. gallinarum*. This fact may be responsible for the higher antitrichomonal activity of *D. barteri* than of *D. convexa*. The prenylated and geranylated chalcones were found to be as active as the prenylated flavones, 6-prenylapigenin and the diprenylated derivative 6,8-diprenyleridictyol. They were, however, about five times lower in activity than bartericins A and B, quercetin and quercitrin.

Moderate antioxidant capacity (EC50 >50 µg/ml) and high antioxidant capacity (EC50 <50 µg/ml) were found in the leaf and twig extracts of *D. barteri* and compounds tested (Table 3). The concentration needed to decrease the remaining DPPH by 50% (the initial substrate concentration EC50) is a parameter widely used to measure antioxidant power (25,26). The lower the EC50, the higher the antioxidant power. The values found in our study are shown in Table 3. According to Dufall et al. (27), the potency of the scavenging...
Antitrichomonal activities of *Dorstenia* species

...ing activity of some compounds isolated from *D. manni* has the following range: dorsmanin C > 6,8-diprenyleridictyol > dorsmanin F. We also found that the order of potency was similar in our study: 6,8-diprenyleridictyol (32.12 µg/ml) > dorsmanin F (53.89 µg/ml). The order of potency was as follows: ascorbic acid > quercitrin > 6,8-diprenyleridictyol > bartericin A > dorsmanin F > stigmasterol > isobavachalcone > 6-prenylapigenin. The higher antioxidant property exhibited by the *D. barteri* twig extract than the leaf extract may be due to the relative presence or distribution of active components in the extracts.

One third of the World’s cancer cases are caused by chronic infections (28). In Asia and Africa, hepatitis B and C viruses infect about 500 million people and are a major cause of hepatocellular carcinoma (29). Schistosomiasis is another major chronic infection which is widespread in Africa and China. The African schistosomal worm lays eggs in the colon, producing inflammation that often leads to colon cancer (30). There is evidence that this disease may be on the increase in the southwestern part of Nigeria (31,32). The common link between oxidants and inflammatory reactions, infection and other disorders has been well established (33,34). In chronic infection and inflammation, release of leukocytes and other phagocytic cells readily defends the organism from further injury. The cells do this by releasing free oxidant radicals, NO, O₂⁻, H₂O₂, and OH⁻, as powerful oxidant mixtures (28,33). Antioxidants appear to inhibit the actions of some of the oxidants generated in inflammation (28). No wonder, therefore, that the extracts of *D. barteri* exhibited both antitrichomonal and antioxidant properties in this study. The antioxidant properties of these chemical constituents of *D. barteri* extracts could be used to explain, at least in part, the anti-inflammatory and antinociceptive activities obtained in our earlier study (35).

Endogenous enzymatic antioxidants offer protective defenses in the body (28) to limit the levels of reactive oxidants and the damage they inflict. In addition, consumption of dietary antioxidants appears to be associated with a lowered risk of degenerative diseases. Prenylated flavonoids have been shown to influence cyclooxygenase and lipoxygenase activity (36,37) and to inhibit platelet aggregation (38). The former action may account for the anti-inflammatory and antitrichomonal action of plants containing such compounds. The antioxidant activity shown by the extracts and compounds tested in this study may lend credence to the use of *Dorstenia* species as anti-infective agents in folk medicine.

The prenylated flavonoids and the isolated compounds with antioxidant properties reported here probably account for the antioxidant and antitrichomonal actions of these extracts.

**Acknowledgments**

Thanks are extended to Prof. A. Afolayan of the Department of Biochemistry for spectrometry facilities.

**References**

amongst students of a higher institution in Nigeria. *Applied Parasitology*, 34: 19-25.


