Flavonoids and fractions from *Saccharum officinarum* L. juice: antinociceptive agents and molecular docking evaluations with $\mu$-opioid receptor

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**ABSTRACT**

Opioid receptors mediate antinociceptive effects. Methanolic fractions from sugarcane varieties (MFSCf) were evaluated in classic nociception models. Interactions between bioactive compounds and the $\mu$-opioid receptor ($\mu$OR) through docking analysis were also studied. Five methanolic fractions of sugarcane juice were obtained and analysed by LC-ESI-MS/MS. The fractions and standards of phenolic compounds were evaluated in a nociception model using the formalin test. All MFSCfS exhibited antinociceptive activity in the first phase of the formalin test. Docking analyses corroborates with the *in vivo* test results, suggesting that the phenolic substances are able to activate $\mu$OR. These results, for the first time, implicate phenolic constituents from sugarcane juice and other phenolic compounds in the activation of $\mu$OR. The antinociceptive activity of fractions from sugarcane juice suggests the potential pharmacological use of this species, widely cultivated in Brazil.
1. Introduction

Morphine, a prototype of opioid agonists, has long been known for its ability to relieve severe pain with remarkable efficacy (Krishnamurti and Rao 2016). Currently, commercial opioids used to pain management act mostly as agonists of μ-opioid receptors (μOR) (Schellack et al. 2018). These compounds present several unwanted side effects, such as respiratory depression, induction of tolerance, physical and psychological dependence, besides causing nausea, sedation, and cognitive dysfunction, among others, as a consequence of their low selectivity (Panlilio et al. 2013). Due to these side effects, several studies have been conducted over the years using natural products of different classes, such as flavonoids, terpenoids and alkaloids (Dias et al. 2020; Justino et al. 2020; Sheikholeslami et al., 2021).

In our previous work, we reported that a methanolic fraction from sugarcane (Saccharum officinarum) juice, constituted by flavone heterosides, phenylpropanoids and stilbenes, and, mainly, the isolated flavonoid tricin-7-O-(2′″-α-L-rhamnopyranosyl)-α-D-galacturonide displayed significant antinociceptive effects in in vivo models through a possible interaction with the opioid system. In addition, the fractions presented no cytotoxic effect in isolated murine peritoneal macrophages (Gomes et al. 2021). These promising results, encouraged us to further investigate fractions from different varieties of sugarcane juice. Thus, this work aimed to identify phenolic substances present in fractions from different varieties of sugarcane juice, in addition to phenolic compound standards, and to explore their antinociceptive activity in in vivo models. We also investigated the interaction between these substances and the μOR receptor using molecular docking simulations to understand their probable antinociceptive mechanism.

2. Results and discussion

Data representing retention time, molecular ions, main fragment ions in MS² and tentative compound identification, as well as chromatograms and MS spectra, obtained by LC–ESI–MS/MS are given in the Supplementary Material.

Through LC–ESI–MS analysis (Table S1, Figures S1, S5, S8, S10 and S11), we verify the presence of phenolic substances in all evaluated varieties: MFSCf RB 92579, MFSCf RB 935744, MFSCf RB 867515, MFSCf SP 813250 and MFSCf SP 911004. Phenolic substances represented by the [M-H]− 563 and 651 ions are present in all varieties. We can tentatively propose that the [M-H]− 563 ion refers to the isomeric pair schaftoside and isoschaftoside (Colombo et al. 2008; Gomes et al. 2021). After the chromatographic separation process of variety MFSCf RB 92579, a fraction containing the combination of isoschaftoside and shaftoside isomers as major compounds was obtained, as tentatively identified through the LC-ESI/MS (Figure S4). The ion [M – H]− 651 has already been described in our previous study for the variety SP 711406 (Gomes et al. 2021) as tricin-7-O-(2″-α-L-rhamnopyranosyl)-α-D-galacturonide. Phenolic substances represented by [M – H]− 475 and 609 ions were detected in the composition of MFSCf RB 935744 (Figure S5). The [M – H]− 475 ion was described in sugarcane juice by Colombo et al. (2006) as 4′,S″-dimethyl-luteolin-8-C-glucoside. In Alves et al. (2012), ion [M – H]− 609 was observed in an extract of the species Cenostigma macrophyllum
(Leguminosae) and elucidated as quercetin 3-O- (6″-O-E-p-coumaroyl)-β-D-glucopyranoside. It is also present in MFSCf SP 911004. Ion [M – H]– 535 was identified in MFSCf RB 867515 suggesting the molecular formula C_{26}H_{32}O_{12}. We can tentatively propose that the glycosylated stilbene present in this variety is resveratrol-3-O-glucosyl-(1-2)-rhamnoside. This is the first to report a stilbene attached to a glucopyranosyl-rhamnopyranoside moiety in sugarcane.

The antinociceptive potential of sugarcane fractions and phenolic standards was evaluated in the formalin test. All sugarcane fractions and morphine, used as assay control, were able to significantly inhibit the first phase of the formalin test in mice. MFSCf RB 92579, RB 935744, RB 867515, SP 911004 and SP 813250 in the screening dose of 100 mg/kg (p.o.), as well as morphine (10 mg/kg i.p.), inhibited nociceptive behaviour in mice by reducing the licking time by 39.7%, 40.52%, 57.60%, 35.90%, 53.3% and 72.8%, respectively. MFSCf RB 867515 and SP813250 exhibited a significant reduction in licking time when compared to the control group, as was observed for morphine (Figure S12A). Although statistically similar to the other fractions, MFSCf RB 867515 presented the highest percentage of paw licking time inhibition among the fractions, and for this reason, it was selected for evaluation with naloxone (2 mg/kg, i.p.), which is a nonspecific opioid receptor antagonist (Sachan and Singh 2013). Once administered with naloxone, the antinociceptive effect of MFSCf RB 867515 was abolished, suggesting a possible participation of the opioid system in the antinociception produced by this fraction in the neurogenic phase of the formalin test (Figure S12A).

In the second phase of the formalin test, no significant effect of the fractions was observed insofar as reducing the licking time (Figure S12B), reproducing the results obtained for the methanolic fraction of the SP711406 variety, as described in our previous study (Gomes et al. 2021). These data suggest that the antinociceptive effect triggered by sugarcane fractions is not related to the release of inflammatory mediators, but rather to a central action. In this same work, the major flavonoid, tricin-7-O-(2″-α-L-rhamnopyranosyl)-α-D-galacturonide, was especially involved in the antinociceptive effect of the methanolic fraction from the mentioned sugarcane variety, and it is also present in the composition of all fractions tested here. In order to evaluate the involvement of phenolic compounds of different classes in antinociceptive activity, quercetin, hesperidin, resveratrol, caffeic and chlorogenic acids and the schaftoside/isoschaftoside isomer pair (flavone), isolated from fraction MFSCf RB 92579, were also tested in the formalin assay, using the same dose as that described previously (100 mg/kg p.o.) (Figure S13). In the first phase of the test (Figure S13A), hesperidin, quercetin, resveratrol and the schaftoside/isoschaftoside pair reduced the licking time (52.5%, 66.53%, 59.06% and 54.7%, respectively) of the animals tested. Caffeic and chlorogenic acids showed no activity. To the best of our knowledge, this is the first report of the direct involvement of schaftoside and/or isoschaftoside in antinociception. Our work indicates that phenolic substances from different classes and subclasses were able to act in the neurogenic phase of the formalin-induced pain model, suggesting that such substances may produce their antinociceptive effect by modulating the direct activation of nociceptive fibers.

To investigate possible interactions between the phenolic compounds and μOR, we conducted molecular docking simulations using the crystal structure of the complex
\(\mu\text{OR-BU72}\) (PDB ID: 51CM) (Huang et al. 2015). The ligand BU72 is a synthetic agonist with high affinity with the receptor (Neilan et al. 2004). The redocking of BU72 (Figure S14) allowed us to validate our protocol (RMSD of 1.58 Å, smaller than the accepted limit in the literature, 2 Å) (Hevener et al. 2009). Figure S15 shows the two- and three-dimensional diagrams with the interactions established between the redocked BU72 and \(\mu\text{OR}\). Our protocol reproduced the interactions with the relevant amino acids for \(\mu\)-opioid receptor activation (Asp147, His54, Val300, Val236 and Ile296), most of them of hydrophobic nature (Huang et al. 2015; Noha et al. 2017). To further investigate the \(\mu\text{OR}\) activation, we also simulated interactions with its prototype agonist, morphine. Apart from the interactions with Asp147 and His54 residues, we observed the important hydrophobic interactions with Val300 and Val236 residues (Figure S16). The surface hydrophobicity maps for the agonists BU72 and morphine indicate the predominance of hydrophobic interactions between the agonists and the \(\mu\text{OR}\) active site (Figure S17), in accordance with the literature (Huang et al. 2015).

Docking results with the other phenolic compounds (Figure S19) support evidence from the formalin test. Resveratrol, which showed antinociceptive effect, is able to establish hydrogen bond with Tyr148 a His54, and by \(\pi\) interactions with Val300, Ile296 and Ile322. For the inactive caffeic acid, on the other hand, we observe a low interaction energy with the \(\mu\text{OR}\) (Table S2). Only a \(\pi\) interaction with Ile322 is present with no occurrence of other relevant interactions with amino acids involved in \(\mu\text{OR}\) activation, such as Val236, Val300, Asp147, His54 or His297. For chlorogenic acid, an ester of caffeic acid, a conventional HB involving His54 and carboxylate group of quinic acid moiety and hydrophobic interaction between the aromatic ring and residue of Val300 are present (Huang et al. 2015; Noha et al. 2017). In vivo, however, chlorogenic acid is metabolized by the action of esterases, releasing caffeic acid (Lafay et al. 2006), which, as mentioned before, does not perform important interactions for \(\mu\text{OR}\) activation, explaining the absence of antinociceptive effect in the formalin test. Our molecular docking results support the in vivo studies and strongly suggest the involvement of phenolic substances, especially those found in fractions from sugarcane juice, in antinociception, acting at the CNS by activating the \(\mu\text{OR}\).

3. Conclusions

Fractions from different sugarcane juice varieties displayed the same antinociceptive profile in the formalin test, suggesting that one or more constituents in common may be responsible for such effect, highlighting tricin-7-O-(2\(^\prime\)-\(\alpha\)-L-rhamnopyranosyl)-\(\alpha\)-D-galacturonide, schaftoside and isoschaftoside, which are present in all varieties studied. Our molecular docking studies support the in vivo studies and strongly suggest the involvement of flavonoids, found in fractions from sugarcane juice, in antinociception by activating the \(\mu\text{OR}\). To the best of our knowledge, this is the first report regarding the evaluation of interactions between flavonoids from sugarcane and phenolic compounds, such as resveratrol, quercetin, hesperidin, caffeic acid and chlorogenic acid, and \(\mu\text{OR}\), using the molecular docking approach.
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Disclosure statement

The authors declare no conflict of interest.

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