Antimalarial compounds from *Schefflera umbellifera*

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Abstract

The organic extract of the leaves of *Schefflera umbellifera* exhibited good antimalarial activity when tested against the chloroquine-susceptible strain (D10). Bioassay-guided fractionation of the dichloromethane fraction of the dichloromethane/methanol extract yielded an active compound, betulin, which exhibited good antiplasmodial activity with an IC50 value of 3.2 µg/ml. The reference compound, chloroquine gave an IC50 value of 27.2 ng/ml. Two other compounds were also isolated from the dichloromethane extract namely, 7-hydroxy-6-methoxycoumarin and ent-kaur-16-en-19-oic acid. These two compounds did not exhibit any significant antiplasmodial activity.

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1. Introduction

*Schefflera umbellifera* is a semi-deciduous tree, widely distributed in Malawi, Mozambique and Zimbabwe as well as in South Africa (Mbambezeli, 2006). This is the only South African member of the genus which grows in warm, tropical regions and is very closely related to *Cussonia*, even the Xhosa and Zulu common names are the same. According to Palmer and Pitman (1972), the specific epithet *umbellifera* refers to the umbellate arrangement of the flowers. The genus *Schefflera* J.R.Forst. & G.Forst. has about 650 species and was named in 1776 by G. and J.R. Forster in honour of J.C. Scheffler of Danzig (Mbambezeli, 2006).

The leaves of *S. umbellifera* have been used traditionally to treat rheumatism, colic and insanity and for malaria, a bark extract is drunk (Watt and Breyer-Brandwijk, 1962). The Vhavenda people use the roots of this plant as a diuretic and laxative, for bathing, for weaning infants and for malaria, venereal diseases and nausea (Hutchings et al., 1996). According to Watt and Breyer-Brandwijk (1962) the bark is used for stomach ulcers and magical purposes. In Tanzania, leaves are used for indigestion while roots are used for fevers and venereal disease, in emetics for nausea and in cold infusions for skin irritation in new-born babies (Hutchings et al., 1996). Root bark decoctions are administered for mental illness (Chhabra et al., 1984). The secondary metabolite characteristic of the Araliaceae family is triterpene glycosides, polyacetylenes, saponins (Gunzinger et al., 1986) and caffeic acid derivatives (Li et al., 2005). Limited information is known about the antimalarial properties of *S. umbellifera* (Tetyana et al., 2002) and no phytochemical studies of this plant have been reported. For this study, three major compounds were isolated from the active dichloromethane extract.

2. Materials and methods

2.1. Plant material

Plant material was collected at Mariepskop, Mpumalanga, on the road towards the top above an electrical substation. This area was a forest with a well-drained, rocky soil and humus clay. A voucher specimen deposited at the South African National Biodiversity Institute (SANBI) was identified as *S. umbellifera* (Sond.) Baill.
2.2. Extraction and isolation of compounds

The wet leaves (6.00 kg) were dried in an oven at 60 °C for overnight. 2.90 kg was recovered as a dry matter and finely ground. 2.2 kg of the dried, ground leaves were extracted using methanol/dichloromethane (1:1) for 12 h, filtered and reduced to dryness using a rotovaporator. Methanol/dichloromethane extract was then partitioned between hexane, dichloromethane and water. The dichloromethane layer was dried and applied to a silica column, chromatographed using silica gel (0.040–0.063 mm), eluted with 5% acetone/hexane followed by 100% acetone. This yielded eighteen fractions and some of these fractions were later combined on the basis of their TLC profiles. The first fraction (3.20 g) was further fractionated using flash silica gel with 2% EtOAc/hexane as mobile phase and afforded a white pure compound 1 (23 mg, 0.72% yield). The second fraction (0.66 g) was further fractionated on flash silica gel using 40% EtOAc/hexane as the mobile phase and yielded a pure white crystalline compound 3 (16 mg, 2.24% yield). The third fraction (0.10 g) was also further chromatographed on flash silica using 5% acetone/dichloromethane as mobile phase and yielded a pure bright blue fluorescing compound 2 (6 mg, 6% yield).

The structures of all three compounds (Fig. 1) were determined using $^1$H NMR and $^{13}$C NMR data recorded on a Varian 400 MHz Unity spectrometer at the CSIR. All compounds were dissolved in either deuterated chloroform or methanol and the chemical shifts were recorded in ppm, tetramethylsilane (TMS) was used as an internal standard and spectra were recorded at room temperature. The mass spectral data were obtained from a coupled HPLC-UV/MS instrument with a triple pole Quattro LC Micro mass spectrometer which was set to operate both in ESI$^-$ and ESI$^+$ modes. High-resolution mass spectra (HREIMS) of compounds were obtained from the University of Witwatersrand using a VG 70SEQ HRMS instrument, operating mainly on EI$^+$ mode using 8 kV as standard ionization energy. A Perkin Elmer 241 polarimeter at 589 nm using a sodium lamp as a light source and a cell with path length of 1 dm was used in all experiments. These experiments were done using either methanol or chloroform as solvent depending on the solubility of the compound. Melting points were determined using a Reichert Koffler hotstage apparatus and are uncorrected.

2.3. Description of the in vitro antimalarial assay

Compounds or extracts were assayed against Plasmodium falciparum strain, namely chloroquine-sensitive (CQS) (D10). Continuous in vitro cultures of asexual erythrocyte stages of P. falciparum were maintained using the method of Trager and Jensen (1976). The parasites were maintained at a 5% haematocrit with RPMI 1640 (Biowhittaker) medium supplemented with Albumax II (lipid rich bovine albumin) (GibcoBRL) (25 g/l), hypoxanthine (44 mg/l), HEPES [N-(2-hydroethyl)-piperazine-N′-(2-ethansulfonic acid)] (Sigma Aldrich) (50 mg/l). The cultures were incubated at 37 °C in an atmosphere of 93% N$_2$, 4% CO$_2$ and 3% O$_2$.

Quantitative assessment of in vitro antimalarial activity was determined via a parasite lactate dehydrogenase (pLDH) assay (Makler et al., 1993). Chloroquine (CQ) was used as a reference drug in all experiments and the IC$_{50}$ values were obtained using a non-linear dose-response curve fitting analyses via Graph Pad Prism v.4.0 software.

3. Results and discussion

3.1. Identification and characterization of isolated compounds

Compound 1 was obtained as a white crystalline solid, crystallized using an acetone/hexane solvent mixture. This compound is not UV active but shows an intense pinkish colour upon spraying the TLC plate with vanillin. Compound 1 showed very intense fragments at m/z 302.4011, which corresponds to a molecular formula of C$_{30}$H$_{50}$O$_2$ and some distinctive fragment peaks at m/z 288 due to [M–CH$_2$]$, ^+$, m/z 242 which corresponds to a loss of both a carboxyl and a methyl group. After careful inspection of the NMR data the compound was identified as ent-kaur-16-en-19-oic acid and the structure confirmed by comparison of the $^1$H and $^{13}$C NMR data with literature values (Buckingham, 1996; Lobitz et al., 1997).

Compound 2, was isolated as a pure bright blue fluorescing powder. Electro-spray mass spectrometry data gave a molecular ion signal M$^+$ at m/z 302.411, which corresponds to a molecular formula of C$_{30}$H$_{50}$O$_2$ and some distinct fragment peaks at m/z 288 due to [M–CH$_2$]$, ^+$, m/z 242 which corresponds to a loss of both a carboxyl and a methyl group. After careful inspection of the NMR data the compound was identified as 7-hydroxy-6-methoxyxycumarin, also known as scopoletin. NMR data corresponded with the reported data (Sun et al., 2006).

Compound 3 was obtained as a white crystalline solid using flash chromatography and was crystallized from an ethyl acetate/hexane mixture. This compound showed a very intense pinkish colour upon spraying the TLC plate with vanillin. Compound 3 was identified as betulin [lup-20(29)-en-3β,28-diol] with a molecular formula of C$_{36}$H$_{50}$O$_2$. The mass spectra also showed very intense fragments at m/z 411 due to [M–CH$_2$OH]$^+$, m/z 426 due to [M–OH]$^+$ and fragment peaks at m/z 235, 250.
202, 193 which are characteristic of fragmentation patterns of a lupine-type of compound with an angular hydroxyl methylene group. NMR data of compound 3 compares very well with authentic betulin (Mahato and Kunda, 1994; El Deeb et al., 2003).

3.2. In vitro antimalarial activity of compounds isolated from *S. umbellifera*

The dichloromethane/methanol extract of the leaves of *S. umbellifera* exhibited good antimalarial activity (IC$_{50}$ 5.0 µg/ml) when tested against the chloroquine-susceptible strain (D10). This extract was then partitioned between hexane, dichloromethane and water and these fractions tested for antimalarial activity. Only the dichloromethane fraction exhibited potent antiplasmodial activity against the chloroquine-sensitive strain (D10) with an IC$_{50}$ of 3.7 µg/ml. This result is regarded as potent based on the criteria set by the Novel Drug Discovery Platform (NDDP) (potent<5 µg/ml; Clarkson et al. (2004). The dichloromethane fraction was further fractionated and because of limited plant material three major compounds were targeted. From the three compounds isolated, only betulin (compound 3) exhibited significant *in vitro* antimalarial activity (3.2 µg/ml) against the *P. falciparum* chloroquine-susceptible strain (D10). This activity compares well to that of the dichloromethane fraction (Table 1) and therefore betulin can be considered as an active ingredient. Although isolation and biological activity of betulin is well published, no information is known regarding its isolation from *S. umbellifera*.

Although antimalarial activity of betulin has been reported, limited information is known about the antimalarial activity of *S. umbellifera*. According to Ziegler et al., 2004, betulin was isolated from several plant families such as Rhamnaceae (*Ziziphus vulgaris*) and Labiatae (*Zataria multiflora*) that have been tested against *P. falciparum* strains and showed moderate activity (IC$_{50}$< 12 µg/ml and<27 µM, respectively).

*Badisa et al.* (2002) and Henry et al. (2006) isolated betulin from a Tanzanian plant, *Uapaca nitida* and reported it to be inactive at 500 µg/ml. However, *Monte et al.* (1988), reported betulin to inhibit *P. falciparum* strains with an IC$_{50}$ of 12 µg/ml. According to literature studies conducted, antiplasmodial activity of *ent*-kaure-16-en-19-oic acid has not been reported previously. It should also be noted that betulin does not exhibit significant antimalarial activity except when it is converted into betulinic acid. Antimalarial activity of betulinic acid against chloroquine-sensitive strains (T9-96) was reported to be 25.9 µg/ml whereas no activity was demonstrated by betulin (500 µg/ml) (Yogeeswari and Siriram, 2005).

The results of this study confirms the traditional use of the plant *S. umbellifera* and bioassay-guided fractionation of the dichloromethane fraction of the dichloromethane/methanol extract yielded an active compound, betulin, which exhibited good antiplasmodial activity against the chloroquine-susceptible strain D10, with an IC$_{50}$ value of 3.2 µg/ml. Considering that a large percentage of South African plants have not been investigated chemically or pharmacologically, they remain a potential source of leads for drug development.

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**Table 1**

Antiplasmodial activity of the dichloromethane fraction of *S. umbellifera*, *ent*-kaure-16-en-19-oic acid (1), scoopoletin (2) and betulin (3) against chloroquine-susceptible strain (D10).

| Extract/compound | Antiplasmodial activity, D10 IC$_{50}$ (µg/ml) |
|------------------|---------------------------------------------|
| CH$_2$Cl$_2$/MeOH extract | 5.0 |
| CH$_2$Cl$_2$ fraction | 3.7 |
| Compound 1 | 32.2 |
| Compound 2 | 28.2 |
| Compound 3 | 3.2 |
| Chloroquine | 27.2 ng/ml |
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