SHORT COMMUNICATION

Anti-inflammatory and anti-arthritic effects of compounds from Buddleja coriacea

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ABSTRACT

Buddleja coriacea (B. coriacea) commonly known as ‘Kiswara’ is used as infusions for stomach and joint pain. This study aims to evaluate the \textit{in vitro} and \textit{in vivo} anti-inflammatory and anti-arthritic activity was evaluated by measuring inflammatory parameters (TNF-α, C-reactive protein and Fibrinogen) in murine models of the aqueous extract and isolated compounds of \textit{B. coriacea}. A bio-guided phytochemical analysis based on NMR/MS was performed identifying three (1-3) compounds of the aqueous extract. All compounds inhibited the production of TNF-α in RAW 264.7 cell line, with IC₅₀ of 13.44 (1), 1.13 (2) and 0.57 μM (3), respectively. In addition, compounds 2 and 3 decreased the levels of TNF-α, C-reactive protein and fibrinogen at a concentration of 5 mg/kg in murine models. Our research shows that the compounds isolated from \textit{B. coriacea} have anti-inflammatory and anti-arthritic properties, providing scientific evidence for the traditional use of this plant species.

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2022.2025593.

ARTICLE HISTORY
Received 30 September 2021
Accepted 26 December 2021

KEYWORDS
Loganiaceae; Buddleja coriacea; anti-inflammatory; anti-arthritic; TNF-α
1. Introduction

Inflammation is a bodily response to any injury caused by physical trauma, noxious chemicals or microbial agents. In relation to this, rheumatism is a chronic inflammatory disease that has become a major problem affecting morbidity worldwide (Aishwarya et al. 2017). Rheumatoid arthritis (RA) involves the breakdown of cartilage at joints and as a result joint are then packed with white blood cells which secretes sub-stances like interleukins and tumour necrosis factor-alpha (TNF-α) that also results in pain, joint swelling and damage (Kaur et al. 2012).

Although various types of treatments are available today, like NSAIDs, corticosteroids, and DMARDs, these do not target the pathological origin and its long-term use causes adverse reactions (Craig and Cappelli 2018). In this context, natural products can control arthritic inflammation through multiple pathways, for example, through the inhibition of effector molecules (for example, pro-inflammatory cytokines and che-mokines) (Astry et al. 2015).

Thus, the present study analyses the species Buddleja coriacea Remy (B. coriacea), known as ‘Kiswara’, belonging to the Loganiaceae family, is native from Bolivia and has traditionally been used in the form of infusion for the treatment of ulcers, stomach pain, liver problems, urinary tract inflammation and rheumatism (Paniagua-Zambrana and Bussmann 2020). Taking this information into account, the present work was carried out to evaluate the anti-inflammatory and anti-arthritic activities of the aqueous extract of B. coriacea and its isolated compounds in order to confirm the traditional use of this species.

2. Results and discussion

(11β,16α)-16,17-[Butyldiene(bis(oxy))-11,21-dihydroxypregna-1,4-diene-3,20-dione (1), (E)-3-(3-(2-(dimethylamino)ethyl)-1H-indol-yl)-N-(4-methoxybenzyl) acrylamide (2) and (1β,11β,12α)-1,11,12-Trihydroxy-11,20-epoxypicrasa-3,13(21)-diene-2,16-dione (3) (Figure 1) were isolated from the n-hexane sub-extract prepared of aqueous extract from B. coriacea.

The cytotoxic effect of the compounds was evaluated by the LDH assay in RAW 264.7 cells for 12 h. Compounds 1 (CC50 = 88.86 μM), 2 (CC50 = 69.89 μM) and 3 (CC50 = 54.55 μM) were less cytotoxic than the actinomycin D (ACT, CC50 = 0.008 μM) (**p < 0.001) (Supplementary material, Figure S40).

No previous studies on the cytotoxicity of compounds 1 and 2 have been found, however, compound 3 showed cytotoxicity at 0.25–0.52 μM on different tumor cell lines (Yang et al. 2014). The results from this study and ours cannot be compared since they have been carried out in different cell lines. On the other hand, these compounds inhibited the production of the TNF-α in RAW 264.7 cells with IC50 of 13.44 (1), 1.13 (2) and 0.57 μM (3), respectively, when compared to the positive control (C87, IC50 = 0.11 μM) (**p < 0.001) (Supplementary material, Figure S41).

Regarding the anti-inflammatory activity, there are reports on the anti-inflammatory effects of compound 1 (Barrette et al. 2016), being used in chronic inflammatory diseases (Bayiha et al. 2020). On the other hand, the mechanism of action of compounds...
2 and 3 on the TNF-α pathway is unknown, but we can suggest that it is similar to that of C87.

As shown in Figure S42A (Supplementary material), the sub-plantar injection of λ-carrageenan (CARR) induced oedema that reached its maximum expression (acute inflammation) within 2 h after CARR injection. This effect increases in inflammatory mediators after the CARR injection (Makni et al. 2019). In this sense, treatment with compounds 1 and 2 (5 mg/kg) decreased the size of the circumference of the paw oedema by 32.66% and 51.29%, respectively (\(p < 0.001\)). Likewise, mice treated with compound 3 (5 mg/kg) showed higher anti-inflammatory activity (64.29%) than the IND (5 mg/kg) that inhibited the oedema by 59.79% (\(p < 0.001\)).

Regarding the anti-arthritic activity, the sub-plantar injection of Freund’s complete adjuvant (CFA) led the paw to swell gradually for more than 14 days. The curves of oedema rate as a function of time were divided into two phases. In the first phase, oedema rate of the injected footpad increased and reached a peak during the first three days. Thereafter, the swelling slowly subsided until the seventh day when the paw began to swell again and peaked in the second week (second phase); this effect increased in inflammatory mediators after the CFA injection (Sharma et al. 2018). The administration of the compounds 1 and 2 (5 mg/kg) significantly inhibited the development of joint swelling induced by CFA by 30.08% and 50.52%, respectively, when compared to the 5 mg/kg IND (57.08%) on the eighth day after CFA injection (Supplementary material, Figure S42B) (\(*p < 0.01\)). Finally, the mice treated with the compound 3 (5 mg/kg) showed the highest anti-arthritic activity (61.96%) on the eighth day after the CFA injection.

The compounds decreased the TNF-α level in the serum when reaching the fifth hour after the CARR injection (\(***p < 0.001\)). Nevertheless, compounds showed a
higher decrease of the TNF-α level in serum when reaching the fifth hour after the CARR injection (**p < 0.001) when compared to the IND group (5 mg/kg) (Supplementary material, Figure S43A). Additionally, the compounds (5 mg/kg) decreased the TNF-α level in the serum during the first three days after the CFA injection more than the IND group (5 mg/kg) (**p < 0.001) (Supplementary material, Figure S43B).

In relation to the C-reactive protein (CRP) level, this decreased significantly (**p < 0.001) in the groups treated with the compounds 1 (23.34 mg/mL), 2 (14.95 mg/mL), 3 (8.11 mg/mL) and IND (13.37 mg/mL) when compared to the CARR group (25.43 mg/mL) (Supplementary material, Figure S44A). Lastly, concerning the fibrinogen rate, this decreased significantly (**p < 0.001) in the groups treated with the compounds 1 (5.79 g/L), 2 (2.62 g/L), 3 (1.58 g/L) and IND (3.48 g/L) when compared to the CARR group (5.73 g/L) (Supplementary material, Figure S44B).

Instead, the CRP level decreased significantly (**p < 0.001) in the groups treated with the compounds 1 (36.78 mg/mL), 2 (16.64 mg/mL), 3 (11.66 mg/mL) and IND (27.69 mg/mL) when compared to the CFA group (38.74 mg/mL) (Supplementary material, Figure S45A). Finally, concerning the fibrinogen rate, this decreased significantly (**p < 0.001) in the groups treated with the compounds 1 (15.62 g/L), 2 (12.88 g/L), 3 (11.48 g/L) and IND (13.75 g/L) when compared to the CFA group (16.38 g/L) (Supplementary material, Figure S45B).

In relation to inflammatory parameters, haematological changes that occur after the administration of CARR or CFA confirm the resulting acute or chronic inflammation. These changes led to an impressive increase by 89% of the blood cells at the site of inflammation due to the release of inflammatory cytokines. Likewise, the inhibition of inflammation in the mice treated with the isolated compounds (5 mg/kg) is confirmed by the significant decrease in fibrinogen and CRP levels compared to the mice treated with IND (**p < 0.001).

### 3. Conclusions

In conclusion, we found that in assays of short duration (oedema induced by CARR) and of longer duration (arthritis induced by CFA), the aqueous extract of *B. coriacea* significantly inhibited the swelling of the mice feet (at a dose of 5 mg/kg). Similarly, the isolated compounds, especially compound 3, have a potent suppressive effect in pro-inflammatory responses, proving to be an alternative for the treatment of inflammatory diseases.

Regarding the mechanism of action of the isolated compounds, even if the inhibition of TNF-α is through the down regulation of the gene expression of NF-κB, more specific and selective methods need to be used, such as the cytometry flow that will allow us to determine if these compounds can act simultaneously on different inflammatory targets (Lehmann et al. 2009). On the other hand, although the effects of the compounds on rheumatoid arthritis have been studied, it is necessary to study them on other diseases that present chronic inflammation, such as asthma, diabetes, inflammatory bowel disease. This is because the compounds were isolated from the *B.
coriacea species, a plant species that is traditionally used as an infusion to treat stomach pain, liver problems and urinary disorders (Paniagua-Zambrana and Bussmann 2020).

**Disclosure statement**
The authors declare no conflict of interest.

**Funding**
This work was supported by the Fundación de la Universidad Autónoma de Madrid (FUAM).

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