Antiplasmodial Activity of Compounds from the Surface Exudates of
Senecio roseiflorus

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From the surface exudates of Senecio roseiflorus fourteen known methylated flavonoids and one phenol were isolated and characterized. The structures of these compounds were determined on the basis of their spectroscopic analysis. The surface exudate and the flavonoids isolated showed moderate to good antiplasmodial activity with 5,4'-dimethoxyflavone having the highest activity against chloroquine-sensitive (D6) and resistant (W2) strains of Plasmodium falciparum, with IC50 values of 3.2 ± 0.8 and 4.4 ± 0.01 μg/mL respectively.

Keywords: Senecio roseiflorus, Asteraceae, Surface exudates, Antiplasmodial activity.

Senecio roseiflorus R.E Fries (Asteraceae) is an erect herb or weak shrub, with very sticky leaves. It is endemic to Kenya and is found in the drier alpine zone, at an altitude of 3100-4200 m [1]. The genus Senecio is composed of about 1500 species, of which about 33 are found in Kenya [1,2]. Plants in this genus are known to yield mainly pyrrolizidine alkaloids, sesquiterpenoids mainly with an eremophilane skeleton, diterpenoids and flavonoids, with hepatotoxic, carcinogenic, insecticidal, antimicrobial, antitumor, antiviral, antilucre, and immunosuppressing activities [3-6]. The main characteristic feature of this plant is the leaf surface exudate (up to 30% dry leaf weight) due to deposition of surface compounds, which are usually methylated flavonoids in a terpenoid milieu. Flavonoids are known to have good antiplasmodial activity against chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2) Q strains of Plasmodium falciparum [7,8]. As a continuation of our search for new antiplasmodial compounds from plants with surface exudates, the phytochemistry of the aerial parts of S. roseiflorus, as well as the antiplasmodial activity of the exudates and pure compounds, were determined. This is the first phytochemical work on this plant.

Fourteen flavonoids: 5-hydroxy-3,7,4'-trimethoxyflavone [9]; 3,5-dihydroxy-7,4'-dimethoxyflavone [9]; 5,4'-dihydroxy-3,7,3'-trimethoxyflavone [10]; 3,5-dihydroxy-3',4',7-trimethoxyflavone; rhamnazin [10]; rhannocitran [11]; 5,4'-dihydroxy-7-dimethoxyflvanone [12]; 5-hydroxy-3,6,7,4'tetramethoxyflavone [11]; retusin [13,14]; rhamnocrin [11]; isokaempferide [10]; 5,7-dihydroxy-3,3'-dimethoxyflavone [11]; quercetin-3,4'-dimethyl ether [10]; and rhannazin [10], and one phenol: 4-hydroxy-methylenbenzoate [15] were isolated from the exudates of S. roseiflorus. The antiplasmodial activity of some of these isolated compounds is summarized in Table 1. The flavanone: 5,4'-dihydroxy-7-dimethoxyflavanone was the most potent against chloroquine-sensitive (D6) and resistant (W2) strains of Plasmodium falciparum, with IC50 values of 3.2 ± 0.8 and 4.4 ± 0.01 μg/mL, respectively. The other flavonoids showed moderate activities. The activities of all the compounds tested against the two strains of P. falciparum were comparable, with no significant differences. The surface exudates of the aerial parts of S. roseiflorus showed no antiplasmodial activity (IC50 > 90.0 ± 9.8 μg/mL) against the chloroquine-sensitive (D6) strain of P. falciparum.

Table 1: The in vitro activity (IC50) of flavonoids from surface exudates of S. roseiflorus against D6 and W2 strains of Plasmodium falciparum

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC50 in μg/mL</th>
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<tbody>
<tr>
<td></td>
<td>D6</td>
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<tr>
<td>5,4'-dihydroxy-3,6,7-trimethoxyflavone</td>
<td>18.2 ± 3.5</td>
</tr>
<tr>
<td>5,7-Dihydroxy-3,4'-dimethoxyflavone</td>
<td>8.9 ± 1.7</td>
</tr>
<tr>
<td>Quercetin-3, 4'-dimethyl ether</td>
<td>18.2 ± 3.5</td>
</tr>
<tr>
<td>Rhamnazin</td>
<td>18.6 ± 7.7</td>
</tr>
<tr>
<td>Retusin</td>
<td>10.7 ± 5.7</td>
</tr>
<tr>
<td>5,4'-Dihydroxy-3,3',7-trimethoxyflavone</td>
<td>10.9 ± 2.1</td>
</tr>
<tr>
<td>5,4'-Dihydroxy-7-dimethoxyflavanone</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>0.43 ± 0.002</td>
</tr>
<tr>
<td>Quinine</td>
<td>0.07 ± 0.001</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>0.004 ± 0.001</td>
</tr>
</tbody>
</table>

Experimental

General: For analytical TLC, Merck pre-coated silica gel 60 F254 plates were used. Column chromatography (CC) was carried out using silica gel 60 (70–230 mesh) and Sephadex LH-20. EIMS were recorded at 70 EV, on a SSQ 710 Finnigan MAT mass spectrometer. 1D and 2D NMR were run on an AVANCE-500 (Bruker) spectrometer.

Plant material: The fresh leaves of S. roseiflorus were collected from Mt Kenya Forest, Meru, Kenya at about 1300-1500 m, on 30th August 2009. The plant was identified with the assistance of Mr S.G. Mathenge of the University Herbarium, School of Biological Sciences (SBS), University of Nairobi, where a voucher specimen (Mathenge-030/August 2009) is deposited.

Extraction and isolation: Fresh leaves of S. roseiflorus were extracted by dipping into fresh portions of ethyl acetate for a short period (ca 15 secs). The solvent was removed in vacuo using a
rotary evaporator resulting in a pale brown gummy solid (223 g). A portion of the extract (50 g) was adsorbed onto silica gel (50 g) and subjected to CC (SiO₂, 500 g CH₂Cl₂-n-hexane 1:1). Separation was carried out by stepwise gradient elution using mixtures of CH₂Cl₂-n-hexane followed by MeOH-CH₂Cl₂ in increasing polarities. The fraction eluted in 60% CH₂Cl₂-n-hexane was purified by CC (Sephadex-LH 20, MeOH-CH₂Cl₂; 1:1) to give 5-hydroxy-3',4',7-trimethoxyflavone (33 mg), 3,5-dihydroxy-7,4'-dimethoxy-flavone (54 mg), 5,4'-dihydroxy-3,7,3'-trimethoxyflavone (103 mg) and 3,5-dihydroxy-3',4',7-trimethoxyflavone (21 mg). Yellow crystals of 5,4'-dihydroxy-7-methoxyflavone (1.480 g) precipitated out of the fraction eluted in neat CH₂Cl₂ (24 mg). White crystals of 5,4'-dihydroxy-7-methoxyflavonanone (1.480 g) precipitated out of the fraction eluted in neat CH₂Cl₂ (24 mg). The mother liquor was purified by CC (Sephadex-LH 20; MeOH- CH₂Cl₂; 1:1) to yield yellow crystals of 3,5,4'-trihydroxy-7-methoxyflavone (rhamnocitrin) (24 mg). White crystals of 5,4'-dihydroxy-7-methoxyflavonanone (1.480 g) precipitated out of the fraction eluted in neat CH₂Cl₂ (24 mg). The mother liquor was purified by CC (Sephadex-LH 20; MeOH- CH₂Cl₂; 1:1) coupled with PTLC (SiO₂) 90% CH₂Cl₂-n-hexane multiple development to yield 20 mg of 5-hydroxy-3,6,7,4'-tetramethoxyflavone and retusin (28 mg). The fraction eluted in 2% CH₂Cl₂-MeOH after purification by CC (SiO₂, 1% MeOH-CH₂Cl₂) followed by PTLC (SiO₂, CH₂Cl₂) multiple development yielded rhamnocitrin (15 mg) and isokaempferide (24 mg). The fraction eluted with 3% CH₂Cl₂-MeOH afforded 104 mg of 5,7-dihydroxy-3,4'-dimethoxyflavone and 53 mg of quercetin-3,4'-dimethyl ether. The mother liquor was purified by preparative TLC (SiO₂, CH₂Cl₂ multiple development) to yield rhamnazin (35 mg) and 4-hydroxy-methylbenzene (22 mg).

In vitro antiplasmodial activity assay: The extracts and the pure compounds were assayed using an automated micro-dilution technique to determine 50% growth inhibition of cultured parasites [16,17]. Two strains of Plasmodium falciparum parasites, from the Walter Reed Army Institute of Research, that are commonly used in drug sensitivity assays were cultured. The chloroquine sensitive Sierra Leone I (D6) and chloroquine- resistant Indo-China I (W2) strains were grown in continuous culture supplemented with mixed gas (90% nitrogen, 5% oxygen, 5% carbon dioxide), 10% human serum, and 6% hematocrit of A+ red blood cells. Once cultures reached a parasitemia level of 3% with at least a 70% ring stage development, parasites were transferred to a 96 well microtitre plate with wells pre-coated with sample. The samples were serially diluted across the plate to provide a range of concentration used to accurately determine IC₅₀ values. Plates were incubated in a mixed gas incubator for 24 h. Following the specified incubation time, [³H]-hypoxanthine was added and parasites allowed to grow for an additional 18 h. Cells were processed with a plate harvester (Tom Tec) onto a filter paper and washed to eliminate unincorporated [³H]-hypoxanthine. Filters were measured for activity in a microtiter plate scintillation counter (Wallac). Data from the counter was imported into a Microsoft Excel spreadsheet and subsequently into an Oracle database/program to determine (IC₅₀) values.

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References