# FLAVONOIDS OF *MICONIA ALYPIFOLIA* AND THEIR ANTIOXIDANT ACTIVITY

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**Summary**

The chemical and biological study of a methanol extract of leaves of *Miconia alypifolia* (Melastomataceae) revealed the presence of four flavonoids: apigenin-7-O-glucoside, kaempferol-3-O-diglucoside, kaempferol-3-O-galactoside and quercetin-3-O-galactoside. These compounds appear to be of chemotaxonomic significance in the genus and shows antioxidant properties *in vitro.*

**Key Words:** *Miconia alypifolia;* Flavonoids; Chemotaxonomy; Antioxidant activity.

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## Introduction

The study of plants used in traditional medicine is a privileged way for discovering new active compounds. In a series of studies on plants used in traditional medicine of Peru (1, 2, 3 ), we studied the flavonoid fraction of a methanol extract of leaves *Miconia alypifolia* Naud. (Melastomataceae). This plant, traditionally called "*Hierba del susto*", is prescribed in popular medical practices of Northern Peru as a general tonic, in states of weakness and for the treatment of respiratory disorders (4).
No phytochemical or pharmacological studies are available in the literature on *M. alypifolia*. However, several species of the genus *Miconia* have been investigated. Triterpenes have been isolated from the wood of *M. albicans* Steud. (5), *M. stenostachya* DC. (6) and *M. fallax* DC. (7), some of which showing trypanocidal activity *in vitro* (7). The benzoquinone primin and its quinol miconidin isolated from some *Miconia* species, have been reported for their antibacterial, cytotoxic (8, 9) and antifeedant activities (10). Primin was also reported for its cytotoxicity in M109 and A2780 tumor cell lines (11) and showed antineoplastic activity in patients with basic cellular carcinoma (12). Fatty acid synthase inhibitors were isolated from *M. pilgeriana* Ule. (13). Flavonoids have been isolated from *M. trailii* Cogn. (14) and *M. myriantha* Benth., these latter inhibitors of *Candida* aspartic proteases (15). Moreover, extracts of some *Miconia* species have been reported for their analgesic effects (16, 17, 18). Moreover, in spite of Melastomataceae are among the most abundant and diversified groups of plant throughout the tropics, their intrafamily relationships and morphological evolution are poorly understood (19). In this paper we studied the flavonoid fraction of a methanol extract of leaves of *M. alypifolia* and we evaluated the antioxidant activity *in vitro* of isolated compounds.

## Materials and methods

***Plant material***. Leaves of *Miconia alypifolia* were collected in Laguna Prieta, Ayabaca Province, Departement of Piura (Peru) in September 1998. The plant was identified by Prof. V. De Feo. A voucher specimen of the plant, labelled as DF/P/88/20, is deposited in the Herbarium of Medical Botany Chair at the State University of Salerno.

***Extraction and isolation.*** Air-dried leaves of M. alypifolia (587 g) were extracted sequentially at room temperature with $CH_2Cl_2$, $CHCl_3$ and MeOH. The extracts were concentrated in vacuo, yielding 45.47, 4.47, and 60.14 g of residues, respectively. Part of the methanol extract (3.40 g) was fractionated by gel permeation chromatography on a Sephadex LH-20 column using MeOH as eluent. Fractions of 8 ml were collected and combined according their similarity TLC in n-BuOH-AcOH-$H_2O$ (60:15:25) and $CHCl_3$-MeOH-$H_2O$ (70:30:3). Fifty-eight fractions were collected and combined in 12 main fractions (I-XII). Fraction IV was purified by RP-HPLC on a C-18 μ-Bondapak column, using MeOH-$H_2O$ 1:1 as eluent and yielded apigenin-7-O-glucoside (7.5 mg). From fraction V (eluent MeOH:$H_2O$ 1:1) kaempferol-3-O-diglucoside, kaempferol-3-O-galactoside (11.7 mg) and quercetin-3-O-galactoside (14.1 mg) were obtained.
The structures of the isolated coumpounds were established by $^{1}H$ and $^{13}C$ NMR data and by comparison with literature (20).

***Antioxidant activity.*** The antioxidant potentials of pure compounds were measured by ABTS (2,2'-azinobis (3-ethylbenzothiozoline-6-sulfonate) radical cation (ABTS•+) scavenging test. The ABTS•+ cation radical was produced by the reaction between 7 mM ABTS in $H_2O$ and 2.45 mM potassium persulfate (final concentration). The reaction mixture was allowed to stand in the dark at room temperature for 12-16 h before use. The ABTS•+ solution was then diluted with phosphate buffered saline (PBS), pH 7.2, to an absorbance of 0.70 at 734 nm and equilibrated at 30 °C. At the beginning of the analysis day, the $ABTS^{\bullet+}$ radical cation stock solution was diluted in 5mM PBS to reach an $A_{\lambda 734} = 0.7$ and equilibrated at 30°C Pure compounds were solubilized in a minimal volume of DMSO and diluted with PBS (DMSO final concentration in stock solutions never exceeded 10%). Trolox (6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) was used as antioxidant standard. 2.5 mM Trolox was prepared in PBS plus DMSO (10% final concentration) and stored at -20°C. Fresh working standard dilution (0.25 – 2.5 mM) were prepared daily by diluting this stock solution with PBS. Controls without $ABTS^{\bullet+}$ are used to allow for any absorbance of test compounds. One milliliter of PBS (instead of $ABTS^{\bullet+}$ solution) is mixed with the test compound and the absorbance at 734 nm is read after 1 min. At all tested compound dilutions the absorbance were neglegible. The antioxidant potential was measured at 30° C. 10 μL of sample or Trolox (2 - 20 μM, final concentration) dilutions were added to 1 mL of $ABTS^{\bullet+}$ working solution ($A_{\lambda 734} = 0.7$).

The absorbance of the $ABTS^{\bullet+}$ solution was measured exactly 4 min following reagent mixing. The percentage inhibition was calculated for *each* concentration relative to a blank absorbance (solvent blank contained the same amount of DMSO presents in sample and Trolox assay tubes). Values were plotted as a function of the concentrations of test compounds or of Trolox (standard curve was comparable to that reported in the literature). TEAC value is definite as the concentration of the compound with equivalent inhibition to 1 mM standard Trolox solution Determinations were performed at least in triplicate over two different days for each sample (21).

## Results and Discussion

This investigation permitted the structural determination of 4 flavonoids, three flavonol glycosides, of quercetin and kaempferol and apigenin, a flavone glucoside. Generally, according to the observations of Wollenweber and Dietz (1981)(22), methoxylated derivatives of flavones or flavonols with one sole hydroxyl on the B-ring (apigenin and kaempferol patterns, respectively) are more common than methoxylated derivatives of compounds with di- or tri-hydroxylated B-rings.

The compounds present in *M. alypifolia* could be useful for a better knowledge of the chemistry of Melastomataceae, have been found also in other two genera of Melastomataceae, *Lavoisiera* and *Microlicia* (23). In these genera flavonoids have been proposed as a chemosistematic marker (23). *Lavoisiera* species could be split into species that produce exclusively flavones and species that produce either flavones and flavonols. *Microlicia* may be divided chemically into two groups: species with only flavonols and species with both flavonols and flavones but there is a large number of flavonols and few flavone derivatives. Presence of flavonols has long been regarded as a marker of primitiviness, often associated with woody habits, in opposition to flavones, more characteristic of purported advanced and herbaceous taxa (24). The composition of flavonoidic fraction in *M. alypifolia* could be useful for a better knowledge of the chemistry of Melastomataceae. Flavonoids of *M. alypifolia* were also evaluated for their antioxidant activity *in vitro.*

**Table 1.** Relation between the inibition of adsorbance of the radical cation ABTS and TEAC.

| Compound | %Inhibition | | | TEAC |
|---|---|---|---|---|
| | 30 μM | 70 μM | 100 μM | |
| Quercetin-3-O-galactoside | 21% | 49% | 70% | 0,20 |
| Apigenin-7-O-glucoside | 19% | 32% | 41% | 0,14 |
| Kaempferol-3-O-diglucoside | 32% | 53% | 96% | 0,48 |
| Kaempferol-3-O-galactoside | 17% | 29% | 43% | 0,17 |

As shown in Table 1, the scavenger effect of tested compounds is dose dependent. TEAC values, calculated on the basis of inhibition (%) of $ABTS^{\bullet+}$ solution absorbance at 30 μM concentration of each compound, shows that the order of potency of isolated flavonoids is kaempferol-3-O-diglucoside > quercetin-3-O-galactoside > apigenin-7-O-glucoside; kaempferol-3-O-galactoside.

Antioxidant activity of compounds found in *M. alypifolia* agree partially with data of literature. In fact, the available literature reports some observations regarding structural requirements of flavonoids to exert antioxidant activity. The antioxidant activity of flavonoids and their metabolites *in vitro* depends upon the arrangement of functional groups, in particular hydroxyl groups, about the nuclear structure. In fact, free radical scavenging capacity is attributed to the high reactivities of hydroxyl substituents (25). A 3',4'-cathecol structure in the B-ring enhances lipid peroxidation inhibition. Flavones, like apigenin-7-O-glucoside, lacking catechol or o-trihydroxyl systems form relatively unstable radicals and weak scavengers (25). A-ring substitution correlates little with antioxidant activity. A 5-OH may contribute to antioxidant effects as well as a free 3-OH (flavonols), that increase the stability of the flavonoid radical. The torsion angle of the B-ring with respect to the rest of the molecule strongly influences free radical scavenging ability. Flavonols with a 3-OH are planar, while the flavones lacking this feature are twisted. Flavonoids with a 2-3 double bond in conjugation with a 4-carbonyl group exhibit lower $IC_{50}$ values (indicative of stronger antioxidant activity) in a microsomal system compared to the those with saturated heterocycles (25).

Generally aglycones are more potent antioxidant than their corresponding glycosides, even if the position and structure of the sugar play an important role. This negative effect may stem from the properties of the sugar itself. An A-ring sugar results in a greater diminution of activity than 3-glycosylation in the heterocycle (25). O-glycosylation at carbon 7, but not carbon 3, weakens the antioxidant effect of flavonoids (apigenin-7-O-glucoside) in rat mitochondria (25). It is also important to acknowledge that a glycosyl substituent, regardless of position and structure, seldom confers an antioxidant advantage over the aglycone. O-glycosilation interferes with the coplanarity of the B-ring with the rest of the flavonoid and the ability to delocalize electrons (25).

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