SHORT REPORT

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Phytochemical Study of *Myrtopsis corymbosa*, Perspectives for Anti-dengue Natural Compound Research

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Abstract: In order to find new molecules for anti-viral drug design, we screened 102 ethyl acetate extracts obtained from 51 plants native from New-Caledonia for an antiviral activity against the dengue 2 virus RNA dependant RNA polymerase (DENV-NS5 RdRp). Leaf and bark extracts of *Myrtopsis corymbosa* which strongly inhibited the DENV-NS5 were selected for chemical investigation. We present here the first chemical study of *M. corymbosa* which led us to isolate three coumarins, namely ramosin (**1**), myrsellinol (**1**) and myrsellin (**3**), and three alkaloids, namely skimmianine (**4**), γ-fagarin (**4**) and haplopin (**6**). These compounds were identified as major compounds from the active extracts of the plant. However, they demonstrated only weak antiviral activity on the dengue virus. Further studies are necessary to know if the antiviral activity is due to a synergy between several compounds or due to the presence of other minor compounds.

Keywords: Rutaceae; Myrtopsis corymbosa; dengue virus; alkaloids; coumarins.

1. Plant Source

Leaves and barks of *Myrtopsis corymbosa* Guill. were harvested in April 2009 in Goro region (22°16'43" S; 166°50'18" E), in the South province of New Caledonia (harvesting authorization N°10919-2009). A voucher specimen (Cou 12) was deposited at Herbarium of the IRD center of Noumea. Barks and leaves of the plant have been dried at 37°C and ground separately.

2. Previous Studies

The dengue virus (DENV) is responsible for dengue fever (DF) and dengue hemorrhagic fever (DHF), which are increasingly important public health problems in tropical and subtropical regions [1]. World Health Organization estimates that 2.5 billion people are exposed to the DENV. 500

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million infections occur each year, including 500 000 hospitalizations and 20 000 deaths [2]. Despite the importance of this disease, no vaccine or specific antiviral is available. For the development of an effective therapeutic agent against DENV, the RNA dependent RNA polymerase (DENV-NS5 RdRp) is considered as a promising target for new drugs. The polymerase activity is essential for viral replication and human host cells are devoid of such RdRp activity [3]. Bioguided fractionation of active extracts on the DENV-NS5 was previously validated and already led to isolation of new candidates for anti-dengue drug design [4,5].

3. Present Study

As previously described, ethyl acetate (EtOAc) crude extracts of barks and leaves of *M. corymbosa* were prepared with 50 g of pulverized dried powder with an automated solvent extraction system (ASE, Dionex). 100 mg of each crude AcOEt extract was filtered on polyamide column and tested against the DENV-NS5 RdRp [5]. The DENV polymerase activity was assayed by monitoring the incorporation of radiolabeled guanosine into a homopolymeric cytosine RNA template, as previously described [4,5]. The d-GTP was used as positive control. Cytotoxic assays were performed *in vitro* according to previously published procedures [6] on the human diploid embryonic lung cells MRC5 (MRC5-SV2, ECACC) with taxotère® as positive control.

Table 1. Antidengue potential and cytotoxicity of crude extracts of *M. corymbosa*.

Part of the plant	Inhibition of the DENV-NS5 RdRp at			Cytotoxicity on MRC5 cells at	
	50 µg/mL	10 µg/mL	1 µg/mL	10 µg/mL	1 µg/mL
L1	89 ± 2	78 ± 4	50 ± 16	0	0
L2	-	25 ± 6	20 ± 7	-	-
L3	-	69 ± 3	64 ± 4	-	-
B1	96 ± 2	92 ± 5	87 ± 4	0	0
B2	-	76 ± 2	68 ± 3	-	-
B3	-	62 ± 3	33 ± 12	-	-
C	-	100	100	100	99

L: leaf extract; B: bark extract; 1, 2 and 3 correspond respectively to extraction with EtOAc, with CH_2Cl_2 and alkaloidal extract. C: positive control were respectively measured with 3'-dGTP and Taxotere® for evaluation of DENV-NS5 RdRp inhibition and Cytotoxicity.

Among 102 EtOAc extracts prepared with the different parts (mainly barks and leaves) of 51 native plants from New Caledonia, extracts obtained from *Myrtopsis corymbosa*, an endemic species of the Rutaceae family, were particularly active on the DENV-NS5 RdRp: Crude EtOAc extracts of barks (B1) and leaves (L1) of *M. corymbosa* were tested at three different concentrations against DENV polymerase, MRC5 cells and three microorganisms (*E. coli*, *S. aureus* and *C. albicans*). Neither significant cytotoxicity (see **Table 1**) nor antimicrobial activity (data not shown) was measured. On the contrary, a remarkable activity on the DENV-NS5 RdRp is observed with the leaf (L1) and bark (B1) extracts which respectively inhibit 78 and 92% of the viral RNA polymerase activity at 10 µg/mL. The bark extract is the strongest and even inhibits 87% of DENV polymerase at 1 µg/mL.

In view of the phytochemical screening of those active extracts and also in accordance with chemotaxonomy of Rutaceae [7], we hypothesized that alkaloids and/or coumarins could be responsible of the antidengue potential of the plant. Thus, crude CH_2Cl_2 and alkaloids extract (obtained with CH_2Cl_2 with ammonia followed by liquid-liquid fractionations) of leaves (L2 and L3) and barks (B2 and B3) were realized and tested on the DENV-NS5 RdRp. Results in **Table 1** indicate that B2 and L3 conserve the antidengue potential and therefore may contain antidengue compounds. Thus, major compounds of B2 and L3 were purified and tested for their antidengue potential. Purity of every compound (> 95%) was assessed by HPLC-UV/MS. Structure elucidation of all isolated compounds is based on spectral analysis. 1D and 2D NMR spectra were recorded on a Bruker AM-300 and Bruker AM-400 instruments. HRESIMS spectra were recorded on a Bruker MicroTOF Q-II spectrometer. Spectral data which allowed the structural elucidation are identical to those available in literature [8-11].

Compounds **1**, **2**, and **3** were isolated from the CH_2Cl_2 extract of barks of *M. corymbosa* (B2) and respectively identified as ramosin, myrsellinol and myrsellin (**Figure 1**). The purification procedure and spectral data are available in supplementary data.

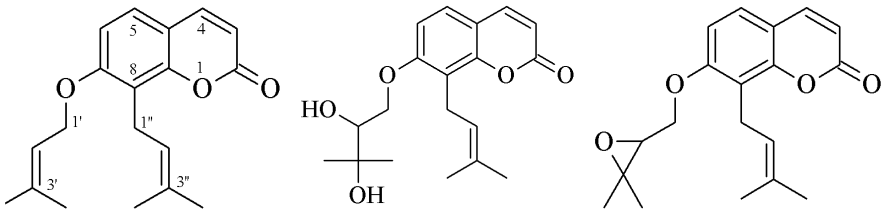

Figure 1. Chemical structure of coumarins **1-3** isolated from barks of *M. Corymbosa.*

HPLC profile of bark crude extract (**Figure 2**) shows that compounds **1-3** are major constituents of the CH_2Cl_2 bark extract which conserve the antidengue potential (**Table 1**). Thus, compound **1-3** were thought to be responsible of the strong antiviral activity measured with bark extracts. However, the isolated coumarins (**1-3**) only demonstrated weak activity on the DENV-NS5 RdRp (see **Table S5**).

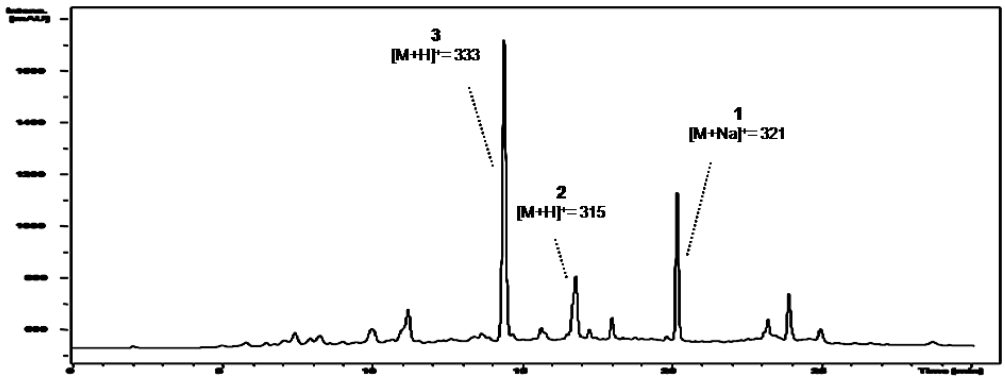

Figure 2. Chromatogram HPLC-UV/MS of CH_2Cl_2 bark extract of *M. corymbosa* at 330 nm

Alkaloids content of leaves were also investigated in view of the activity of L3 (**Table 1**). Compounds **4**, **5** and **6**, were respectively identified as skimmianine, γ-fagarin and haplopin (**Figure 3**). Here again, isolated alkaloids were only slightly active against the DENV-NS5 RdRp when tested separately. The purification procedure and spectral data are available in supplementary data.

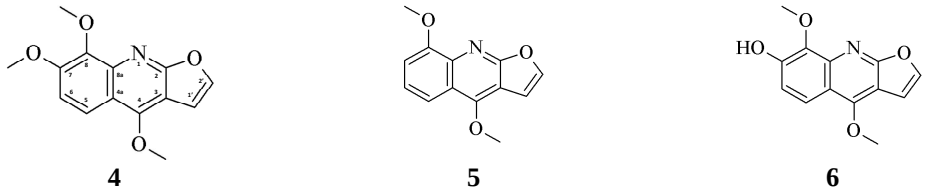

Figure 3. Chemical structure of alkaloids **4-6** isolated from leaves of *M. corymbosa*.

The present study is the first pharmacological oriented phytochemical study of *M. corymbosa*. Crude AcOEt extracts from this plant, filtered on polyamide column, are strong non cytotoxic inhibitors of DENV-NS5 RdRp with a dose dependant effect. This is particularly true for the bark extract which inhibit 87% of DENV polymerase activity at 1 µg/mL. This is one of the first record of antidengue potential of a Rutaceae, a family which was supposed to contain antiviral compound [12,13]. According to our results, *M. corymbosa* may contain potent inhibitors of DENV-NS5 which could be proposed for antiviral drug design. In accordance with chemotaxonomy of Rutaceae [7], three prenylated coumarins (**1-3**) and three furoquinoleic alkaloids (**4-6**) were isolated respectively in barks

and leaves. ramosin (**1**) and haplopin (**6**) are described for the first time in the *Myrtopsis* genus whereas compounds **2-5** were previously isolated in other *Myrtopsis spp* [8]. Alkaloids and coumarins which exhibited antiviral activity on other Flaviviridæ in literature [14], were presumed to be responsible of inhibitory activity of leaves and bark extracts on DENV-NS5 RdRp. However, all isolated compounds were only slightly active against the DENV-NS5 when tested alone. The antidengue potential could be due to other minor compounds or synergy of several molecules.

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