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# New Inhibitors of the DENV-NS5 RdRp from *Carpolepis laurifolia* as Potential Antiviral Drugs for Dengue Treatment

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**Abstract:** Since a few decades the dengue virus became a major public health concern and no treatment is available yet. In order to propose potential antidengue compounds for chemotherapy we focused on DENV RNA polymerase (DENV-NS5 RdRp) which is specific and essential for the virus replication. *Carpolepis laurifolia* belongs to the Myrtaceae and is used as febrifuge in traditional kanak medicine. Leaf extract of this plant has been identified as a hit against the DENV-NS5 RdRp. Here we present a bioguided fractionation of the leaf extract of *C. laurifolia* which is also the first phytochemical evaluation of this plant. Five flavonoids, namely quercetin (**1**), 6-methylapigenin-7-methylether (**2**), avicularin (**3**), quercitrin (**4**) and hyperoside (**5**), together with betulinic acid (**6**), were isolated from the leaf extract of *C. laurifolia*. All isolated compounds were tested individually against the DENV-NS5 RdRp and compared with four other commercial flavonoids: isoquercitrin (**7**), spiraeoside (**8**), quercetin-3,4'-di-O-glucoside (**9**) and rutin (**10**). Compounds **3**, **4**, **6**, **8** and **10** displayed $IC_{50}$ ranging from 1.7 to 2.1 µM, and were the most active against the DENV-NS5 RdRp.

**Keywords:** Myrtaceae; *Carpolepis laurifolia*; Dengue Virus; Flavonoids; Triterpenes.


## 1. Plant Source

The Myrtaceae family is represented by 235 species in New Caledonia (NC), and 234 of them are endemic to this island. Thus, all three species of Carpolepis are endemic to NC and has never been investigated for their chemical content.

The leaves of *Carpolepis laurifolia* J.W.Dawson. were harvested in December 2009 at 480 meters high on the plateau de Tango (20°59'04" S; 165°05'05' E ), in the North Province of NC (harvesting authorization n° 609122852-2009). A voucher specimen (De Rus 27) was deposited at Herbarium of the IRD center of Noumea (NOU) and identified by Dr. Jérome Munzinger (IRD).

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## 2. Previous Studies

Since a few decades the dengue virus (DENV) became the most prevalent and widespread arthropod borne virus affecting human and no treatment is available yet. In order to find new agents for chemotherapy, the viral DENV-NS5 RNA polymerase (DENV-NS5 RdRp) has been identified as a target of choice for antidengue drug research [1]. Previously, few natural compounds were described for their inhibitory activity on the DENV-NS5 RdRp [2,3]. Among those compounds, biflavonoids were the most active [4].

## 3. Present Study

During the screening of medicinal plants for antidengue activity, the crude extract obtained from the leaves of *Carpolepis laurifolia*, an endemic species of Myrtaceae from NC, was found to be particularly active on the DENV polymerase (91% inhibition at 10 µg/mL) and did not show any cytotoxicity on MRC5 cells at 10 µg/mL. This plant was selected for further investigations. A phytochemical screening led on the leaf powder underlined the abundance of flavonoids in leaves of *C. laurifolia* which could be responsible of the antidengue potential of the plant.

340 g of dry leaf powder obtained from *C. laurifolia* were successively extracted at room temperature with petroleum ether (3 × 1 L, 3h each) to obtain the fraction A (8.5 g) and with MeOH 80% (3 × 1 L, 3h each) to obtain fraction B (23.5 g). Methanolic extract (B) was reduced to 1 L and extracted by EtOAc (3 × 1 L) to obtain B.2 (20 g). The petroleum ether extract (A) exhibited low inhibitory activity on DENV-NS5 RdRp while methanol extract (B) and ethyle acetate part of methanol extract (B.2) conserved the antidengue potential measured during the screening (data not shown). The bioguided fractionation of B.2 allowed us to isolate five flavonoids (**1**-**5**) namely quercetin (**1**), 6-methyl-7-methoxyapigenin (**2**), avicularin (**3**), quercitrin (**4**), hyperoside (**5**) together with the triterpene betulinic acid (**6**). Purification was achieved by successive fractionations on silica gel columns, Sephadex LH-20 and preparative HPLC. The purity of all tested compounds (> 95 %) was assessed by HPLC-UV/MS. Spectroscopic data (MS, $^{1}$H and $^{13}$C NMR) of all compounds were consistent with published data [5-7]. Copies of the original spectra are obtainable from the corresponding author.

The biological activity on the DENV-NS5 RdRp of all compounds (**Figure 1**) was evaluated individually and compared with four other commercial flavonoids: Isoquercitrine (**7**), spiraeoside (**8**), quercetin-3,4'-di-O-glucoside (**9**) and rutin (**10**), purchased from Extrasynthèse. The DENV polymerase activity was assayed by monitoring the incorporation of radiolabeled guanosine into a homopolymeric cytosine RNA template, as previously described [8]. The d-GTP was used as positive control.

Among tested compounds, the active flavonoids (**1**, **3**, **4**, **8** and **10**) exhibited $IC_{50}$ against the DENV-NS5 RdRp ranging from 1.7 to 3.6 µM. The avicularine (**3**) was the most active flavonoid tested here ($IC_{50}$= 1.7 µM). According to the results obtained in this study (see **Figure 1**), the structure of the genin significantly modulates the inhibitory activity of the DENV-NS5 RdRp and even small modification of it can influence the activity measured on the DENV polymerase. Thus we can observe that quercetin (**1**) was significantly more active than the C-methylapigenin derivative (**2**). Also, the structure and position of the substituants on the quercetin modulated the inhibitory activity of the DENV-NS5 RdRp. Indeed the avicularin (**3**) and quercitrin (**4**) with a rhamnose and arabinose substituant in C3 respectively, demonstrated high inhibitory activity of DENV polymerase while the activity dramatically decreased for hyperoside (**5**) and isoquercitrin (**7**) with a galactose and glucose in the same position (**Figure 1**). Also, the spiraeoside (**8**) substituted by a glucose in C4', strongly inhibited the DENV-NS5 RdRp ($IC_{50}$= 1.9 µM) whereas the additional glucose induced a loss of activity for quercetin-3,4'-di-O-glucoside (**9**).

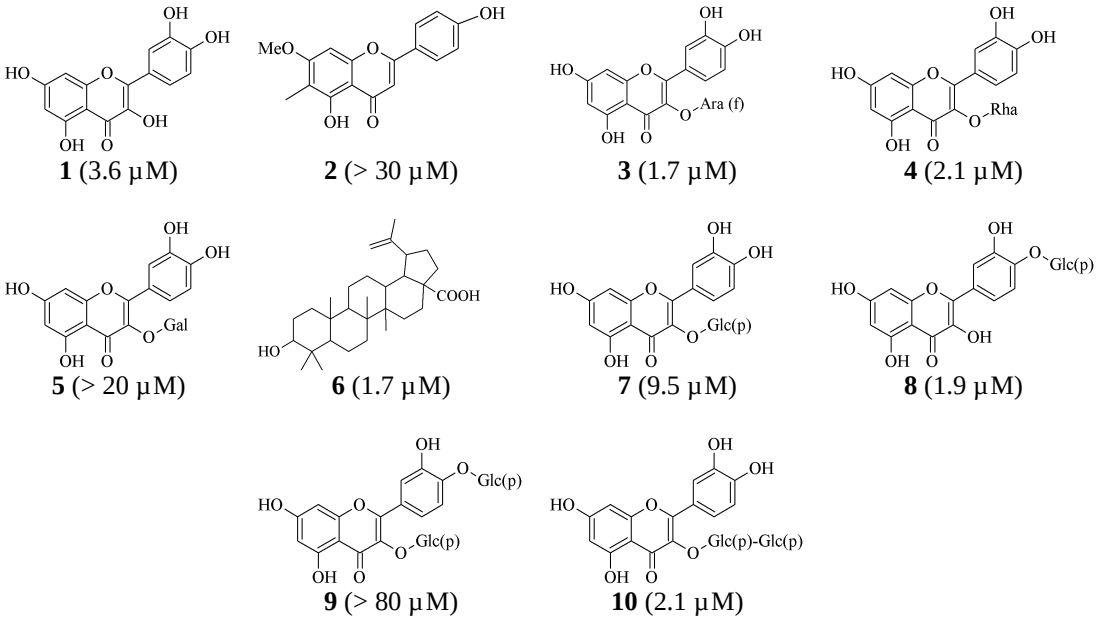


**Figure 1.** Inhibition of the DENV-NS5 RdRp ($IC_{50}$ in µM) by compounds from *C. laurifolia* (**1**-**6**) and other related commercial flavonoids monomers (**7**-**10**).

The flavonoids tested here have been previously described for various biological activities including antiviral [9] but were tested for the first time for antidengue potential. We should note that other flavonoids were previously described for antidengue activity [3, 10, 11]. Interestingly, betulinic acid (**6**) isolated from *C. laurifolia*, was one of the stronger inhibitor of the DENV-NS5 RrRp among tested compounds ($IC_{50}$= 1.7 µM). This compound was previously described for several biological activities including antiviral activity against HIV [12] but it is the first report for antidengue potential of a triterpene. It will be interesting in the future to evaluate the activity of other triterpene derivatives on the DENV-NS5 RdRp.

To the most of our knowledge, this study represents the first phytochemical study of a *Carpolepis* species, an endemic genus in New-Caledonia, from the Myrtaceae family. This plant appeared to be rich in quercetin derivatives (such as **1**, **3**, **4** and **5**). This result is consistent with the literature since those quercetin derivatives are frequently observed in the Myrtaceae species. The presence of C-methyl apigenin derivatives (such as **2**), that are less common in plant kingdom, confirms that *Carpolepis* genus belong to the clade of Metrosideroideæ, in the phylogeny of Myrtaceae. Indeed, those compounds have been isolated in the leaves of several species of this group, including *Metrosideros spp.* [13]. The leaf crude extract of this plant strongly inhibited the DENV-NS5 RdRp and was not cytotoxic. Five flavonoids (**1**-**5**) and betulinic acid (**6**) were isolated from the active fractions of this plant. The activity of all isolated compounds was determined individually on the DENV-NS5 RdRp and compared with the activity of other four commercial flavonoids (**7**-**10**). This study allowed us to identify five flavonoids (**1**, **3**, **4**, **8** and **10**) and a triterpene (**6),** with high potential for antidengue drug development.

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