Full Length Research Paper

Evaluation of anthelmintic activity of the stem bark extract and chemical constituents of *Bridelia ferruginae* (Benth) Euphorbiaceae

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Anthelmintic properties of the stem bark extract and compounds isolated from *Bridelia ferruginae* were investigated. In relation to the traditional use of *B. ferruginae* against gastro-intestinal infections, bioactivity-guided fractionations of the CHCl\(_3\) and CH\(_2\)Cl\(_2\) soluble fractions of the 80% MeOH extract from the stem barks of *B. ferruginae* yielded two known triterpenoids: betuline (1), glucoside of betulinic acid (2) and other two known flavonoids: quercetin (3) and kaempferol (4). Structures of compounds 1 to 4 were elucidated by spectroscopic studies and comparison with related compounds in literature. The time of paralysis and death of the parasitic worms: *Fasciola gigantical* (liver fluke), *Taenia solium* (tape worm) and *Pheritima posthuma* (earthworm, Annelid) were determined at 25, 50, 80 and 100 mg/ml. The stem barks extract of *B. ferruginae* and isolated compounds demonstrated concentration-dependent anthelmintic potencies against parasitic worms assayed. Structural-activity relationship is explained.

Key words: *Bridelia ferruginae*, anthelmintic activity, betulinic acid, betuline, quercetin, kaempferol.

INTRODUCTION

*Bridelia ferruginae* (Benth)-Euphorbiaceae is one of the most popular medicinal plants used in the Northern, South-Western Nigeria and other African countries for gastro-intestinal infections (Addae-Mensah, 1992; Iwu, 1986; Ayensu, 1978). *B. ferruginae* is among the 60 species of the genus *Brindelia* (Oliver-Bever, 1960). Its morphology is well documented (Rashid et al., 2000). *B. ferruginae* is widely distributed in the guinea savannah and coastal parts of Africa, particularly Cote d'Ivoire Ghana, Togo and Nigeria (Addae-Mensah, 1992). In Nigeria, *B. ferruginae* has many vernacular names, depending on the usage and locations. *B. ferruginae* is widely used in traditional Nigerian medicine to treat a range of diseases. The barks of *B. ferruginae* is reported for wound treatment, gonorrhoea infections, antimicrobial potency, gastro-infection treatment, anti-diabetic, anti-inflammatory and radical scavenging activities (Ekanem et al., 2008; Olajide et al., 1999; De-Bruyne et al., 1997; Adeoye et al., 1988; Iwu, 1984). Previous phytochemical attention on *B. ferruginae* has led to characterization of flavonoids, triterpenoids, flavonoid glucosides, bioflavonoids, phenols and tannins from various morphological parts of *B. ferruginae* (Cimmanga, 2001; Rashid et al., 2000; Addae-Mensah, 1985; Irobi, 1994). In spite of numerous pharmacological and phytochemical reports on *B. ferruginae*, there is a dearth of literature report on the anthelmintic potency of the plant. In a preliminary screening of plants used in South-Western Nigeria for the treatment of gastroinfectional disorders, *B. ferruginae* was investigated based on positive screening results of its stem bark extract on selected parasitic worms: *Fasciola gigantical* (liver fluke), *Taenia solium* (tape worm) and *Pheritima posthuma* (earthworm, Annelid). In response to the folkloric usage of the stem barks of *B. ferruginae* in Nigeria traditional medicine and in furtherance of our search for anthelmintic phytochemicals from Nigeria medicinal plants, *B.*
**Materials and Methods**

**Plant material**

The stem bark of *B. ferruginae* used in this study was collected at Olokemeji Forest Reserve in Ibarapa Local Government Area of Oyo State, Nigeria in July, 2009. The plant was botanically authenticated by Mr. Odowo, T. K., a taxonomist in the Forest Research Institute of Nigeria (FRIN) through comparison with authentic samples in the herbarium of FRIN (Accession number FHI 1234).

**Extraction and isolation of plant material**

The stem barks of *B. ferruginae* were air-dried at room temperature. Dried and powdered stem bark (1.5 kg) were defatted by soxhlet extraction with n-hexane (bp 68°C). The dried plant material (marc) was macerated and percolated successively with 80% MeOH. The MeOH extract was concentrated in vacuo at reduced pressure, yielding a residue of 560 g. 500 g of the residue was dissolved in hot water (60°C) and filtered after 24 h. The filtrate was extracted with rotatory evaporator to afford 15, 21 and 27 g of the CHCl₃ and column chromatography (CC) on silica gel (Merck 60, 70 to 230 mesh), eluted with gradient solvent systems of CHCl₃ to 1, 1 to 0, vol/vol), collecting 100 ml fraction each time. The fractions were monitored by TLC and later pooled together into 6 basins of their TLC profile. Fractions A to E was assayed for anthelmintic activity of the stem bark extracts and constituents of *B. ferruginae*. In developing countries like Nigeria, helminth infections are a major health concern because they predispose human to other infections such as bacterial and fungal infections (Cox, 2001). Such helminth infections can lead to serious diseases among poor people due to poor sanitation, poverty and malnutrition (Brooker et al., 2006).

**Animal material**

The worm used for the study: *F. gigantica* (liver fluke, mean weight of (0.06 to 0.08) g, *T. solium* (tape worm, mean weight 2.5 to 2.9 g) were obtained from freshly slaughtered cows at Odo-eran abattoir, Abeokuta, Ogun State, Nigeria. Nigeria earthworm *P. posthuma* (Annelid) were collected from the water logged areas of soil in Oba river, Obantoko, Ogun State, Nigeria. The average size of earthworm was 6 to 8 cm; the worm was washed with cold water to remove dirt. All parasitic worms were authenticated at the Parasitological Research Unit, Zoology Department, University of Agriculture, Abeokuta, Ogun State, Nigeria.
Anthelmintic assay

All chemicals used were of IP/HP specifications. Parasitic worms: F. gigantica (liver flukes), T. solium (tape worms) and P. posthuma (earthworms) of comparable size were used for evaluating anthelmintic activity using piperazine citrate, as standard anthelmintic drug. The anthelmintic procedure followed the method containing four different concentrations (10, 20, 50, 100 mg/ml in distilled water). A solution of each concentrate was prepared in distilled water. Five worms (of the same size) were placed in 9 cm petri dishes in solution of crude extracts and standard drug at the concentrations mentioned above. This was done in duplicate for all the worm types. The control test having five worms in 50 ml of distilled water was equally conducted simultaneously. The average time required for the paralysis and death of worms was recorded. The mean paralysis time (minute) of the worms was recorded when the worm show no movement of any sort except when the worm was shaken vigorously or transferred into a beaker containing hot water at 50°C. The death time was recorded after ascertaining the worms neither move when shaken vigorously nor when dipped in hot water (70°C). The results are presented in Tables 1 and 2.

Statistical analysis

All data were expressed as the mean ± S.E.M., data was subjected to two-way ANOVA followed by Student’s t - test, using microsoft excel® and statistical® computer software packages. Difference in mean were considered significant when P ≤ 0.05.

RESULTS AND DISCUSSION

CHCl₃ and CH₂Cl₂ extracts of B. ferruginae was subjected to silica gel CC to afford two known triterpenoids: betuline (1) and glucoside of betulinic acid (2). Compounds 1 and 2 (Figure 1), though known are reported for the first time from B. ferruginae. Compound 1 was identified as 3β, 24-dihydroxy lup-20(29)-ene (betuline) on the basis of 1H- and 13C-NMR spectral data; along side other physical data with literature values. Betuline was previously isolated from the Polyoporus pinicola and Bentula mandschurica repel plants (Dan and Dan, 1986; Patra et al., 1988). Compound 2 was eluted with 50% hexane-EtOAc and characterized as glucoside of 3β-hydroxyl-lup-20(29)-ene-28-oic acid (a glucoside of betulinic acid) based on 1H- and 13C-NMR spectral data, as well as comparison of their physical data. Compound 2 has been reported from Oplopanax nakai plant tissue (Wang et al., 1996) and from the leaves of Cussonia racemosa (Liva et al., 2002). The flavonoids, quercetin (3) and kaempferol (4) were eluted with 40% hexane-EtOAc and characterized as glucose of 3β-hydroxyl-lup-20(29)-ene-28-oic acid (a glucoside of betulinic acid) based on 1H- and 13C-NMR spectral data, as well as comparison of their physical data with literature values. Compounds 3 to 4 were characterized on the basis of their spectral data; as comparison with other physical data in literature. Compounds 3 and 4 have been widely reported from various plant tissues, including B. ferruginae (Addae-Mensah and Achenbach, 1985; Addae-Mensah and Munenge, 1989; Cimmanga et al., 1999). Anthelmintic screening of extracts and isolated compounds (1 to 4) (Figure 1) was evaluated in-vitro on three parasitic worms: F. gigantica (liver flukes), T. solium (tape-worms) and P. posthuma (earthworms). The CHCl₃ and CH₂Cl₂ extracts of B. ferruginae demonstrated significant anthelmintic activity against the parasitic worms assayed. CH₂Cl₂ extract exhibits higher anthelmintic activity than the CHCl₃ extract of B. ferruginae on the parasitic worms. It is noted that the extracts displayed concentration-related anthelmintic activity (Table 1). The time (minute) of paralysis and death compared favourably with the
Table 2 Anthelmintic activity of isolated compounds from *B. ferruginae*.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (mg/ml)</th>
<th><em>F. gigantica</em></th>
<th><em>T. solium</em></th>
<th><em>P. posthuma</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P D P D P D</td>
<td>P D</td>
<td>P D</td>
<td>P D</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>85±0.8</td>
<td>125±0.9</td>
<td>85±0.7</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>70±0.4</td>
<td>130±0.5</td>
<td>78±0.5</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>66±0.3</td>
<td>92±0.3</td>
<td>67±0.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>57±0.1</td>
<td>79±0.2</td>
<td>54±0.2</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>40±0.2</td>
<td>65±0.4</td>
<td>42±0.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>30±0.1</td>
<td>59±0.1</td>
<td>34±0.1</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>28±0.05</td>
<td>45±0.06</td>
<td>22±0.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10±0.01</td>
<td>39±0.02</td>
<td>14±0.1</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>10</td>
<td>59±0.3</td>
<td>80±0.7</td>
<td>67±0.4</td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

P = mean paralysis time (minute), D = mean death time (minute), conc. = concentration (mg/ml). Control worms were alive after 40 h except *T. solium* which were alive after 20 h.

Figure 1. Structures of triterpenoids and flavonoids isolated from *Bridelia ferruginae*.
standard anthelmintic drug, piperizine citrate at the same concentration. At concentration above 50 mg/ml, the extracts and isolated compounds demonstrated higher anthelmintic potency compared to the reference anthelmintic drug at the same concentration (Table 1). Quercetin (3) and kaempferol (4) were the most potent flavonoids at the same concentration (Table 1). At concentration above 50 mg/ml, the anthelmintic compounds, followed closely by glucoside of betulinic acid (2), and lastly betuline (1) (Table 2).

The function of worm expeller like piperazine citrate is to cause paralysis of the worms such that they are expelled in the faeces of men and animals (Lechat et al., 1978). The extracts and isolated compounds did not only paralyse the worms, but killed them at different concentrations. The intrinsic high anthelmintic potency displayed by compounds (3) and (4) (Figure 1) can be attributed to the presence of reactive –OH (phenolic) groups of flavonoids (Hillwell, 1994; Havsteen et al., 1980). Previous studies have implicated flavonoids in pharmacological activities such as anthelmintic and inflammatory activities (Makkar et al., 2007). The presence of quercetin and kaempferol in B. ferruginae stem barks extract emphasizes its anthelmintic potentials and this justifies the use of the plant in traditional medicine as an anthelmintic plant. Triterpenoids such as betulinic acid isolated from medicinal plant has equally demonstrated high intrinsic anthelmintic potency on parasitic worms (Asuzu et al., 1993; Enwerem et al., 2001). The reactive carbonyl in betulinic acid, coupled with the hydroxyl moiety can be used to justify its anthelmintic potency.

**Conclusion**

The findings in this work agree with the use of *B. ferruginae* in ethno medicinal treatment of worm infection.

**ACKNOWLEDGEMENTS**

Our thanks are due to Mr. Thomas Kolawole Odewo of the Forest Research Institute of Nigeria (FRIN) for the collection and authentication of the plant material used in this study. We thank Prof Benn of the Department of Chemistry, University of Calgary, Alberta, Canada for running the NMR spectral in his laboratory.

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