A new phthalide derivative from Peperomia nivalis

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RESEARCH ARTICLE

A new phthalide derivative from *Peperomia nivalis*

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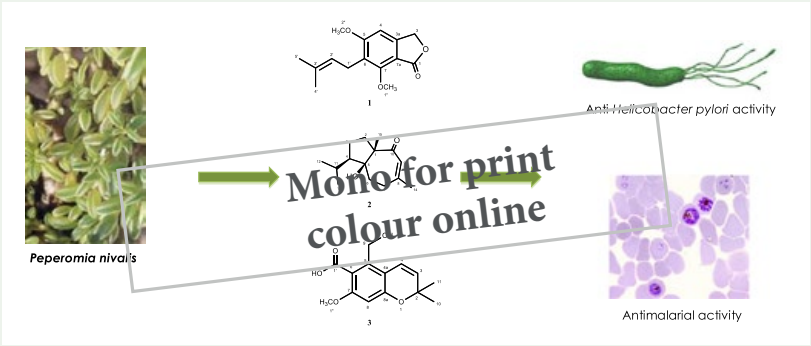
ABSTRACT

One new phthalide (**1**) was isolated from aerial parts of *Peperomia nivalis*, along with known compounds (**2** and **3**), reported in this species for the first time. The structure of the new compound was characterised on the basis of 1D (^{1}H and ^{13}C NMR), 2D (COSY, HMQC, HMBC and NOESY) NMR and high-resolution mass spectral (HRMS) data. Compound **2** was isolated from a natural source for the first time but previously synthesised. Compounds **1**–**3** were evaluated for their anti-*Helicobacter pylori* and anti-*Plasmodium falciparum* activities. Compound **1** showed moderate activities against *H. pylori* (MIC 47.5 µM) and the F32-Tanzania strain of *P. falciparum* (IC_{50} 8.5 µM). Compounds **2** and **3** exhibited weak anti-*H. pylori* activity (MIC 241.3 and 237.6 µM, respectively) and were inactive against *P. falciparum*.



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Peperomia nivalis; phthalide; secondary metabolites; NMR; *Helicobacter pylori*; *Plasmodium*

1. Introduction

Peperomia, a genus of the pepper family (Piperaceae), is one of the two large genera of the Piperaceae family, with more than 1500 recorded species of tropical and subtropical fleshy herbs, annuals as well as perennials. The *Peperomia* genus is the second most abundant source of bioactive compounds in the Piperaceae family (López et al. 2010). From this genus,

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previous works have reported the isolation of bioactive compounds such as lignans, lactones and phenolic (Li et al. 2006; Mahiou et al. 1995). In Peru, numerous species of the *Peperomia* genus, such as *Peperomia galioides*, *P. congona* and *P. hartwegiana*, are used in traditional medicines to treat stomach problems (de la Cruz et al. 2014) or for their antibacterial activity (*P. galioides*) (Langfield et al. 2004; De Feo et al. 2008). However, some compounds such as prenylated phenols with antiparasitic activity were reported from *P. galioides* (Mahiou et al. 1995, 1996). In Cameroon, *P. vulcanica* and *P. fernandopoiana* are used as medicinal plants by traditional medicine practitioners to treat febrile illnesses (Ngemenya et al. 2015). Ngemenya et al. (2004) investigated *P. vulcanica* and observed moderate *in vitro* activity against *Plasmodium falciparum* (70% inhibition by 40 µg/mL crude extract).

Within the scope of our research line on isolation, structural characterisation and evaluation of the bioactivity of secondary metabolites from peruvian medicinal plants (Ruiz et al. 2011; Haddad et al. 2013; Cabanillas et al. 2015; Girardi et al. 2015), we investigated aerial parts of *Peperomia nivalis*. This plant, endemic to Peru, grows in rocky sunny places in high-altitude Andean areas and is used in traditional medicine to treat stomach ulcers, gastritis, internal pain and sensation of burning stomach (Pino et al. 2005). Phytochemical investigation led to the isolation of a new phthalide derivative along with two known compounds (**2**, **3**). Compounds **1**–**3** were evaluated for their anti-*Helicobacter pylori* and anti-*P. falciparum* activities. To the best of our knowledge, this is the first phytochemical report on *P. nivalis*.

2. Results and discussion

Compound **1** ($C_{15}H_{18}O_4$, HR-TOF-MS) was obtained as a white-coloured solid with molecular formula requiring seven degrees of unsaturation. It exhibited UV absorption bands at 248 and 301 nm, while the IR spectrum displayed characteristic absorption bands for a γ-lactone ring at 1760 cm^{-1} and for alkene at 1619 and 1601 cm^{-1}. The ^1H NMR spectrum (Table S1) exhibited resonances for one aromatic (6.44, 1H, s) proton, two equivalent oxymethylene protons (δ_H 5.12, 2H, s), one olefinic methine at δ_H 5.09 (m), two methoxyl groups (δ_H 4.00, 3H, s and 3.95, 3H, s), two equivalent allylic methylene protons at δ_H 3.23 (d, $J = 7.2$ Hz) and two signals indicating terminal methyl at δ_H 1.74 (3H, d, $J = 0.9$ Hz) and δ_H 1.71 (3H, d, $J = 1.4$ Hz), respectively. The ^{13}C NMR spectrum (Table S1) showed one ester carbonyl (δ_C 169.5), six resonances in the aromatic region (δ_C 163.3, 158.4, 148.7, 115.6, 105.4 and 94.6), two olefinic (δ_C 132.9 and 120.8), one methylene (δ_C 68.0), two methoxy (δ_C 56.1, 56.0), one allylic (δ_C 24.4) and two methyl (δ_C 25.7 and 17.7) carbons. Inspection on key HMBC correlations (Figure 1) as well as comparison with the literature data suggested the substitution of the aromatic cycle by two methoxyl groups and one alkenyl chain (Li et al., 2006). These data were confirmed by the HMBC correlations observed between H-1′ (δ_H 3.23, d) and C-6 (δ_H 115.6) and C-5 (δ_c 163.3) that established the connectivity of the alkenyl chain at C-6, in addition to those of H-1″(δ_H 4.00, 3H, s) and C-7 (δ_c 158.4) and between H-2″ (δ_H 3.95, 3H, s) and δ_c 163.3 suggesting that the methoxyl groups are located at C-7 and C-5, respectively.

On the basis of the above results and by comparison of its NMR data with the literature values published for 5,7-dimethoxy-6-(3-methylbut-2-enyl)isobenzofuran-1(3H)-one (Li et al., 2006), and with other phthalides previously isolated from another genus (Li et al. 2012; Venditti et al. 2015), the structure of peperophthalide A was elucidated as 5,7-dimetoxy-6-(3-methylbut-2-enyl)-isobenzofuran-1(3H)-one **(1)** .

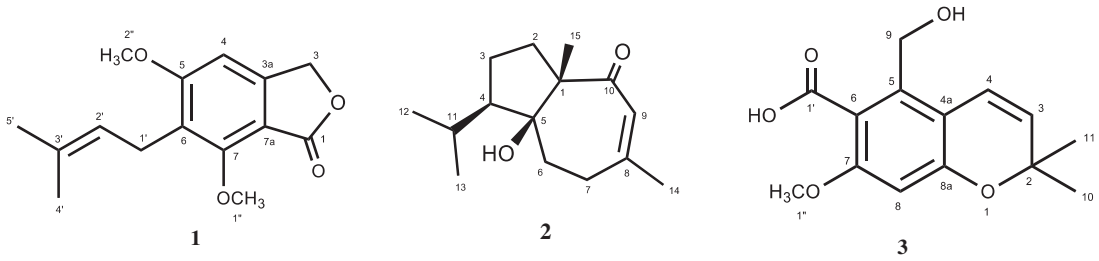

Figure 1. Chemical structures of compounds **1**–**3**.

Since *P. nivalis* is used in traditional medicine to treat stomach ulcers, gastritis, internal pain and sensation of burning stomach and since Marilone A, a phthalide derivative isolated from the sponge-derived fungus *Stachylidium* sp, and some chromenes were shown to have antiplasmodial activity against *Plasmodium berghei* (Oshimi et al. 2009; Almeida et al. 2011) and strong antiprotozoal activity (Harel et al. 2013), compounds **1**–**3** were evaluated for their anti-*H. pylori* and anti-*P. falciparum* activities.

Compound **1** was the most effective product against both biological models used in this work. It showed a moderate activity against *H. pylori* (MIC 47.5 µM) and against the F32-Tanzania strain of *P. falciparum* (IC_{50} 8.5 µM), while compounds **2** and **3** exhibited weak anti-*H. pylori* activity (MIC 241.3 and 237.6 µM, respectively) and were shown to be inactive against *P. falciparum*. According to the WHO guidelines, antiplasmodial activity was classified as weak at $10 < IC_{50} < 100$ µM. These results are in accordance with previous reports. On the one hand, previous screening programme designed to discover active compounds against *H. pylori* allowed the isolation of seven phthalide antibiotics with specific anti-*H. pylori* activity (Dekker et al. 1997; Radcliff et al. 2008). On the other hand, the antiplasmodial activity of peperophthalide **1** (IC_{50} 8.5 µM) was found to be close to the antiplasmodial activity of the phthalide derivative Marilone 1, isolated from the sponge-derived fungus *Stachylidium* sp., with an IC_{50} of 12.1 µM against *P. berghei* (Almeida et al. 2011). Nevertheless, Sommart et al. (2012) isolated seven new phthalide derivatives, microsphaerophthalides A–G, which were shown to be inactive against *P. falciparum* (K1, multidrug-resistant strain). Furthermore, compound **3**, a known chromene derivative, was shown to exhibit strong antifungal activity against *Cladosporium cladosporioides* (Malquichagua Salazar et al. 2005). In addition, some chromene derivatives, isolated from the genus *Peperomia* (Batista et al. 2011), and some chromenes containing structure, have shown to have a strong bacteriostatic activity against *Staphylococcus aureus* (Starks et al. 2014) and strong antiprotozoal activity (Harel et al. 2013). However, chromene **3** exhibited only a weak anti-*H. pylori* activity and no activity against *P. falciparum*. AQ11

3. Conclusion

Investigation of *P. nivalis* aerial part extract afforded the isolation of one new phthalide **1** derivative together with two known compounds. Compound **2** is reported here for the first time as a natural compound, and compound **3** has never been isolated before in *P. nivalis*. We reported for the first time the *in vitro* anti-*H. pylori* and anti-*P. facliparum* activity of *P. nivalis* derivatives. The moderate activity of compound **1** against *H. pylori* can be linked to the traditional use of the plant to treat stomach problem.

Supplementary material

Experimental details relating to this paper are available online.

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Disclosure statement

No potential conflict of interest was reported by the authors

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