

Antispasmodic Activity of *Persea cordata* Vell. Mez. (Lauraceae) Fractions on Guinea Pig Ileum Induced by 5-Hydroxytryptamine and Bradykinin

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Abstract: Problem statement: *Persea cordata* previously demonstrated antispasmodic action against two neurotransmitters. It is important confirm such action against other neurotransmitters. **Approach:** Present study described the antispasmodic activity of polar (EtOAc; n-BuOH) and non polar (n-hexane; dichloromethane) fractions from the bark of *Persea cordata*, against guinea pig ileum contracted by 5-hydroxytryptamine (5-HT) and bradykinin (BK). **Results:** The results indicated that hexane and dichloromethane fractions caused the most significant relaxant activity against both neurotransmitters tested. **Conclusion:** The effects seem to be related to the evidenced presence of steroids and terpenes, but additional studies were in progress to identify the active principles of this plant.

Key words: *Persea cordata*, antispasmodic activity, guinea pig ileum, terpenes and steroids

INTRODUCTION

Persea cordata Vell. Mez (Lauraceae), known in Brazil as “pau-andrade”, “canela-rosa” or “abacateiro-do-mato” is frequently used by rural communities for the treatment of some ailments, including inflammatory and infectious disorders. Our research group confirmed the antibacterial potential of this plant against pathogenic bacteria^[1] and more recently we demonstrated, into a program to search natural product with relaxant potential, that *P. cordata* produce active principles, which exhibit antispasmodic action against acetylcholine and histamine on guinea pig ileum^[2].

In this present study, we have extended our previous studies about the relaxant effects of *P. cordata* and evaluated the fractions (n-hexane, dichloromethane, ethyl acetate and butanol) against other two neurotransmitters, 5-hydroxytryptamine and bradykinin, on guinea pig ileum.

MATERIALS AND METHODS

Plant: The bark of *P. cordata*, collected in Bom Retiro, in the State of Santa Catarina, Brazil, in December

1998, was identified by Dr. Claudete Scharge Nuernberg (Agronomy Faculty/CAV/UDESC, Lages-SC). A voucher specimen was deposited at the herbarium of the Centro Agroveterinário, in Lages, under No. 045.

Preparation of fractions: Air-dried bark triturated (350 g) was extracted with methanol at room temperature (ten days). The solvent was evaporated under vacuum using a rotatory evaporator at 50°C, given the methanolic extract (yield = 21.5 g), which was successively partitioned with n-hexane (yield = 3.4 g), dichloromethane (yield = 1.2 g), EtOAc (yield = 8.6 g) and n-BuOH (yield = 6.6 g), furnishing the respective fractions after solvent removal as described previously^[3]. An aliquot of the fractions was then used for phytochemical evaluation (2-4 µL each plate). The chromatographic profile of the extract was examined by Thin Layer Chromatography (TLC) using Merck silica pre-coated aluminum plates 200 µm in thickness with several solvent systems. Spots were

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visualized by general and specific reagents, according to previously described methodologies^[4].

The specific spray reagents employed included anisaldehyde-sulfuric acid (terpenes and steroids), iron (III) chloride (phenolic compounds, including flavonoids) and Dragendorff (alkaloids) and the general reagents were ultra-violet irradiation and sulfuric acid-methanol^[4].

All the fractions were dissolved in 20% dimethyl sulfoxide (DMSO) and later diluted in normal saline solution 0.9% or distilled water to the desired concentration just before pharmacological use.

Animals: Guinea pigs (*Cavia porcelus*) of both sexes weighing 300-400 g were given access to water and Nuvital chow "ad libitum" unless otherwise indicated. The animals remained in the Central Biotery of UNIVALI until some hours before the experiments in atmosphere with clearly-dark cycle of 12 h and controlled temperature ($22\pm 2^\circ\text{C}$). Each animal was used only one time. All the procedures were carried out according to the rules of experimental and vivisection animal recommended by American Association for Laboratory Animal Science.

Antispasmodic effect on guinea pig ileum: Animals were killed by cervical dislocation and the ileum was removed from the exposed abdominal chamber. Ileal strips of about 10-20 mm in length were taken from the portion situated 15 cm proximal to the ileum-caecal junction^[5]. The intestinal content was removed by washing with Kreb's Heinseleit solution and the mesenteric residues were eliminated. Preparations were set up for recording isotonic contractions under 1g of load in 5ml jacketed organ baths containing Kreb's Heinseleit solution at 37°C , continuously bubbled with a mixture of 95% O_2 and 5% CO_2 . The composition of the Kreb's Heinseleit was as follows (mM): NaCl 118.0; KCl 4.4; CaCl_2 2.5; NaHCO_3 25.0; MgSO_4 1.1; KH_2PO_4 1.2 and glucose 11.0. After an initial equilibration period of about 30-45 min., cumulative concentration-response curves were obtained for 5-hydroxytryptamine (1 pM-100 μM) and bradykinin (0.01 nM-100 μM), in the absence or presence of the following fractions: Hexane, 1-30 $\mu\text{g mL}^{-1}$; dichloromethane 3-60 $\mu\text{g mL}^{-1}$; EtOAc, 10-3000 $\mu\text{g mL}^{-1}$ and n-BuOH, 1-300 $\mu\text{g mL}^{-1}$), incubated for 15 min, beforehand. Six cumulative concentration-response curves were obtained for each preparation, with a 20 min-rest between each. The mean maximal response obtained from the first cumulative

concentration-response curve (in absence of lead compounds) was taken as the 100% response value.

Statistical analysis: The data are shown as mean \pm SEM, except for the IC_{50} (concentration of drugs causing half-maximal responses), which are presented as geometric means accompanied by their respective 95% confidence intervals. The statistical analysis were obtained by the ANOVA test, followed by the Dunnett's test where necessary. $p < 0.05$ or $p < 0.01$ was considered significant. The IC_{50} s were calculated from individual experiments for graphic interpolation on semi-logarithmic paper and analysis using the GraphPad InStat program.

RESULTS

The IC_{50} values ($\mu\text{g mL}^{-1}$) against 5-hydroxytryptamine were 2.59 (1.92-3.49), 11.48 (9.03-14.59), 47.22 (34.61-64.42) and 45.34 (40.4-51.20) with maximal inhibition of $98\pm 1\%$; $84\pm 3\%$; $93\pm 3\%$ and $93\pm 3\%$ for n-hexane, dichloromethane, ethyl acetate and n-butanol fractions, respectively (Fig. 1).

The IC_{50} values ($\mu\text{g mL}^{-1}$) observed for bradykinin were 14.15 (12.76-15.70), 25.58 (22.57-29.00), 330.21 (250.37-430.48) and 1795.32 (351.23-2385.36) with maximal inhibition of $93\pm 2\%$; $97\pm 1\%$; $98\pm 1\%$ and $83\pm 6\%$, for n-hexane, dichloromethane, ethyl acetate and n-butanol fractions, respectively (Fig. 2).

Phytochemical analysis gave positive tests for terpenes and steroids (hexane and dichloromethane extracts) and phenolic compounds (ethyl acetate and butanol fractions).

DISCUSSION

In recent study our research group demonstrated the antispasmodic activity of some fractions from the *Persea cordata* bark, against guinea pig ileum contracted by acetylcholine and histamine^[2]. Now, we have complemented such studies with other two agonists, 5-hydroxytryptamine and bradykinin.

The results shown in Fig. 1 (a-d) indicate that either polar fractions (EA and BU) like the non polar (HEX and DCM) fractions from *P. cordata* bark (1-100 $\mu\text{g mL}^{-1}$) significantly inhibited, in a non-competitive way and concentration-dependent manner, the contractile response elicited by 5-hydroxytryptamine on guinea-pig ileum.

Similarly, they were capable to inhibit in a not competitive way and dependent-concentration the induced contraction for bradykinin in isolated ileum of guinea-pig.

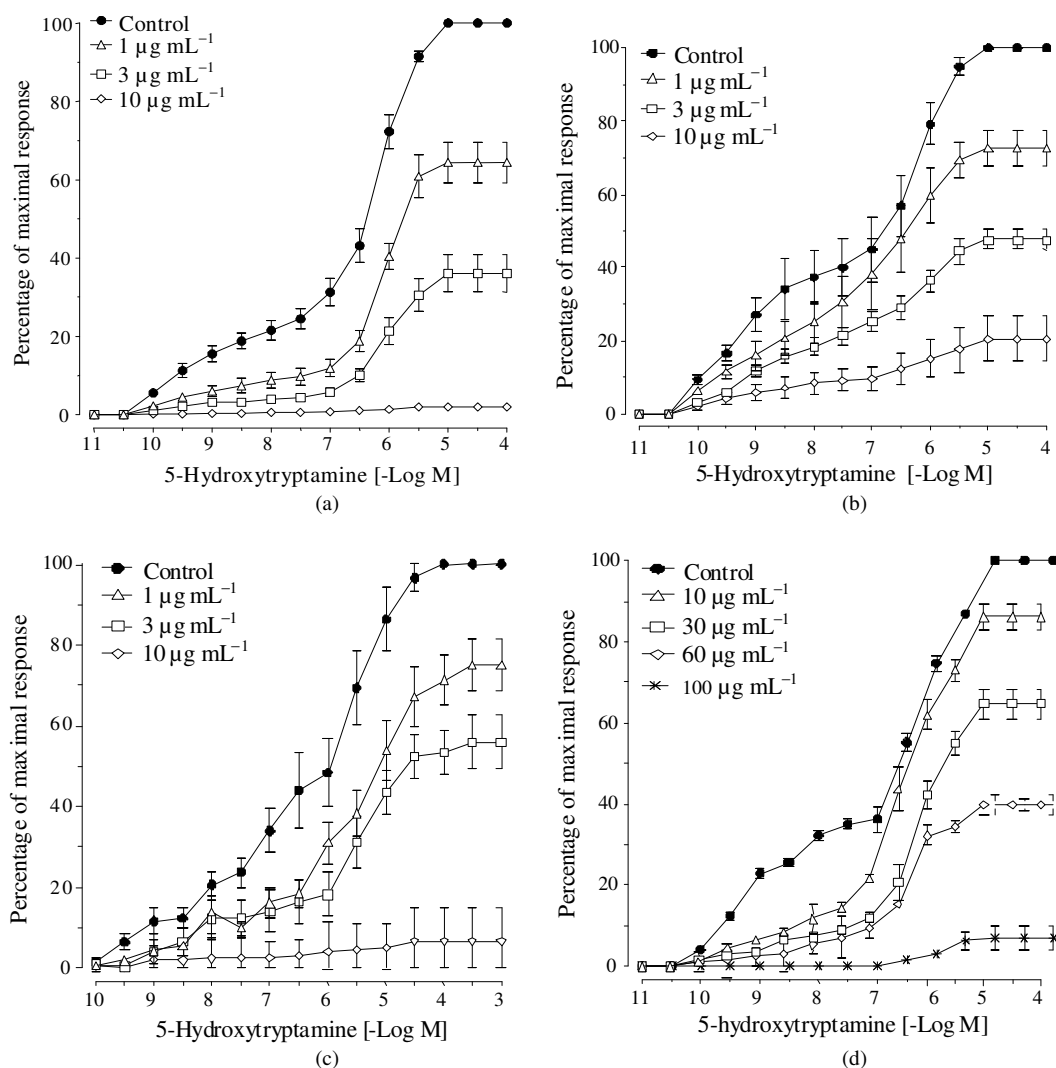


Fig. 1: Inhibitory effect of fractions from *P. cordata* on 5-hydroxytryptamine-induced contractions in isolated guinea pig ileum. Each point represents the mean of 8-10 experiments and the vertical bars represent the SEM ($p < 0.05$). (a): HEX; (b): DCM; (c): EA; (d): BU

Like observed in our previous study using acetylcholine and histamine^[2] also here the non polar fractions (n-hexane and dichloromethane) caused the stronger effects, confirming the presence of promising antispasmodic substances in these fractions. The results obtained until now are not enough to determine the mechanism of action of these fractions.

The use of Thin Layer Chromatography (TLC) together with spectroscopic methods (NMR) and some standards available in our laboratory permitted to evidence the presence of β -sitosterol (hexane fraction) and its glucoside derivative (dichloromethane fraction)^[11]. The presence of these compounds can

explain, at least partially, the relaxant effect of non polar fractions. The last have been reported to spasmolytic^[6,7] whereas the first exhibited Ca(2+) channel-blocking action^[8]. The phytochemical studies are now in progress to determine quantitatively these and others compounds in order to identify the active principles of this plant.

In summary, the present results together with those previously published reports^[2] confirm that *P. cordata* exerts antispasmodic action as evident from its non selective concentration-dependent relaxation in the guinea-pig ileum pre-contracted by different agonists.

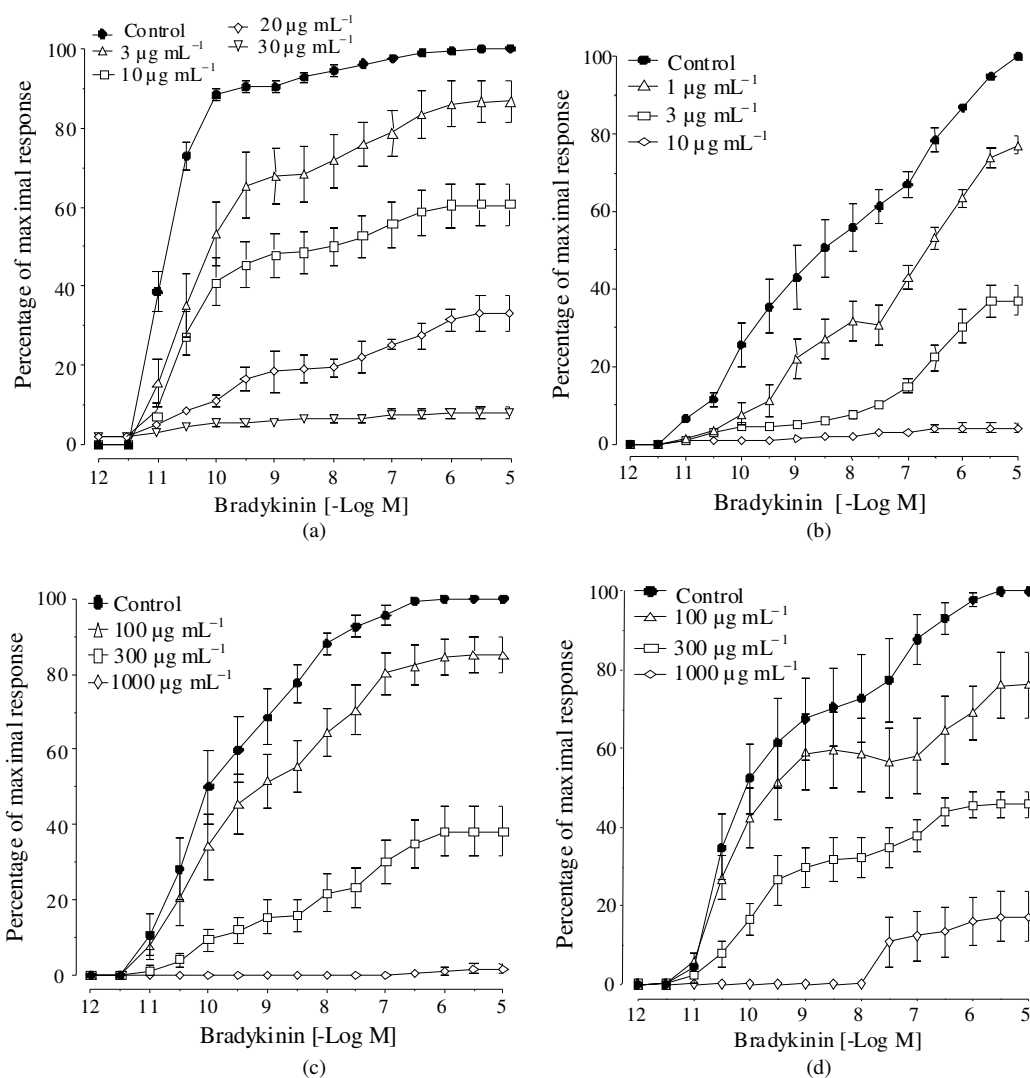


Fig. 2: Inhibitory effect of fractions from *P. cordata* on bradykinin-induced contractions in isolated guinea pig ileum. Each point represents the mean of 8-10 experiments and the vertical bars represent the SEM ($p < 0.05$). (a): HEX; (b): DCM; (c): EA; (d): BU

CONCLUSION

In summary, the present results together with those previously published reports^[2] confirm that *P. cordata* exerts antispasmodic action as evident from its non selective concentration-dependent relaxation in the guinea-pig ileum pre-contracted by different agonists.

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