Subfraction C also presented a higher capacity than the positive control, ascorbic acid.

According Seyoum *et al*.,[34] flavonoids and other polyphenols are great scavengers of free radicals because they easily donate hydrogen atoms due to the presence of OH grouping. Thus, the antioxidant activity present in EE, MF, and subfraction C and D was hypothesized to be due to the presence of flavonoids, including hyperoside and rutin, since they possess hydroxyl groups.

Topical anti-inflammatory effect of *Eschweilera nana*

Evaluation of the topical anti-inflammatory activity of EE and MF was performed using croton oil-induced inflammation, which increases phospholipase A_2 activity,[35] which results in the release of arachidonic acid and biosynthesis of prostaglandins and leukotrienes.[36,37]

The data [Figure 2] showed that EE at a dose of 5 mg/ear significantly inhibited swelling, probably due to the decrease of vascular permeability. Despite being a mixture of compounds, EE inhibited 45% of the edema in 6 h after application of the inflammatory agent. MF at doses of 0.625, 1.25, and 2.5 mg/ear also showed significant inhibition of inflammation [Figure 2], with mean percentages of 41, 52, and 67%, respectively. The positive control, dexamethasone, at a dose of 0.1 mg/ear presented with 93% edema inhibition.

Methanol fraction at a dose of 2.5 mg/ear showed a higher topical anti-inflammatory activity than the EE (an increase of around 49%), indicating that higher the concentration of polar compounds, higher the anti-inflammatory activity. Flavonoids have been correlated with the anti-inflammatory activity of many plant extracts and have been hypothesized to inhibit inflammatory mediators such as cyclooxygenase and/or lipoxygenase, which are involved in arachidonic acid release.[27,38] Furthermore, hyperoside and rutin, the major compounds in EE, demonstrated significant anti-inflammatory activity, which can be related by the inhibition of phospholipase A2 activity, which has an important role in the arachidonic acid cascade.[39-41]

Coutinho *et al*.[42] describes some of the structural factors that positively influencing anti-inflammatory activity of flavonoids, among them are the unsaturation in C-ring (positions 2–3), number and position of the OH groups, carbonyl group at C-4 (B-ring), and the absence glycosylation of the molecule. Most of these items are found in the structure of hyperoside and rutin, which explain the anti-inflammatory activity of them.

The results published previously[43] and obtained in this test indicate that EE and MF had satisfactory inhibitory activity against edema, probably due to the presence of flavonoids, including, the hyperoside and rutin that were identified, previously. The results do not explain the exact pharmacological mechanism involved; however, it may be related to the inhibition of different mediators of the inflammatory response, such as involved in arachidonic acid cascade.

Table 3: Accuracy results of the HPLC-UV-Vis method

	Theoretical amount (µg/mL)	Mean of determined amount ±SEM (µg/mL) (*n*=3)	Recovery ±RSD (%)
Rutin	10.0	9.99±0.05	99.95±0.45
	25.0	25.41±0.59	101.64±2.31
	50.0	50.01±0.48	100.00±0.95
Hyperoside	5.7	5.72±0.06	100.37±1.09
	20.7	21.02±0.46	101.53±2.18
	45.7	45.71±0.36	100.03±0.79

HPLC-UV-Vis: High performance liquid chromatography with ultraviolet/visible; SEM: standard error of the mean; RSD: relative standard deviation

Table 4: Comparison of IC_{50} value obtained of hydroalcoholic EE leaves, MF, subfraction C, subfraction D and ascorbic acid by DPPH method

	IC_{50} (µg/mL)
EE	10.87±0.03[a]
MF	12.63±0.13[c]
Subfraction C	7.38±0.09[d]
Subfraction D	17.06±1.22[e]
Ascorbic acid	9.62±0.01[f]

Values expressed in mean±SEM (*n*=3). Different subscript - One-way ANOVA *post-hoc* Tukey's test, specimens differ at $P<0.05$. EE: extract of *Eschweilera nana*; MF: methanol fraction; DPPH: 1,1-diphenyl-2-picrylhydrazyl; IC_{50}: inhibitory concentration 50%

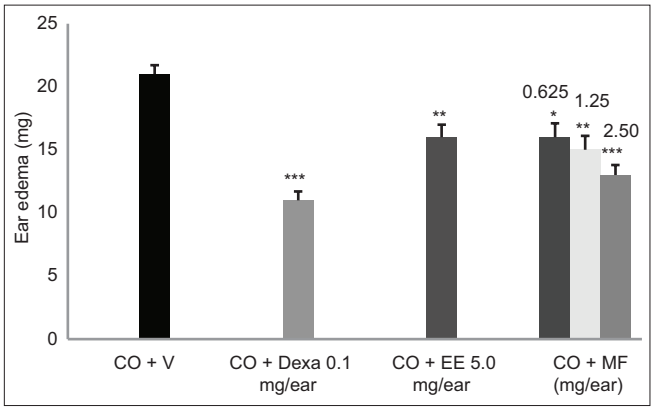

Figure 2: Effect of extract of *Eschweilera nana (EE)*, methanol fraction (MF) and dexamethasone (dexa) on edema of the ear induced by croton oil (CO). (*$P<0.05$, **$P<0.01$, ***$P<0.001$, compared with the control group [CO + V] [analysis of variance, Tukey's *post hoc* test])

CONCLUSION

We identified hyperoside, rutin, α-amirin, β-amirin, β-sitosterol, and stigmasterol in EE, through the first

phytochemical study of this plant species. HPLC-UV-Vis for simultaneous detection and quantification of major compounds, hyperoside and rutin, in EE was validated. Furthermore, *in vitro* and *in vivo* assays demonstrated that EE and MF showed significant antioxidant and topical anti-inflammatory effects, possibly associated with flavonoids.

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