SHORT COMMUNICATION

Antinociceptive activity of the ethanolic extract of Trixis angustifolia DC

Anuar Salazar-Gómeza, Ma. Elena Vargas-Díaz, María Estela Meléndez-Camargob and Saudy Saret Pablo-Pérezab

aDepartamento de Química Orgánica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México, México; bDepartamento de Farmacia, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México, México

ABSTRACT

The antinociceptive activity of the ethanolic extract of Trixis angustifolia DC. (EETx) was investigated using the acetic acid-induced writhing and the hot-plate tests in mice. In the acetic acid-induced writhing test, mice treated with EETx (50, 100 and 200 mg/kg, p.o.) exhibited reduced writhing (38%, 67%, and 74%, respectively). In the hot-plate test, the three doses administrated increased the nociceptive response time. The phytochemical analysis of EETx led to the isolation of three known compounds, hygric acid (1), 5,6-Dihydroxy-7,8,4'-trimethoxyflavone (2) and xanthomicrol (3). Compound 1 was identified for the first time in this species. These results demonstrate that T. angustifolia has potential central and peripheral antinociceptive effects and support the ethnomedicinal use of this plant.

KEYWORDS

Trixis angustifolia; antinociceptive; writhing; indomethacin

CONTACT

Maria Estela Meléndez-Camargo mcamargo@ipn.mx; Saudy Saret Pablo-Pérez spablop@ipn.mx

The authors share co-corresponding authorship

Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2021.2017931.
1. Introduction

Trixis angustifolia DC. (Asteraceae) is an endemic plant of Mexico widely distributed in the central and the north part of the country (Villaseñor 2016). This plant shares morphological characteristics with other plants commonly known as “árnica” or “árnica del país”, which are used as analgesic and anti-inflammatory remedies (Waizel-Bucay and Cruz-Juárez 2014). Consequently, the plant has several established uses in Traditional Mexican medicine. However, only the antibacterial, hypoglycemic and hypolipidemic effects have been demonstrated (Sánchez-Chávez et al. 2019; Salazar-Gómez et al. 2019a, 2019b) and no scientific reports demonstrating its antinociceptive activity have been published so far. A previous phytochemical study of the aerial parts of T. angustifolia resulted in the isolation and characterization of six flavones: pebrellin, salvigenin, gardenin B, xanthomicrol, cirsimaritin, and 3’-demethoxysudachitin (Sánchez-Chávez et al. 2019), some of which have been shown to have anti-inflammatory activity (Shin et al. 2017; Guo et al. 2018). Therefore, the present study was conducted to investigate the antinociceptive potential of the ethanolic extract of T. angustifolia (EETx) and to identify the main components present in the extract.

2. Results and discussion

The oral administration of EETx at 1000 and 2000 mg/kg did not cause any signs of toxicity or mortality during the 14-day acute toxicity test. In addition, no significant pathological changes in vital organs were observed by macroscopic examination. Therefore, the EETx is well-tolerated with LD$_{50}$ > 2000 mg/kg, indicating that it is reasonably safe in vivo (OECD 2001).

The antinociceptive effect of the EETx was evaluated using two classical experimental pain models: acetic acid-induced writhing and hot-plate tests. The intraperitoneal injection of acetic acid induces the release of endogenous mediators that stimulate pain nerve endings, causing abdominal constriction response in mice (Ikeda et al. 2001). Treatment with EETx at the doses of 50, 100, and 200 mg/kg reduced mean writhing by 38%, 67%, and 74%, respectively, compared with the sodium bicarbonate (vehicle) control group (Figure S1, supplementary material). The EETx at 200 mg/kg had a significant protective effect similar to the NSAID indomethacin at 10 mg/kg, which produced 77% decreases in the number of writhing episodes. These results suggest that the ability of EETx to attenuate nociception in the abdominal constriction could derive from the suppression of the synthesis and/or release of prostaglandins by the COX pathway (Satyanarayana et al. 2004) or from inhibition of the production of inflammatory mediators (e.g., TNF-α, IL-1β, and IL-8) released into the peritoneal cavity (Ribeiro et al. 2000).

The hot plate test was used to test the central antinociceptive potential by measuring the nociceptive response time (RT) and percent maximal possible effect (%MPE). All three doses of EETx increased the RT and %MPE in the 2nd and 3rd h compared to the sodium bicarbonate (vehicle) control group (Table S1, supplementary material). The treatment with EETx at 200 mg/kg increased the RT in the 3rd h, similar to acetylsalicylic acid (100 mg/kg; used as a positive control). These results suggest that compounds present in EETx might also have centrally mediated activity.
The phytochemical analysis of EETx led to the isolation and identification of three known compounds: a proline derivative and two O-methylated flavonoids. Compound 1 was isolated as fine needles. Based on ESI (−) mass spectrometry, a molecular ion at m/z 128.0719 was identified in agreement with the molecular formula C₆H₁₁NO₂ (Figure S2, supplementary material). The analysis of ¹H NMR spectrum revealed proline ring \( [\delta 3.81-3.75 \text{ (1 m, 1H), 3.74-3.67 and 3.14-3.05 (2 m, 2H), 2.51-2.42 and 2.15-2.06 (2 m, 2H), 2.15-2.06 and 2.01-1.92 (2 m, 2H)}] \) and a singlet signal at \( \delta 2.91 \) for three hydrogens of N-methyl group (Figure S3, supplementary material). ¹H and ¹³C NMR (Figure S4, supplementary material) assignments were carried out with the aid of the detailed 2D analyses (gHSQC) (Figure S5, supplementary material) and the resulting NMR evidence revealed a compound defined as hygric acid (Aurelio et al. 2003). To the best of our knowledge, the presence of 1 has never been reported before in plants from the *Trixis* genus. Additionally, two previously reported compounds, 5,6-Dihydroxy-7,8,4′-trimethoxyflavone (2) and xanthomicrol (3) were also identified in the EETx by comparisons of their NMR spectroscopy with those reported earlier (Sánchez-Chávez et al. 2019). Previous studies confirmed the association of opioid system in the antinociceptive effect of some O-methylated flavonoids (Shajib et al. 2018) and natural proline derivatives such as N-methyl-(2S,4R)-trans-4-hydroxy-l-proline have been found to possess anti-inflammatory properties (de Aquino et al. 2017). Although there is no possibility at this stage to identify the exact constituent(s) responsible for the antinociceptive activity of *T. angustifolia*, compounds 1, 2, and 3 presented in the EETx could contribute to the biological effect observed in the present study. Thus, further studies are necessary to validate this hypothesis and identify the mechanism of action involved in the antinociceptive activity of EETx.

### 3. Conclusions

The results of the present study reveal the peripheral and centrally acting antinociceptive properties of the ethanolic extract of *T. angustifolia* and support the ethnomedical use of this plant in various painful conditions.

### Acknowledgements

ASG was supported by fellowships from CONACyT (Consejo Nacional de Ciencia y Tecnología) and BEIFI (IPN). ASG, MEVD, MEMC, SSPP thank SNI for support.

### Disclosure statement

No potential conflict of interest was reported by the authors.

### Funding

This work was supported by Comisión de Operación y Fomento de Actividades Académicas, Instituto Politécnico Nacional; Secretaría de Investigación y Posgrado, Instituto Politécnico Nacional.
ORCID

Anuar Salazar-Gomez http://orcid.org/0000-0001-9633-6542
Ma. Elena Vargas-Diaz http://orcid.org/0000-0002-2925-3373
Maria Estela Melendez-Camargo http://orcid.org/0000-0002-6052-0281
Saudy Saret Pablo-Perez http://orcid.org/0000-0001-5745-6437

References

Aurelio L, Box JS, Brownlee RT, Hughes AB, Sleebs MM. 2003. An efficient synthesis of N-methyl amino acids by way of intermediate 5-oxazolidinones. J Org Chem. 68(7):2652–2667.

de Aquino PEA, Magalhães TR, Nicolau LAD, Leal LKAM, de Aquino NC, Dos Santos SM, Neves KRT, Silveira ER, de Barros Viana GS. 2017. The anti-inflammatory effects of N-methyl-(2S, 4R)-trans-4-hydroxy-l-proline from Syderoxylon obtusifolium are related to its inhibition of TNF-alpha and inflammatory enzymes. Phytomedicine. 24:14–23.

Guo S, Wu X, Zheng J, Charoensinphon N, Dong P, Qiu P, Song M, Tang Z, Xiao H. 2018. Anti-inflammatory effect of xanthomicrol, a major colonic metabolite of 5-demethyltangeretin. Food Funct. 9(6):3104–3113.

Ikeda Y, Ueno A, Naraba H, Oh-Ishi S. 2001. Involvement of vanilloid receptor VR1 and prostanooids in the acid-induced writhing responses of mice. Life Sci. 69(24):2911–2919.

OECD. 2001. Guideline for testing of chemicals: No 423: acute oral toxicity fixed dose method. Paris: Organization for Economic Co-operation and Development.

Ribeiro RA, Vale ML, Thomazzi SM, Paschoalato AB, Poole S, Ferreira SH, Cunha FQ. 2000. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur J Pharmacol. 387(1):111–118.

Salazar-Gomez A, Sanchez-Chavez AC, Zepeda-Vallejo G, Chamorro-Cevallos G, Garduno-Siciliano L, Vargas-Diaz E. 2019a. Hypolipidemic effect of Trixis angustifolia aqueous extract on triton WR-1339-and high-fat diet-induced hyperlipidemic mice. Nat Prod Commun. 14(7):1–6.

Salazar-Gomez A, Vargas-Diaz ME, Garduno-Siciliano L. 2019b. Hypoglycemic potential of Trixis angustifolia aqueous extract in alloxan-induced diabetic mice. Bangladesh J Pharmacol. 14(2):74–79.

Sanchez-Chavez AC, Salazar-Gomez A, Zepeda-Vallejo LG, Hernandez de Jesus MLD, Quinto-Escalanente M, Vargas-Diaz ME, Luna-Herrera J. 2019. Trixis angustifolia hexanic extract displays synergistic antibacterial activity against M. tuberculosis. Nat Prod Res. 33(10):1477–1481.

Satyanarayana PS, Jain NK, Singh A, Kulkarni SK. 2004. Isobolographic analysis of interaction between cyclooxygenase inhibitors and tramadol in acetic acid-induced writhing in mice. Prog Neuropsychopharmacol Biol Psychiatry. 28(4):641–649.

Shajib M, Rashid RB, Ming LC, Islam S, Sarker M, Moklesur R, Nahar L, Sarker SD, Datta BK, Rashid MA. 2018. Polymethoxyflavones from Nicotiana plumbaginifolia (Solanaceae) exert anti-nociceptive and neuropharmacological effects in mice. Front Pharmacol. 9(85):1–17.

Shin MS, Park JY, Lee J, Yoo HH, Hahm DH, Lee SC, Lee S, Hwang GS, Jung K, Kang KS. 2017. Anti-inflammatory effects and corresponding mechanisms of cirsimartitin extracted from Cirsium japonicum var. maackii Maxim. Bioorg Med Chem Lett. 27(14):3076–3080.

Villasenor JL. 2016. Checklist of the native vascular plants of Mexico. Rev Mex Biodivers. 87(3):559–902.

Waizel-Bucay J, Cruz-Juarez MDL. 2014. Arnica montana L., planta medicinal europea con relevancia. Rev Mex De Cienc Agric. 5(25):98–109.