SHORT COMMUNICATION

Isolation, chemical characterization, and anti-inflammatory activity of coumarins, flavonoids, and terpenes from Tagetes lucida

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ABSTRACT
Tagetes lucida is widely used in traditional Mexican medicine for several disorders, including those associated with inflammation. In this work, fifteen compounds were identified (1–15) from T. lucida. Some of these compounds (1–8, 10, 12–14) were detected for the first time in the plant, and quercetagетin 7-O-β-(6″-Protocatecoyl) glucopyranoside (13) has been identified for any plant species. The inflammation inhibition effects of these compounds were as follows: Amix (1–2) > 10 > 12a > 13 > 14a > Bmix (3–9) > 12; 12 and 13 showed a dose-response behavior. The mixture of 14 and 15 was not active. This work contributes to the knowledge of the anti-inflammatory capacity of T. lucida and the chemical identity of their bioactive compounds.

1. Introduction
Tagetes lucida (Asteraceae) is a perennial herbaceous plant native to Mexico and Central America that has glossy leaves and aromatic yellow flowers. Since pre-Hispanic
times, it has been used in a magical-religious context, as a food condiment, and to
treat rheumatism and inflammation (Monroy-Ortiz and Castillo-España 2007).
Antifungal and antibacterial activity of the dichloromethane and methanolic extracts
has been reported, and there was a study that isolated and evaluated coumarin-type
compounds, such as 7,8-dihydroxycoumarin, umbelliferone (7-hydroxycoumarin),
esculetin (6,7-dihydroxycoumarin), 6-hydroxy-7-methoxycoumarin, herniarin (7-
methoxycoumarin), scoparone (6,7-dimethoxycoumarin), and scopoletin (6-methoxy-7-
hydroxycoumarin), and flavonoids, such as patuletin, quercetin, and quercetagenin
(Céspedes et al. 2006). The ethanol extract of this plant has been shown to have an
analgesic effect that is attributed to the presence of quercetagenin 7-O-β-D-glucoside
and to coumarin 6,7 dimethoxycoumarin (González-Trujano et al. 2019) as well as to a
known terpene β-caryophyllene (Hernández-Leon et al. 2020). Although T. lucida is
widely used as an anti-inflammatory in Mexico, few studies have focused specifically
on this activity. This plant species has been studied by our working group in recent
years. Previously, the anti-inflammatory effect produced by the hexane extract
obtained from T. lucida was demonstrated (Monterrosas-Brisson et al. 2020). Five cou-
marins were isolated, 7-isoprenyloxycoumarin being the most active. Therefore, the
objective of this work was to continue with the chemical analysis of the hexanic
extract (less polar) and the aqueous extract (polar compounds) for the isolation and
chemical characterization of other compounds present in T. lucida with anti-inflamma-
tory activity in the murine model of auricular edema induced with phorbol ester-TPA.

2. Results and discussion

The GC-MS analysis of TIRbC1-F2 allowed for the identification of the amyrin mixture
(AMix) α and β in a proportion of 85.4% and 9.24%, respectively. In the fraction
TIRbC1-F3, called BMix, a mixture of sesquiterpenes was identified (3–8); the propor-
tion of these was 31.9%, 5.5%, 5.3%, 14.2%, 7.2%, and 17.9%, respectively (Figure 1).
Compounds 9 and 11 (Figure 2) were identified by comparison with standards of 7-
isoprenyloxycoumarin and herniarin using HPLC (Monterrosas-Brisson et al. 2020).
Compounds 10 was obtained as an amorphous white precipitate. The mass spec-
trum (MS) gave a corresponding molecular peak in m/z 276.9 [M]+ to the molecular
formula [C_{15}H_{16}O_{5}]^{+}. According to the analysis of one and two–dimensional NMR data
(Table S3) and the comparison with those described (Heemann et al. 2006) in the lit-
erature, this compound was identified as 7-(2'-hidroxy-3'-methylbut-3'-enoxy)-6-
methoxychromen-2-one, which is known as virgatenol (10, Figure 2).

Compound 12, an amorphous yellow precipitate, was obtained, and after being
developed with reagents specific for flavonoids, it turned orange and absorbed UV
light. In the UV-vis spectrum, it showed a λ_{max} of 214, 260, and 357 nm, which are
characteristic of flavonols. The MS gave a corresponding molecular peak at m/z 479.03
[M-H]^{-} to the molecular formula [C_{21}H_{19}O_{13}]^{-}. A comparative analysis of the spectro-
scopic data (Tables S1 and S2) with those described in the literature (Shahzadi and
Shah 2015) indicates that this compound corresponds to the quercetagenin-7-O-β-glucopyranoside (12). The one-dimensional NMR spectra of the peracetylated derivative
(12a, white precipitate) were obtained, which was identified as quercetagenin-7-O-
β-glucopyranoside nona-acetate (12a, Figure 2) according to the analysis of the spectroscopic data (Tables S1 and S2). The MS gave a molecular ion of m/z 881.03 [M + Na]⁺, which corresponds to the molecular formula [C₃₉H₃₈NaO₂₂]⁺.
Compounds 13 and 14 showed the same NMR signals as compound 12, with the difference that in 13 and 14, it showed additional signals characteristic of an aromatic ring with an ABX system at $\delta$ 6.82 (d, 1.8 Hz), 6.79 (d, 8.4 Hz) and 7.29 (dd, 1.8, 8.4) and $\delta$ 6.73 (d, 1.8 Hz), 6.52 (d, 8.0 Hz) and 6.55 (dd, 1.8, 8.4 Hz) assigned to H-2, H-5, and H-6, respectively. Additionally, in 14, a trans double bond signal was observed at $\delta$ 7.42 (d, 15.7, H-7) and 6.16 (d, 15.7, H-8). The analysis of the NMR spectra of one and two dimensions and the comparison with the data described (Tables S1 and S2) identified the presence of protocatechuic and caffeic acids, respectively. Both 13 and 14 have quercetagetin-7-O-$\beta$-glucopyranoside as their base structure, and acids are attached to carbon 6 of glucose. This was observed by the correlation in the HMBC of methylene H-6a ($\delta$ 3.96) and H-6b ($\delta$ 4.32) with carbonyl at C-7 ($\delta$ 167.2) and H-6a ($\delta$ 4.65) and H-6b ($\delta$ 4.33) with carbonyl at C-9 ($\delta$ 167.7), respectively. According to the analysis of the NMR data (Tables S1 and S2), 13 corresponded to the quercetign-7-O-$\beta$-(6’-protocatecoyl) glucopyranoside and 14 to quercetatin 7-O-$\beta$-(6’-caffeoyl) glucopyranoside (Farshid et al. 2018). The MS of 13 gave a molecular ion of m/z 640 [M-2H]$^-$, which corresponds to the molecular formula [C$_{30}$H$_{24}$O$_{16}$]. One- and two-dimensional NMR data (Tables S1, S2, and S3) of the acetylated derivative of 14 allowed us to identify the mixture of quercetatin 7-O-$\beta$-(6’-caffeoyl) glucopyranoside deca-acetate (14a) and a coumarin known as scoporone (15, Figure 2). The MS gave a molecular ion of m/z 1085 [M+Na]$^+$, which corresponds to the molecular formula [C$_{50}$H$_{46}$NaO$_{26}$]$^+$. In the present work, some of the identified compounds have already been isolated and reported in the species of the genus Tagetes (and other plant families), although not in T. lucida. This was the case of the amyrins. GC/MS showed a higher concentration of $\alpha$-amyrin than $\beta$-amyrin, the latter of which has been reported for T. erecta (Maity et al. 2011). For $\alpha$-amyrin, there are reports of a dose-dependent anti-inflammatory effect in the TPA assay, which is associated with the suppression of PGE2 by blocking the expression of COX-2 and the activation of NF-$\kappa$B (Medeiros et al. 2007). The application of TPA on the mouse ear caused an edema of 10.87 mg, which was inhibited by 77.5% by the administration of IND at 1 mg/ear (Table S4). The treatments obtained from T. lucida showed a significant inhibition of inflammation when compared to the VEH group ($^*p<0.05$), with varied percentages. The order of edema inhibition was as follows: AMix (1–2) > 10 > 12a > 13 > 14a > BMix (3–8) > 12 > (14 + 15), which did not show anti-inflammatory activity. The compounds quercetatin-7-O-$\beta$-glucopyranoside (12) and quercetatin 7-O-$\beta$-(6’-Protocatecol) glucopyranoside (13) both decrease local inflammation at doses of 0.5 and 1.0 mg/ear, which were statistically different from the VEH group ($^*p<0.05$). Figure S1 shows the dose-dependent behavior and the $E_{max}$ and $ED_{50}$ values for each treatment. The mechanism of TPA-induced edema is a series of cellular events, which includes the activation of phospholipase A2 proteins and protein kinase C (PKC), increased expression of cyclooxygenase-2 (COX-2), which catalyzes the biosynthesis of prostaglandins (PGs), produces edema, stimulates migration of polymorphonuclear leukocytes, increases levels of pro-inflammatory mediators such as IL-1$\beta$, and increases the expression of the enzyme cyclooxygenase 2 (COX-2) and the transcription factor NF-$\kappa$B (Chung et al. 2007).
3. Experimental
See supplementary data.

4. Conclusion
The present work contributes to the knowledge of the chemical composition of *T. lucida*, which consists of flavonoids, terpenes, and coumarins that had not been previously isolated for this species, as well as the isolation and structural elucidation of a new flavonol called quercetagetin 7-O-β- (6‴ - Protocatecoyl) glucopyranoside (13). In addition, to show the anti-inflammatory activity of such compounds, we propose the plant as a candidate in the study of pharmacological models of chronic diseases associated with inflammation.

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Disclosure statement
The authors declare no conflicts of interest.

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