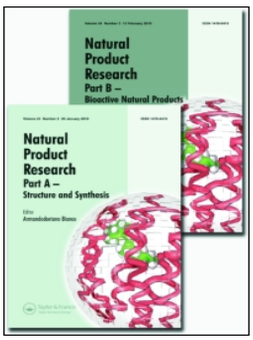

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

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To cite this article: Marisa C. Gaspar, Diogo A. Fonseca, Manuel J. Antunes, Christian Frigerio, Nelson G. M. Gomes, M. Vieira, Armanda E. Santos, Maria T. Cruz, Maria D. Cotrim & Maria G. Campos (2018) Polyphenolic characterisation and bioactivity of an Oxalis pes-caprae L. leaf extract, Natural Product Research, 32:6, 732-738, DOI: 10.1080/14786419.2017.1335728

To link to this article: https://doi.org/10.1080/14786419.2017.1335728

NATURAL PRODUCT RESEARCH, 2018
VOL. 32, NO. 6, 732–738
https://doi.org/10.1080/14786419.2017.1335728

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SHORT COMMUNICATION

Polyphenolic characterisation and bioactivity of an *Oxalis pes-caprae* L. leaf extract

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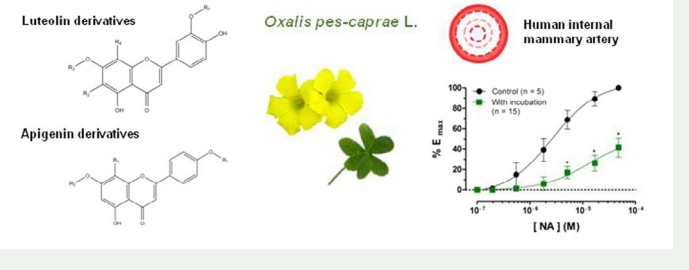
ABSTRACT

The present work is focused on the characterisation of the polyphenolic content of an *Oxalis pes-caprae* L. leaf extract and on the evaluation of its bioactivity with particular interest on its vascular activity and antioxidant potential. The polyphenolic content was characterised by HPLC-DAD and LC-MS/MS. The vascular activity was evaluated according to the influence on the serotonergic and adrenergic systems of the human internal mammary artery (HIMA). Antioxidant and neuroprotective studies were also conducted. Several luteolin and apigenin derivatives were identified as main constituents of the extract, which did not present any contractile effect nor had any effect on the serotonergic system of HIMA. However, it showed antagonistic effect on the adrenergic system, inhibiting the contraction to noradrenaline (reduction of 58.44% of maximum contraction). The extract showed antioxidant activity and standardised luteolin and apigenin derivatives showed neuroprotective potential, particularly homoorientin.



ARTICLE HISTORY
Received 19 April 2016
Accepted 18 May 2017

KEYWORDS
Oxalis pes-caprae L.; polyphenols; vascular activity; human internal mammary artery; noradrenaline; antioxidant potential; neuroprotection

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2017.1335728.

1. Introduction

Oxalis pes-caprae L. (Oxalidaceae) is an invasive weed from temperate and mediterranean zones (Vilà et al. 2006; DellaGreca, Previtera, et al. 2007; Ross et al. 2008; Ferrero et al. 2015). Its sour taste derives from the high content in oxalic acid, a toxic compound that might cause nervous system paralysis in large herbivores when consumed in great quantities (Campos and Proença 2001; Van Wyk et al. 2002; Herbert and Dittmer 2016). Several *Oxalis* species have been used in the folk medicine, particularly the roots, owing to their diuretic properties, and the leaves due to their antihypertensive effects (Güçlütürk et al. 2012; Aprilita et al. 2013). Some bioactive compounds have been identified from extracts of *O. pes-caprae* L. Ester and phenyl cinnamate derivatives, aromatic compounds and phenols were found in the leaves and twigs (DellaGreca, Previtera, et al., 2007; DellaGreca et al. 2008; DellaGreca et al. 2009; DellaGreca et al. 2010). Polyphenolic compounds from extracts of aerial parts were identified and showed high antioxidant activity (Güçlütürk et al. 2012). In related species (e.g. *Oxalis corniculata*) flavonoids have been reported from leaves, namely the glycosylflavones, isoorientin, isovitexin and swertisin (Mizokami et al. 2008; Aprilita et al. 2013). Polyphenols are associated with beneficial cardiovascular effects and antioxidant properties (Pandey and Rizvi 2009). The reports on the polyphenolic content (particularly in the leaves) and antioxidant activity, as well as its abundance, highlight *O. pes-caprae* L. as an interesting and inexpensive source of bioactive compounds. This work evaluated for the first time the potential bioactivity of an *O. pes-caprae* L. leaf extract focusing on the vascular, antioxidant and neuroprotective activity.

2. Results and discussion

2.1. Polyphenolic characterisation of the O. pes-caprae L. leaf extract

2.1.1. HPLC-DAD

Considering the retention time (RT) of each compound (Table S1) and the respective UV spectra, using the theory developed by Campos and Markham (2007), compounds **1**–**3** were identified as luteolin derivatives (Figure S1) and compounds **4**–**6** as apigenin derivatives (Figure S2).

Luteolin derivatives, as for the main flavones, displayed two main bands of UV absorption: Band I at λ_{max} 350.0 nm (with higher UV absorption than Band II) and a Band II with a double peak, Band IIa at λ_{max} 267.0 nm and Band IIb at λ_{max} 257.0 nm characteristic of 3′ and 4′ substitution in ring-*B* (Campos and Markham, 2007). When Band I at this wavelength present a lower absorption compared to Band II indicates a flavonol structure with a radical at 3-*O* position (what is not the casa) and when this band presents a higher absorbance than Band II, a flavone nucleus, without 3-*O* substitution, is a more probably configuration, as it is the case of all compounds in this extract, **1**–**6**.

Other relevant information includes the intensity of the double peaks in Band II (IIa and IIb). An higher absorbance of Band IIa relatively to Band IIb suggests a 6- or 8-*C* derivative, possibility, as it is the case of compounds **1**–**3**.

Compounds **4**–**6** presents a Band I at λ_{max} 337.4 nm that was more intense than Band II (λ_{max} 268.0 nm) suggesting a flavone nucleus as explained above. Compounds **5** and **6** also presented a higher absorbance in Band I (λ_{max} 338.8 nm and λ_{max} 342.5 nm, respectively) relatively to Band II (λ_{max} 269.4 nm and λ_{max} 268.0 nm, respectively).

2.1.2. LC-MS/MS

Compound **1** have a full MS spectrum with a $[M - H]^-$ ion at *m/z* 638.4 which was compared to *m/z* 622.05 of 2″-glycosylvitexin (standard) in a first approach indicates an additional *m/z* 16 $[M - H]^-$. Fragments at *m/z* 518 $[(M - H)-120]^-$ and 476 $[(M - H)-162]^-$, indicating the presence of an 8-*C*-hexose linked with another hexose, and a fragment *m/z* 447 $[M - H]^-$ that correspond to a luteolin-*C*-glucoside. All this date lead to the identification of compound **1** as 7,3′-dimethoxyl-2″-*O*-glycosylorientin.

For compound **2**, have also a full MS spectrum with a $[M - H]^-$ ion at *m/z* ion at *m/z* 638.4, but fragments at *m/z* 492 $[(M - H)-146]^-$ and 402 $[(M - H)-146-90]^-$, indicate a 6-*O*-desoxyhexose and an 8-*C*-hexose, respectively. Thus, it corresponds to 7,3′-dimethoxyl-6-desoxyhexoseorientin.

For compound **3**, a molecular ion at *m/z* 638.6 and fragments at *m/z* 488 $[(M - H)-150]^-$ and *m/z* 447 $[(M - H)-191]^-$, indicate a 6-*C*-hexose and an 8-*O*-acetylhexose, respectively. Therefore, it corresponds to 6-hexosyl-8-acetylhexosylluteolin.

For compound **4**, a molecular ion at *m/z* 622.0 and fragments at *m/z* 502 $[(M - H)-120]^-$ and 459 $[(M - H)-162-1H]^-$, indicate the presence of a 2″-*C*-hexose. Thus, it corresponds to 7,4′-dimethoxyl-2″-glycosylvitexin.

Compound **5**, a molecular ion at *m/z* 576.9 and fragments at *m/z* 413 and 293, indicate the presence of a 2″-*O*-rhamnose. Thus, it corresponds to 2″-*O*-rhamnosylvitexin. For compound **6**, a molecular ion at *m/z* 431.0 and fragments at 341 and 311, indicate the presence of an 8-*C*-glucose. Thus, it corresponds to 8-glycosylapigenin, also known as vitexin. See Figures S3 and S4 and Table S1 for details.

2.2. Vascular activity

The extract (0.0023– 0.63 mg/mL) did not show any intrinsic activity as it was not able to induce any effect on the human internal mammary artery (HIMA), results not shown. This lack of intrinsic activity may result from a bulky effect of the 6-*C* and 8-*C* substituents in luteolin derivatives and 7-*O* and 8-*C* in apigenin derivatives, in the receptors. A 30 min pre-incubation with the extract (0.66 mg/mL) did not alter the response of the HIMA to 5-hydroxitriptamine (serotonin, 5-HT)-induced cumulative concentration-response curves (results not shown). However, the same concentration of the extract induced a statistically significant reduction (58.44%, $p < 0.05$) of the maximum contraction to noradrenaline (NA) (41.56 ± 9.32% contraction after extract incubation) (Figure 1). A parallel shift of the NA-induced cumulative concentration-response curve to the right was observed with a change of the pEC_{50} from 5.579 ± 0.140–4.894 ± 0.330, which showed a reduction in the potency of NA-induced response of the HIMA. These results suggest a non-competitive antagonism on the adrenergic receptors once there is no intrinsic activity and a reduction in the maximum contraction (excluding the competitive antagonism) and in the potency of the agonist. Such results are in agreement with other studies. In isolated rat and guinea pig organs (e.g. aorta and uterus), Afifi et al. (1999) suggested that luteolin presents antispasmodic activity and homoorientin (6-*C*-glycosylluteolin) decreases the frequency and amplitude of the phasic contractions of uterine segments. Other authors studied the relaxant responses and the ability of the flavones/flavonols to inhibit contraction in rat isolated thoracic aorta (Woodman et al. 2005). Duarte et al. (1993) suggested that luteolin relaxes the contractions induced by NA in rat

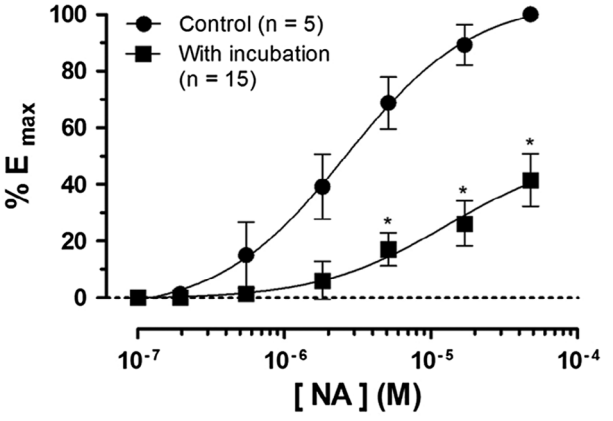

Figure 1. Cumulative concentration-response curves to NA in the absence ($n = 5$) and presence ($n = 15$) of *O. pes-caprae* L. on HIMA. Results were presented as Mean ± SEM (*$p < 0.05$ vs. control).

aorta strips and Abdalla et al. (1994), reported a concentration-dependent relaxation of the tone of ileum, adrenaline-pre-contracted pulmonary artery.

2.3. Anti-inflammatory activity

Macrophages activated by the *Toll-like* receptor 4 (TLR4) agonist, lipopolysaccharide (LPS), produce large amounts of the pro-inflammatory mediator nitric oxide (NO) and constitute a well-described *in vitro* model of inflammation, useful for the screening of molecules with anti-inflammatory activity. The production of NO could be measured by the accumulation of nitrites in the culture supernatants, using the Griess reagent, as previously reported (Francisco et al. 2011). Our results demonstrated that LPS strongly induced NO production, but the extract did not modulate NO production triggered by LPS (data not shown). These results can be due to other compounds present in the extract that have immunostimulatory activity. Therefore, they may act as inhibitors of the anti-inflammatory activity of luteolin and apigenin derivatives. In fact, previous studies evidenced the anti-inflammatory properties of luteolin, apigenin and its derivatives. Using a similar approach, homovitexin inhibited the inducible NO synthase mRNA expression in LPS-activated macrophages (Conforti et al. 2009). Other mechanisms have also been reported for homovitexin, such as the inhibitory effect on cyclooxygenases (Dongmo et al. 2007). Using a model of acute inflammation, other authors demonstrated that luteolin presents anti-inflammatory activity in rats (Simões et al. 1988) and homoorientin also demonstrated anti-inflammatory activity in mice (Kupeli et al. 2004).

2.4. Antioxidant activity

The screening for free radical scavenging bioactivity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) displayed an $IC_{50} = 17.93$ µg/mL of dried extract, confirming the extract is an interesting source of antioxidant compounds being in agreement with the results from other natural extracts. One from spring leaves of *Oxalis acetosella* L. has demonstrated to be an important

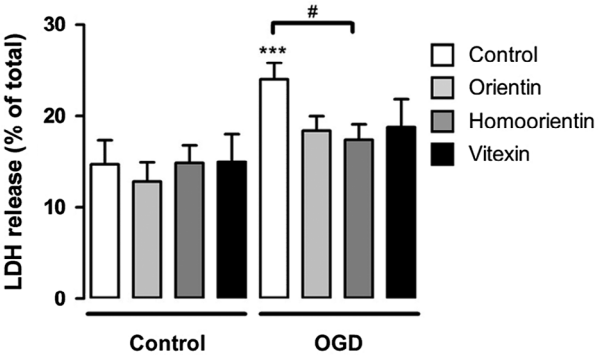

Figure 2. Effect of vitexin, homoorientin and orientin, on the viability of rat brain hippocampal neurons submitted to OGD. Percentage of LDH release relatively to the total content of LDH ($n = 7$; ***$p < 0.001$ vs. control; $^{\#}p < 0.05$ vs. OGD).

source of antioxidants being rich in β-carotene, ascorbic acid and flavonoids (Šircelj et al. 2010). Extracts from the aerial parts of *O. pes-caprae* L. have already been reported as containing high levels of antioxidant compounds, such as luteolin glucoside and cernuoside (Güçlütürk et al. 2012). Through a DPPH radical scavenging assay and a β-carotene-linoleic acid test, orientin demonstrated antioxidant properties (Wu et al. 2009).

2.5. Neuroprotective activity

The neuroprotective potential of homoorientin, orientin and vitexin was studied. Our results demonstrated that homoorientin (structurally similar to two flavonoids of the extract) decreased the lactate dehydrogenase (LDH) release induced by oxygen-glucose deprivation (OGD), thus, conferred neuroprotection against the ischaemic stimulus. Orientin and vitexin showed a trend to decrease the OGD-induced LDH release (Figure 2). The tested flavonoids did not cause hippocampal neurons death, since control neurons are incubated in the presence or in the absence of these flavonoids the release of LDH was similar (Figure 2).

3. Conclusions

Three luteolin derivatives and three apigenin derivatives were identified from the polyphenolic fraction of an *O. pes-caprae* L. leaf extract, which inhibited the NA-induced contraction of HIMA suggesting a non-competitive antagonism and presented antioxidant properties. Interestingly, homoorientin showed a neuroprotective effect. These results suggest the potential use of this extract as a source of bioactive compounds. Further studies need to be carried out to unveil the structure–activity relationship of each compound of the extract.

Acknowledgements

The authors thank Dr. Leonilde and other colleagues from Cardiothoracic Surgery, University Hospital of Coimbra, Portugal for the help in the collection of samples and financial support through "Projeto Estratégico - RG-Centro-177-3717" da "Fundação para a Ciência e Tecnologia" (FCT, Portugal), FCT UID/QUI/00313/2013 and POCI-01-0145-FEDER-007630.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by the Fundação para a Ciência e a Tecnologia [grant number FCT UID/QUI/00313/2013,POCI-01-0145-FEDER-007630].

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