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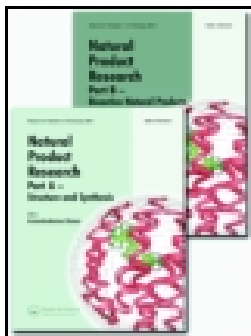
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SHORT COMMUNICATION



Anti-inflammatory and hemostatic effects of *Linaria reflexa* Desf

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ABSTRACT

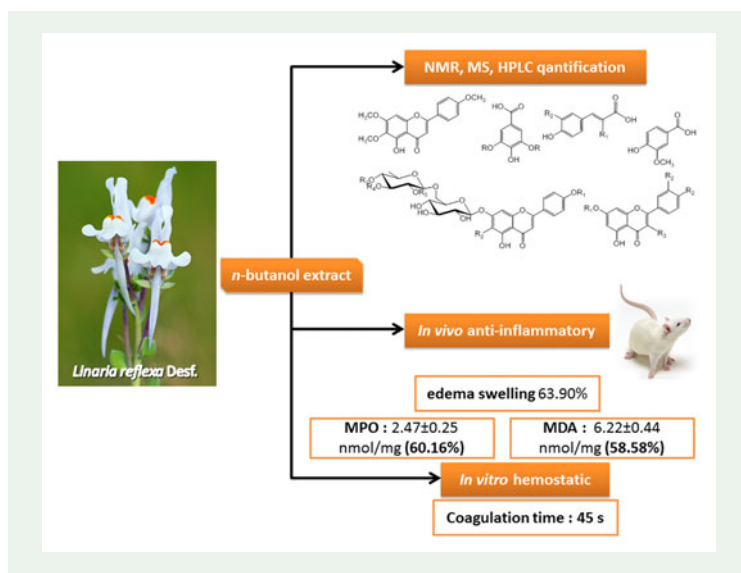
The work presented here was aimed to investigate the *in vivo* anti-inflammatory and *in vitro* hemostatic activities of *Linaria reflexa* extract and to establish the relationship between its bio-activity and chemical composition. Twenty-three secondary metabolites were identified, most of them are good anti-inflammatory agents, in line with data by carrageenin-induced rat paw edema assays of the *n*-butanol extract showing high anti-inflammatory inhibition (63.90%) of edema swelling in the rat paw at the dose 200 mg/kg after 4 h. Furthermore, both extent of inflammatory response and tissue injury were prevented keeping the levels of rate myeloperoxidase (60.16%) and of malondialdehyde, which is the final product of lipid peroxidation generated by free radicals (58.58%). The same extract showed also a remarkable hemostatic effect established by measuring the coagulation time of decalcified plasma (45 s), related to its flavonoid glycosides content.

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flavones; acids; anti-inflammatory; hemostatic; *Linaria reflexa*



1. Introduction

Linaria is a known genus of the Plantaginaceae family reported to contain iridoids (Bianco et al. 2004; Frezza et al. 2019), flavonoids (Cheriet et al. 2017) and terpenoids (Venditti et al. 2015) as major constituents. *Linaria reflexa* Desf. is a small medicinal herb used in the North African folk medicine under the name “Oum lajrah” (mother of wounds) because of its healing power, in the topical treatment of certain dermatoses (Boukef 1986). Significant antidiabetic (Cheriet et al. 2017) and anti-acetylcholinesterase (Loizzo et al. 2007) activities were observed for the plant extracts, related to their chemical composition rich in flavonoids and terpenoids (Tundis et al. 2005; Cheriet et al. 2014). Keeping on our studies about the Algerian *Linaria* species (Cheriet et al. 2015; Hanfer et al. 2017; 2018; Ahmed-Chaouch et al. 2019), we report here the results by *in vivo* anti-inflammatory and *in vitro* hemostatic activities of *L. reflexa* extracts, and by the identification of twenty-three metabolites, of which seven described for the first time in the genus.

2. Results and discussion

The inflammatory response is a series of well-coordinated events involving the increase in vascular permeability, edema and leukocyte recruitment and accumulation, and inflammatory mediator release. Carrageenan injection into the mouse paw induces a biphasic edema. The first phase is characterized by an edema of little intensity and unrelated to the dose of carrageenan used, while the second phase develops after 24 h, displaying more pronounced edema with a maximum effect between 48 and 72 h (Henriques et al. 1987). This is consistent with the results obtained in our study, where the carrageenan induced an inflammation that resulted in massive edema formation, increasing between the first and fourth hour, then remaining high for several hours in the control group (Table S1). The treatment with *n*-butanol extract from

Linaria reflexa (BELR) at a dose of 100 and 200 mg/kg significantly reduced the swelling in both the early phase (1–2 h) which may reflect the liberation of pro-inflammatory agents (Hanfer et al. 2017) and the late phase (4–24 h) of carrageenan-evoked edema. The highest inhibition of swelling (63.90%) was observed at the dose 200 mg/kg after 4 h which corresponds to the phase of prostaglandin release (Temponi et al. 2012) and maintained for 24 h. The inhibition of edema observed in the carrageenan model may be due to the ability of BELR to inhibit the inflammatory mediators, such as histamine and serotonin (Perianayagam et al. 2006). This effect is clearly compared to the value obtained by Diclofenac-sodium (77.95%) at 10 mg/kg, which inhibits the second phase of carrageenan-induced edema *via* the inhibition of prostaglandin synthesis by the cyclooxygenase isoenzymes (COX-1 and -2) (Temponi et al. 2012; León-Reyes et al. 2016).

Paw edema induced by carrageenan produces an inflammatory response associated with edema formation and leukocytes infiltration through the phospholipase A2 activation, and hence the biosynthesis of leukotrienes, prostaglandins, and cytokines (Otuki et al. 2005). Myeloperoxidase (MPO) is an enzyme present in the intracellular granules of neutrophils, used as a specific marker for acute inflammation and which reflects polymorphonuclear leukocyte infiltration of the parenchyma (Loria et al. 2008). Table S2 shows that carrageenan produced an inflammatory response as seen by the significant increase of MPO activity (6.2 ± 0.66 U/mg tissue) in the tissue of the rat paw, which confirmed the accumulation of inflammatory leukocyte infiltration into inflamed tissue at the delayed phase. BELR at 200 mg/kg dose reduced inflammation and MPO activity in paw tissue (60.16%) more than the dose 100 mg/kg (39.83%), although less effective than Diclofenac-sodium (79.45%, 10 mg/kg).

The high level of MPO may contribute to the increased oxidative stress observed in carrageenan paw edema-group (Chaves et al. 2013). It is well-known that in chronic and sub-acute inflammation, reactive oxygen species (ROS) play a relevant role in modulating the extent of inflammatory response and tissue injury. In some pathophysiological processes associated with inflammation, ROS have been proposed to mediate cellular damage. This happens *via* several mechanisms involving the initiation of lipid peroxidation (Winterbourn et al. 2006) and including the formation of malondialdehyde (MDA) as one of the final metabolic products of lipid peroxidation generated by ROS species. In our study, injection of carrageenan in rat hind paw was significantly prevented (58.58% and 62.93%) by both BELR (200 mg/kg) and Diclofenac-sodium (10 mg/kg), respectively in MDA levels (Table S2). Therefore, we can assume that the ROS-scavenging power and/or inhibitory activities of BELR on lipid peroxidation and MDA production is probably due to the inhibition of leukocyte migration to the inflammation site of damage and/or could be due to therapeutic potential for inflammatory diseases. The remarkable anti-inflammatory activity of BELR has been here related to the chemical composition of the extract itself rich in polyphenols, well known to exert potent anti-inflammatory effects (Hussain et al. 2016).

Hemostatic mechanism involves normal functions of blood vessels, platelets and blood coagulation. The latter one is a complex process by which blood forms clots, and represents a crucial part of hemostasis where a damaged blood vessel wall is covered by a platelet and fibrin-containing clot to stop bleeding and begin repair of the

damaged vessel (Satish et al. 2012). At a dose of 10 μ L, petroleum ether extract of *Linaria reflexa* showed the remarkable coagulation time (255 s) followed by lower effects for *n*-butanol (330 s), chloroform (360 s) and ethyl acetate (420 s) extracts (Table S3). The best coagulation times were observed for BELR at both the doses 100 and 200 μ L, with values of 60 and 45 s, respectively. These data indicate a potent hemostatic effect if compared with literature data of known medicinal plants used to stop bleeding (Ohkura et al. 2015) and is attributable to the high amount of flavonoid glycosides in the extract. These metabolites are known for some hemostasis effects, playing pivotal roles in the regulation of hemostasis upon vascular injury through blood clotting (Ghoshal and Bhattacharyya, 2014). Moreover a recent study has shown that a free hydroxyl group in the structures of some natural and synthetic flavones is essential for their antiplatelet activity (Ravishankar et al. 2018).

NMR and ESI-MS data analysis (Supp. Material) led to the identification of salvigenin (1), scutellarein-4'-methoxy-7-O-rutinoside (2), hispidulin-7-O-rutinoside (3), linarin (5) and scutellarein-7-O-rutinoside (6), beside pectolarin (4), linariin (9), isolinariin A (7), B (8), D (10) and E (11) previously isolated from *L. reflexa* methanolic extract (Cheriet et al. 2017). The HPLC profile of BELR (RP18, acetonitrile/water 60:40, 254 nm) was analyzed using a series of molecules as control references. The correspondence of retention time values allowed to identify: gallic acid (12), hydroxycaffeic acid (13), caffeic acid (14), syringic acid (15), vanillic acid (16), rutin (17), luteolin (18), ferulic acid (19), luteolin-7-O-glucoside (20), coumaric acid (21), quercetin (22) and chrysin (23). The quantitative evaluation of these metabolites in the extract was established using a calibration curve built for each standard compound (Figure S2), obtaining the data reported in table S4. Compounds 1–3, 6, 13, 15 and 17 are new for the genus *Linaria*.

3. Conclusion

The Algerian medicinal plant *L. reflexa* Desf. has been studied correlating its phytochemical profile to both *in vivo* anti-inflammatory and *in vitro* hemostasis activities. Twenty-three metabolites including sixteen flavones and seven acids were identified. The biological results prove that the plant can be considered a good anti-inflammatory and hemostasis agent and are promising for further studies on its applicative potential.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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