



## Chemotaxonomic relevance of the constituents from the leaves of *Rothmannia merrillii*

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### ABSTRACT

Phytochemical investigation on *Rothmannia merrillii*, an endemic Philippines Rubiaceae species, had led to the isolation of vomifoliol (1) and *p*-hydroxybenzaldehyde (2). Their chemotaxonomic relevance to other Rubiaceae species is discussed.

**Keywords:** Rubiaceae, *Rothmannia merrillii*, chemotaxonomy, vomifoliol, isolation

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### INTRODUCTION

The plant family Rubiaceae is considered to be the fourth largest angiosperm composed of about 600 genera and 13,000 species distributed worldwide [1]. The Philippines is home to a wide variety of endemic Rubiaceae species whose properties, both phytochemical and medicinal, awaits scientific investigations. One of these endemic species is *Rothmannia merrillii* (Elmer) Chavez and Alejandro [2]. *R. merrillii* is widely distributed in the islands of Palawan and Mindoro and known locally as bagawi, niyog-niyog, balitungyun and dayano. Although the phytochemical studies on the genus *Rothmannia* is limited, the presence of iridoids and iridoid glucosides have previously been identified [3]. In our continuing research related to the endemic Rubiaceae species of the Philippines [4], we herein report the isolation and identification of vomifoliol (1) and *p*-hydroxybenzaldehyde (2) from *Rothmannia merrillii* and their chemotaxonomic relevance. This research also characterizes the first phytochemical study on *Rothmannia merrillii*.

### EXPERIMENTAL SECTION

#### General considerations

<sup>1</sup>H and <sup>13</sup>C NMR were measured using JEOL JNM ECP 400 (400 MHz) or JEOL JNM ECP 600 (600 MHz) spectrometer using CDCl<sub>3</sub> as solvent and TMS as reference. Silica gel (Merck 7734 or 9385) and aluminum-backed Silica gel 60 F<sub>254</sub> TLC plates (Merck) were used for chromatography. UV<sub>254</sub> followed by vanillin-H<sub>2</sub>SO<sub>4</sub> spray and heating were used to visualized the components for TLC.

**Plant collection**

Fresh leaves of *Rothmannia merrillii* were collected from Brgy. Poblacion, Puerto Galera, Mindoro Philippines, in May 2010. Voucher specimens (GN003, GN004, GN006, GN019) were identified by one of the authors (GJDA) as a Philippine Rubiaceae specialist and deposited at the UST-Herbarium, Research Center for the Natural and Applied Sciences.

**Extraction and isolation**

The air-dried, ground leaves of *R. merrillii* (996 g) were percolated with distilled MeOH (total of 9.5 L) for three consecutive days at room temperature. The combined extract was concentrated under reduced pressure to give the crude MeOH extract (75 g). The crude extract was suspended in water (300 ml) and partitioned sequentially with hexane (3 x 100 ml), CH<sub>2</sub>Cl<sub>2</sub> (4 x 100 ml) and *n*-BuOH (2 x 100 mL) to afford the semi-crude extracts.

The CH<sub>2</sub>Cl<sub>2</sub> extract (5.6g) was initially separated over silica gel gravity column chromatography (GCC) using CHCl<sub>3</sub> with increasing MeOH (5 – 100%) to give four pooled fractions (RmA – RmD) after TLC. Fraction RmC (0.187g) was subjected to silica gel GCC (3:7 hexane-EtOAc; 1.5:8.5 hexane-EtOAc; neat EtOAc) to give three fractions (RmC1 – RmC3) after TLC. Fraction RmC1 was purified by silica gel GCC (9:1 CHCl<sub>3</sub>-EtOAc) to afford compound **2** (1.5 mg, light-yellow solid).

Fraction RmD (0.401g) was subjected to silica gel GCC (9:1 EtOAc-MeOH) to give four fractions (RmD1 – RmD4) after TLC. RmD1 was purified by silica gel GCC (neat EtOAc) to give an isolate (6.2 mg) which was further purified by silica gel CC (3:7 hexane-EtOAc) to afford compound **1** (1.1 mg, colorless oil).

**RESULTS AND DISCUSSION**

Chromatographic separation of the crude extract from the leaves of *Rothmannia merrillii* had resulted in the purification of two compounds which were identified as vomifoliol (**1**) and *p*-hydroxybenzaldehyde (**2**) based on NMR analyses and comparison with the literature data [5].

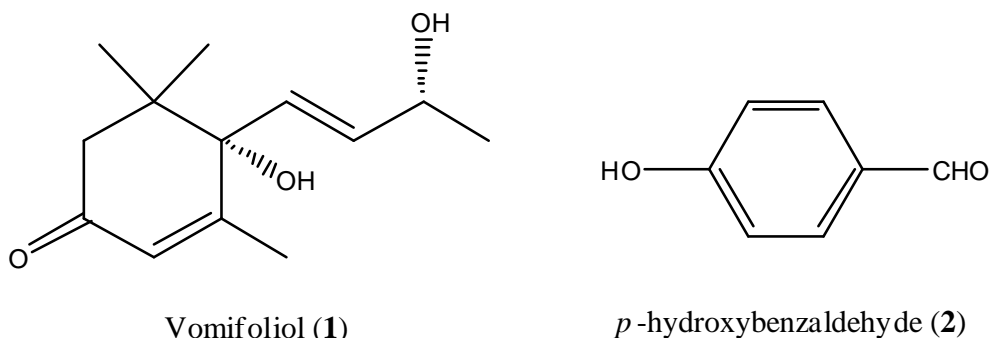


Figure 1. Isolated compounds from *Rothmannia merrilli*

This research represents the pioneering phytochemical study on *Rothmannia merrillii*, one of the endemic Rubiaceae species in the Philippines. It also reports for the first time the isolation of **1** and **2** from the genus *Rothmannia*. Iridoids have been ubiquitous in the previous phytochemical studies on genus *Rothmannia*. The isolation of **1** and **2** from the present study also reaffirms the abundance of these types of compounds from genus *Rothmannia*. The presence of these two compounds in *Rothmannia* is the second record of plant representative aside from *Villaria odorata* [6,4b] belonging to the subfamily Ixoroideae. The former belongs to the tribe Gardenieae and the latter in Octotropideae. Both tribes of Ixoroideae are closely related in the recent phylogeny of the family [7] possessing secondary pollen presentation. Previously, **1** has been found dominant in the subfamily Rubioideae represented by *Hedyotis corymbosa* [8], *Palicourea alpine* [9], *Psychotria gitingensis* [4a], *Psychotria yunnanensis* [10] and *Morinda citrifolia* [11]. However, this compound was also identified from *Ochreinauclea maingayii* [12] from the subfamily Cinchonoideae. It seems that **1** is present in the currently recognized three subfamilies of Rubiaceae. Although compound **2** is present in many plant species, interestingly this has been identified also in *Morinda citrifolia* [13]. The diversity on which the iridoids have been isolated from the complex Rubiaceae family may prove their chemotaxonomic importance in conjunction with morphology and molecular markers.

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