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


1Drug Research and Production Unit and Department of Pharmacology, Faculty of Pharmacy, and Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria
2Department of Organic Chemistry, University of Yaoundé, Yaoundé, Cameroon
3Department of Chemistry, University of Botswana, Gabardine, Botswana
4Department of Pharmacology, University of Darfur-Beauville, South Africa

Abstract

*Dorstenia barteri* and *D. convexa* extracts and some isolated components of the former were investigated for effectiveness against *Trichomonas gallinae* 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-diprenylapicicolin. The order of the antitrichomononal activity of the compounds at 24 h was, quercetin (0.121 μg/ml) > quercitrin (0.244 μg/ml) > isobavachalcone > bavachalcone (0.35 μg/ml) > bavachalcone A (0.73 μg/ml) > stigmastanol (0.98 μg/ml) > 6,8-diprenylapicicolin = bavachalcone = quercetin = quercitrin = 6-prenylapigenin. *D. barteri* extracts, quercetin, and bavachalcone 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 antitrichomononal and antioxidant activities shown by the extracts and compounds in this study are consistent with the ethnomedical 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 goit and various skin diseases (3). Trichomoniasis affects men and animals causing untold economic losses 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 Kissama 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 vaginalis affects birds including poultry, causing high morbidity and mortality especially in young birds. There is no information available in the literature concerning the antioxidant 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, Yaoundé, Cameroon. Combined CH2Cl2/MeOH (1:1) extracts of Dorstenia convexa and D. barreri were obtained as previously described (14). The extraction and isolation of butenolides A and B, stigmastanol, isobavachalcone, and 4-hydroxy-7-oxochaparrin have been previously described by Ngameni et al. (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. Dorsamin P and 6,8-diprenylidendecyl (Table 2) were isolated from D. naesius as previously described (15-17). Quercetin (18), quercetin (19), amethystoflavone (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 vaginalis was cultivated in Ringer’s egg-bovine medium according to the method of Beek and Debroglie (22) as modified by Levine (23). According to Meinsasser 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, 0.8, 1, 3, 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 (in ethanol) in a cuvette. The time-course of the change in absorbance at 517 nm was monitored for 20 min.
tant 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:

\[
\text{%DPPH}_{\text{rem}} = \frac{[\text{DPPH}]_0 - [\text{DPPH}]_{\text{rem}}}{[\text{DPPH}]_0} \times 100
\]

where \([\text{DPPH}]_0\) is the remaining concentration of the stable radical without the antioxidant and \([\text{DPPH}]_{\text{rem}}\) 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 \(\mu\)g 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-hydroxyisobavachalcone (0.048%) from the twigs and 18 mg (0.03%) from the leaves; 35 mg baxtericin A (0.058%) from the twigs and 46 mg (0.077%) from the leaves, while 30 mg baxtericin B (0.03%) was obtained from the twigs but was not detected in the leaves.

**Antirrhinomalous assays**

The MLCs for the extract of *D. barteri* leaves and twigs were found to be 15.625 and 15.625 \(\mu\)g/ml, respectively. *D. convexa* leaf extract with an MLC of 125 \(\mu\)g/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-hydroxyisobavachalcone, baxtericin A and B, and stigmasterol, were compared with quercetin and quercitrin isolated from *Mellotus oppositifolium*, 6-prenylapigenin isolated from *D. kameruniana* and 6,8-diprenylenol, d orcumin F from *D. manii*, amethyflavone from *Cannarium shweeufurthi*, and gedunin from *Carapa grandiflora* (Table 2). The order of antirrhinomalous activity of the compounds is: quercetin (0.121 \(\mu\)g/ml) > quercitrin (0.244 \(\mu\)g/ml) > baxtericin B (0.244 \(\mu\)g/ml) > baxtericin A (0.73 \(\mu\)g/ml) > stigmasterol (0.98 \(\mu\)g/ml) > 6,8-diprenylenol = isobavachalcone = d orcumin F (31.25 \(\mu\)g/ml). Some of these compounds were more effective than metronidazole (0.625 \(\mu\)g/ml). Quercetin, with an MLC of 0.121 \(\mu\)g/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} < 5 \(\mu\)g/ml).

<p>| Table 1: Antirrhinomalous activities of Drostonia extracts. |
|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Product</th>
<th>Plant name</th>
<th>MLC ((\mu)g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td><em>D. convexa</em></td>
<td>125 ± 0</td>
</tr>
<tr>
<td>24 h</td>
<td><em>D. barteri</em></td>
<td>125 ± 0</td>
</tr>
<tr>
<td>48 h</td>
<td><em>D. convexa</em></td>
<td>437.5 ± 125</td>
</tr>
<tr>
<td>416 ± 144</td>
<td><em>D. convexa</em></td>
<td>125 ± 0</td>
</tr>
<tr>
<td><em>Leaves + twigs</em>: <em>D. barteri</em></td>
<td>125 ± 0</td>
<td></td>
</tr>
<tr>
<td><em>Leaves + twigs</em>: <em>D. convexa</em></td>
<td>15.625 ± 0</td>
<td></td>
</tr>
<tr>
<td><em>Leaves + twigs</em>: <em>D. barteri</em></td>
<td>15.625 ± 0</td>
<td></td>
</tr>
</tbody>
</table>

Data are reported as means ± SD for assays in triplicate. MLC = minimum lethal concentration.

Braz J Med Biol Res 38(7) 2005
Table 2. Comparison of antimicrobial activity of Dorstenia compounds with other active compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Name</th>
<th>MLC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Isobavachalcone</td>
<td>31.25</td>
<td>31.25</td>
</tr>
<tr>
<td>*4-Hydroxyforisin</td>
<td>800 ± 0</td>
<td>500 ± 0</td>
</tr>
<tr>
<td>*6-Prenylloganin</td>
<td>93.6 ± 0</td>
<td>31.25 ± 0</td>
</tr>
<tr>
<td>*6,8-Diprenyldeoxyol</td>
<td>31.25 ± 0</td>
<td>31.25 ± 0</td>
</tr>
<tr>
<td>*Dorstenin F</td>
<td>31.25 ± 0</td>
<td>31.25 ± 0</td>
</tr>
<tr>
<td>Amorphaflavone</td>
<td>500 ± 0</td>
<td>500 ± 0</td>
</tr>
</tbody>
</table>

Continued on next page.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Name</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Geranium</em></td>
<td>500 ± 0</td>
<td>500 ± 0</td>
<td></td>
</tr>
<tr>
<td><em>Bacterin B</em></td>
<td>0.244 ± 0.2</td>
<td>0.121 ± 0.2</td>
<td></td>
</tr>
<tr>
<td><em>Bacterin A</em></td>
<td>0.73 ± 1.0</td>
<td>0.121 ± 0.2</td>
<td></td>
</tr>
<tr>
<td><em>Quercetin</em></td>
<td>0.121 ± 0.2</td>
<td>0.121 ± 0.2</td>
<td></td>
</tr>
<tr>
<td><em>Quercetin</em></td>
<td>0.244 ± 0.2</td>
<td>0.121 ± 0.2</td>
<td></td>
</tr>
<tr>
<td><em>Stigmasterol</em></td>
<td>0.98 ± 0.88</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-diprenylfisetinyl > bartericin A > dorstenalin 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 antichlamydial activities. The results of the present study have shown that the extracts of D. barteri and D. convexa possess antichlamydial activity. The active components (bartericin 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 antichlamydial activity of D. barteri than of D. convexa. The prenylated and geranylated flavones were found to be as active as the prenylated flavones, 6,8-prenylflavanon and the diprenylated derivative 6,8-diprenylfisetinyl. They were, however, about five times lower in activity than bartericin A and B, quercetin and quercitrin.

Moderate antioxidant capacity (EC$_{50}$ < 50 μg/ml) and high antioxidant capacity (EC$_{50}$ < 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 EC$_{50}$) is a parameter widely used to measure antioxidant power (25, 26). The lower the EC$_{50}$, the higher the antioxidant power. The values found in our study are shown in Table 3. According to DuFaul et al. (27), the potency of the scaveng-

![Figure 1](image-url)

**Figure 1.** Upper panel, Antioxidant activities of Dorstenia barteri leaves and twigs (10-100 μg/ml). Lower panel, Antioxidant activities of selected compounds (10-100 μg/ml). The percentage of remaining DPPH is an index of antioxidant activity equal to DPPH used at time 0 over the DPPH used at time zero. DPPH = 1,1-diphenyl-2-picrylhydrazyl.

<table>
<thead>
<tr>
<th>Extract/compound</th>
<th>EC$_{50}$ (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartericin A</td>
<td>47.85 ± 2.15</td>
</tr>
<tr>
<td>6,8-Diprenylfisetinyl</td>
<td>32.12 ± 1.10</td>
</tr>
<tr>
<td>Quercetin</td>
<td>28.16 ± 0.94</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>19.93 ± 0.3</td>
</tr>
<tr>
<td>D. barteri leaves</td>
<td>60.46 ± 1.09</td>
</tr>
<tr>
<td>D. barteri twigs</td>
<td>40.12 ± 2.97</td>
</tr>
<tr>
<td>Dorstenalin F</td>
<td>53.89 ± 1.84</td>
</tr>
<tr>
<td>6-Prenylflavanon</td>
<td>89.43 ± 0.76</td>
</tr>
<tr>
<td>Isoflavone</td>
<td>84.30 ± 0.27</td>
</tr>
<tr>
<td>Leaves and twigs</td>
<td>31.67 ± 1.19</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>62.18 ± 0.64</td>
</tr>
</tbody>
</table>

Data are reported as means ± SD.* Only one replicate was tested because the compound was not sufficient to run additional tests.
Antichromosomal activities of Dorsetania species

The antichromosomal activity of some compounds isolated from *D. marrui* has the following range: dorsmanin C > 6,8-diprenylidectidol > dorsmanin F. We also found that the order of potency was similar in our study: 6,8-diprenylidectidol (32.12 μg/ml) > dorsmanin F (53.89 μg/ml). The order of potency was as follows: ascorbic acid > quercetin > 6,8-diprenylidectidol > bartercin A > dorsmanin F > sigmastanol > 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 300 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 (28,35). 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 antichromosomal 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. Preynlated 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 antichromosomal action of plants containing such compounds. The antioxidant activity shown by the extracts and compounds tested in this study may lead credence to the use of *Dorsetania* species as anti-inflammatory agents in folk medicine.

The preynlated flavonoids and the isolated compounds with antioxidant properties reported here probably account for the antioxidant and antichromosomal 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. *Aequid Palati-
olog 34: 19-25.
6. Bakare HA, Ashipu JO, Adeyemo-Dero FA, Ekeweza CC, Oni AA, 
Okosola AO & Adeibayo JA (1996). Non-protective urothelial (BUU) 
due to trichomonas vaginatis in ibadan, West African Journal of 
Medicine, 16: 64-68.
7. Nnora CP, Egwurutie AO & Ejemo DO (2001). Survey of uter-
inary schistosomiasis and trichomoniasis in a rural community in 
ing the incidence of Trichomonas vaginatis amongst pregnant women 
in Jos area of Plateau State, Nigeria. Angewandte Parasitologie, 32: 198-204.
9. Obiiao OK, Ikamoto M, Dallman N, Kemp J, Ikokwu-Wonodu C, 
managing sexually transmitted infections among Nigerian adoles-
cent females. Bulletin of the World Health Organization, 79: 301- 
305.
superoxide radicals, hydrogen peroxide, hydroxyl radicals, and sing-
let oxygen. Journal of Agriculture and Food Chemistry, 48: 5077- 
5088.
demic Press, Orlando, FL, USA.
Huirung BM (Editor). Trichomoniasis Parasitology Mini, Springer 
Verlag, New York, 341-241.
and fenbendazole on metazooal-resistant Trichomonas vaginalis: 
(2004). Peroxynitrite and chlorines and other constitutents from 
the twigs of Dostenia bartetti var. Subtruxtinula. Pharmachemistry, 68: 147- 
152.
15. Naphefu BM, Dorigo M, Dorigo E, Kapcha GW & Algbab BM 
(1998). Generalised and prenylated flavonoids from the twigs of Dostenia 
16. Naphefu BM, Dorigo M, Dorigo E, Kapcha GW & Algbab BM 
Phytochemistry, 60: 1401-1406.
17. Naphefu BM, Dorigo M, Dorigo E, Kapcha GW & Algbab BM 
(2003). Prenylated flavonoids from the aerial parts of Dostenia maritima. 
Phytochemistry, 65: 915-919.
20. Zembou H, Fitoso S, Ntukali FT, Dorigo M, Dorigo E & Algbab BM 
BL, Tenoloy DO & Raimondi ES (1994). Limonoids from Canarium gran-
parasites. Proceedings of the National Academy of Sciences, USA, 11: 236-238.
Levine ND (Editor), Protozoal Parasites of Domestic Animals and Man. Burgess 
Publishing Company, Minneapolis, MN, USA, 277- 
293.
resistant to metronidazole and other nitroimidazoles. Antimicro-
bial Agents and Chemotherapy, 16: 254-257.
free radical method to evaluate antioxidant activity. Lebensmittel-
drome to measure the antioxidant efficiency of polyphenols. Journal of 
The Science of Food and Agriculture, 76: 2700-270.
Antioxidant activity of prenylated flavonoids from the West African 
28. Amou S, Swagana MG & Hagen TM (1980). Oxidants, anti-
oxidants and the degenerative diseases of aging. Proceedings of the 
National Academy of Sciences, USA, 90: 7015-7022.
haemoglobin infection. A review. Tropical Diseases Bulletin, 86: 2- 
56.
due to schistosomiasis: review of the recent literature. Tropical Diseases 
31. Adewummo CO, Furo P, Christensen NO, Mrazik BB & Fakbori M 
(1996). Endemicity and seasonality of transmission of human schis-
tosomiasis in Ille-Ife, Southwest Nigeria. Tropical Medicine and Para-
sitology, 41: 443-444.
32. Adewummo CO, Gebranmengi G, Becker W & Okonofua FO. Dorfer 
G & Adewummo TA (1996). Schistosomiasis and intestinal parasites in 
rural villages in southwest Nigeria: An indication for expanded 
programme on drug distribution and integrated control programme in 
Nigeria. Parasitology and Medicine Proctology, 48: 147-152.
G (1997). In vitro antioxidant and curative activity of extracts of 
Baccharis dracunculoides DC. Journal of Ethnopharmacology, 58: 157-
169.
34. Wang H, Nair MG, Strasburg GM, Chou-Chang Y, Booran AM, 
of anthocyanins and their aglycone, occasioning from Tart Cherry. 
Journal of Natural Products, 62: 249-256.
35. Omosara NOA, Adewummo CO, Jivese EO, Ndjjaguie BT, Wataghung 
J, Algbab BM & Ceyyole JAC (2004). Antioxidant and anti-
anti-inflammatory effects of Dostenia bartetti (Menispermaceae) leaf and twig 
phenolic compounds from the mulberry tree on scutellitarin ma-
37. Chi TC, Jeng HS, Sng HC, Chang HW & Kim HP (2001). Effects of 
naturally occurring prenylated flavonoids on enzymes metabolizing 
estrone:onic acid, Cytochrome P-450 and 5-kinogenase Biochemical 
Pharmacology, 62: 1189-1191.
biological activities of constituents from Manus australis. Biochimi-
ca at Biophysica Acta, 1428: 263-290.