PHARMACOLOGICAL ANALYSIS OF TWO ETHANOLIC EXTRACTS FROM SPECIES OF FRIDERICIA GENUS

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ABSTRACT:
The present study aimed to evaluate the biological activity of the species *Fridericia platyphylla* e *Fridericia florida*, through pharmacological tests. The doses of FpEE and FfEE (20 and 100 mg/kg) were used to assess anti-inflammatory activity (carrageenan-induced mice paw edema and formalin test) and analgesic activity (acetic acid-induced writhing and hot plate) by both extracts. In the carrageenan-induced paw edema the highest activity was observed by the FfEE at dose of 20 mg/kg in the third hour (26.63%), followed by FpEE (23.42%) at same dose in the first hour. In the acetic acid-induced writhing, the highest activity was observed by both doses of FpEE, while in hot plate test, FfEE has more pronounced activity than FpEE in times 60 and 90 minutes by both doses. In formalin test, only FfEE was significant at neurogenic pain phase inhibiting the licking time by 29.58%, whereas, FpEE has the highest activity at inflammatory pain phase (84.67%). This study showed that ethanolic extract of *F. platyphylla* and *F. florida* plants induced anti-inflammatory and analgesic effect in different tests carried out with Swiss mice albine.
Keywords: Medicinal plants. Analgesic. Anti-inflammatory. Fridericia.

1. Introduction

Even with the incentive of the pharmaceutical industry for the use of medicines industrialized, much of the population still uses medicinal plants for health care, to alleviate or even to cure some diseases (Badke et al., 2011).

Fridericia platyphylla (Cham.) L. G. Lohmann is a species of the Bignoniaceae family, previously identified as *Arrabidaea brachypoda*, popularly known as "tinteiro" or "cipó-una", wich has a wide geographic distribution, found in all Brazilian biomes (Lohmann, 2006). A few studies about the species *F. platyphylla (A. brachypoda)* were made and have pharmacological activities attributed to the species, particularly as anti-inflammatory (Rocha et al., 2011; Rocha et al., 2015), antimicrobial, including action against

fungi (Alcerito et al., 2002) and protozoa (Pereira et al., 2012; Rocha et al., 2014).

However, the reports in the scientific literature, about the *Fridericia florida* (*Arrabidaea florida*) species, understand the knowledge of the floristic and its territorial distribution (Tuffi et al., 2013; Inoue et al., 2012; Udulutsch et al., 2010).

Knowing the importance of the scientific study of plants used in folk medicine, the present study aimed to evaluate the biological activity of the species *F. platyphylla* e *F. florida*, through pharmacological tests that evaluate the analgesic and anti-inflammatory effects.

2. Material e Methods
2.1 Plant material

Leaves from both plants were collected in December 2012 at Federal University of Acre (UFAC), located in Rio Branco - Acre, Brazil. The voucher specimen were obtained in loco and deposited in the Herbarium of the Zoobotanical Park with registration numbers: F. platyphylla (voucher n. 590) and F. florida (voucher n. 11894.) and identified by the botanist of herbarium.

2.2 Extraction

The dried plant material was submitted to exhaustive extraction by maceration using as extracting solvent ethanol (70%) for 72 hours at room temperature and repeated for 3 times. The extracts were concentrated on a rotary evaporator under reduced pressure and subsequently taken to an oven with forced air circulation at 40° C until complete drying.

2.3 Pharmacological activities
2.3.1 Animals

Adult male Swiss mice albine weighing 20–25 g (n = 6) animals obtained from Vivarium of Agriculture, Livestock and Supply Ministry (MAPA), kept in Laboratory of Physiopharmacology of UFAC, under controlled light (12:12 h light–dark cycle; lights on at 6 a.m.) and temperature conditions (23 ± 1° C) with access towater and food ad libitum. All experiments and pharmacological approach were carried out in accordance with the Declaration of Helsinki and followed the guidelines of Animal Care, with approval of the ethic commiittee of the instituition.

2.3.2 Acute toxicity

The *F. platyphylla* ethanolic extract (FpEE) and *F. florida* ethanolic extract (FfEE) was orally administered at doses of 500 and 5000 mg/kg. The mice were observed for three days and during this period was available water and food *ad libitum*. The animals were observed clinically and behaviorally: sedation, agitation, motor alteration, modification in excreta and urine and intake diet, itching, bleeding and death.

2.3.3 Carrageenan-induced mice paw edema

Vehicle (10 mL/Kg), FpEE, FfEE (20 and 100 mg/kg) or indomethacin (10 mg/kg) was orally administered 1 h before the intraplantar

(i.pl.) injection of carrageenan (10 mg/mL 50 µL) under the ventral surface of the right hindpaw. Paw volume was measured at 0, 60, 120, 180 minutes and 24 hours with a plethysmometer (PanLab® LE 7500 model) (Winter et al., 1962).

2.3.4 Acetic acid-induced writhing

Oral treatments (p.o.) with vehicle (10 mg/kg), FpEE or FfEE (20 and 100 mg/kg) were given 1 h prior to acetic acid injection. Then, acetic acid (0.8% v/v, 0,1 mL/10g) was injected into the peritoneal cavities of mice. The intensity of nociceptive behavior was quantified 10 minutes after the injection by counting the total number of writhes occurring during 20 minutes (Koster et al., 1959).

2.3.5 Hot plate

The hot plate was heated at a constant temperature of $55 \pm 5°$ C. Mice were placed on the heated surface within the Plexiglas walls to constrain their locomotion on the plate.

The latency to a discomfort reaction (licking of the paws or jumping) was recorded for each animal 0, 30, 60, 90 and 120 minutes after FpEE, FfEE (20 and 100 mg/kg), vehicle (10 mg/kg) or morphine via i.p (5 mg/kg) administration (Yamamoto et al., 2002).

2.3.6 Formalin test

Oral treatments (p.o.) with vehicle (10 mg/kg), FpEE or FfEE (20 and 100 mg/kg) were given 1 h prior to formalin solution injection (2,5% in 0.9% saline; 20 µL/paw) into the hind paw plantar surface (i.pl.). The animals were individually placed in mirrored observation chambers and the time spent in licking the injected paw was recorded and expressed as the total licking time in the early phase (0–5 min) and the late phase (15–30 min) after the formalin injection (Santos and Calixto, 1997).

2.3.7 Statical analysis

Results were expressed as mean ± SEM and the statistically significant differences between groups were calculated by an analysis of variance (ANOVA) followed by Bonferroni's test using the GraphPad Prism 5.00. *P*-values less than 0.05 ($p < 0.05$) were considered as indicative of significance.

3. Results and discussion
3.1 Carrageenan-induced mice paw edema

The treatment with FpEE showed similar activity at both tested doses by reducing paw edema by 23.42% and 21.76%, respectively, in the first hour ($p<0.05$) overcoming the activity of indomethacin (16.97%), whilst, in the third hour, only FpEE 100 mg/kg – 22.11% ($p<0.05$) – showed to be more effective than positive control (17.09%). When compared with vehicle, the treatment FpEE shows mild anti-inflammatory activity, while the treatment with FfEE showed significant activity ($p<0.05$) at both doses, only in the third hour, by reducing edema by 26.63% and 19.10%, respectively, as similar to standard anti-inflammatory (indomethacin) used as positive control (Table 01). Thus, the highest activity was observed by

the FfEE at dose of 20 mg/kg in the third hour (26.63%), followed by FpEE (23.42%) at same dose in the first hour (Table 01). Only indomethacin showed significant activity after 24 hours from the induced mice paw edema – 23.11% ($p<0.05$).

In rats, the inflammatory response induced by carrageenan is sustained by the release of prostaglandins and nitric oxide with a peak at 3 h, produced by inducible isoforms of COX (COX-2) and nitric oxide synthase (iNOS), respectively (Seibert et al., 1994; Morris, 2003). The anti-inflammatory activity of both extracts maybe are related to the low inhibition of one those compounds.

3.2 Acetic acid-induced writhing

The treatment with FpEE showed the highest analgesic activity in both doses used, reducing the number of writhing by 64.73% and 68.16%, respectively, ($p<0.01$ and 0.001), when compared to the control group, indicating a dependent dose response between the doses.

The treatment with FfEE showed similar analgesic activity, significantly reducing the writhing at both doses by 55.78% and 43.96%. Thus, unlike the treatment with FpEE, the dose of 20 mg/kg of FfEE showed lightly more effective than 100 mg/kg (Table 01).

Rocha et al. (2011) found similar results with the extract of *A. brachypoda*, whereupon the writhing induced by acetic acid was strongly reduced at dose of 30, 100 and 300 mg/kg, as well as, Veloso et al. (2012) whose treatment with hydroethanolic extract of *Pyrostegia venusta* (Bignoniaceae) at doses of 100 and 300 mg/kg significantly reduced the the number of writhes by 43.20% and 69.10% ($p<0.001$), respectively.

Table 01 – Effects of the administration of the *F. platyphylla* (FpEE; 20 and 100 mg/kg, p.o.), *F. florida* (FfEE; 20 and 100 mg/kg, p.o.) or indomethacin (10 mg/kg, p.o.) on mice paw edema induced by intraplantar carrageenan injection. Values are expressed as mean ± S.E.M. The asterisks denote the significance levels when compared with the control group according to ANOVA followed by Bonferroni's test: *$p<0.05$

Treatment (p.o.)	Paw edema (Mean ± S.E.M) and % Inhibition				
	Basal	1st h	2nd h	3rd h	24th h
Vehicle (10 mL/kg)	0,352 ± 0.008	0,363 ± 0.006	0,358 ± 0.006	0,398 ± 0.034	0,278 ± 0.009
FpEE (20 mg/kg)	0,286 ± 0,013	0,278 ± 0,013 * (23.42%)	0,322 ± 0,015 (10.06%)	0,326 ± 0,013 * (18.09%)	0,240 ± 0,005 (13.67%)
FpEE (100 mg/kg)	0,272 ± 0,018	0,284 ± 0,006 * (21.76%)	0,302 ± 0,010 (15.64%)	0,310 ± 0,010 * (22.11%)	0,272 ± 0,011 (2.16%)
FfEE (20 mg/kg)	0,304 ± 0.004	0,330 ± 0.011 (9.17%)	0,318 ± 0.012 (11.26%)	0,292 ± 0.015 * (26.63%)	0,254 ± 0.002 (8.74%)
FfEE (100 mg/kg)	0,296 ± 0.005	0,316 ± 0.005 (13.03%)	0,314 ± 0.009 (12.37%)	0,322 ± 0.004 * (19.10%)	0,260 ± 0.004 (6.59%)
Indomethacin (10mg/kg)	0,308 ± 0.025	0,302 ± 0.010 * (16.97%)	0,306 ± 0.024 (14.60%)	0,330 ± 0,013 * (17.09%)	0,212 ± 0.022 * (23.11%)

Source: Personal archive

Table 02 – Analgesic activity of ethanolic extracts from leaves of *F. platyphylla* (FpEE) and *F. florida* (FfEE) in acetic acid-induced abdominal writhing in mice. Values are expressed as mean ± S.E.M of cumulated writhings in 30 minutes for each experimental group. The asterisks denote the significance levels when compared with the control group according to ANOVA followed by Bonferroni's test: **$p<0.01$, ***$p<0.001$.

| Treatment (p.o.)	Nº of writhes (Mean ± S.E.M)	% Inhibition
Vehicle (10 mL/kg)	87.43 ± 2.73	-
FpEE (20 mg/kg)	30.83 ± 1.14 **	64.73
FpEE (100 mg/kg)	27.83 ± 0.74 ***	68.16
FfEE (20 mg/kg)	38.66 ± 0.77 **	55.78
FfEE (100 mg/kg)	49.00 ± 2.60 **	43.96

Source: Personal archive

This model, which is a visceral pain model, releases arachidonic acid via cyclo-oxygenase (COX); prostaglandins biosynthesis plays a notably important role in the nociceptive mechanism (Duarte et al., 1988). The results of the present study indicate that the analgesic effect of the FpEE may possibly be triggered by the inhibition of the synthesis or action of prostaglandin, indicating that this species is a potential source of antinociceptive and analgesic substances.

3.3 Hot plate

Contrary to the results obtained in the test of acetic acid-induced writhing, in this analysis the treatment with FpEE showed low analgesic activity, but significant ($p<0.05$) only at dose of 100 mg/kg (Figure 01).

The most pronounced effect was observed in time 60 and 90 minutes, where the group has its latency time on the hot plate without presenting characteristic signals of increased expression of pain.

Figure 01 – Effects of *F. platyphylla* (FpEE) administered orally in the hot plate test. Each point represents the mean ± S.E.M. The asterisks denote the significance levels when compared with the control group according to ANOVA followed by Bonferroni's test: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
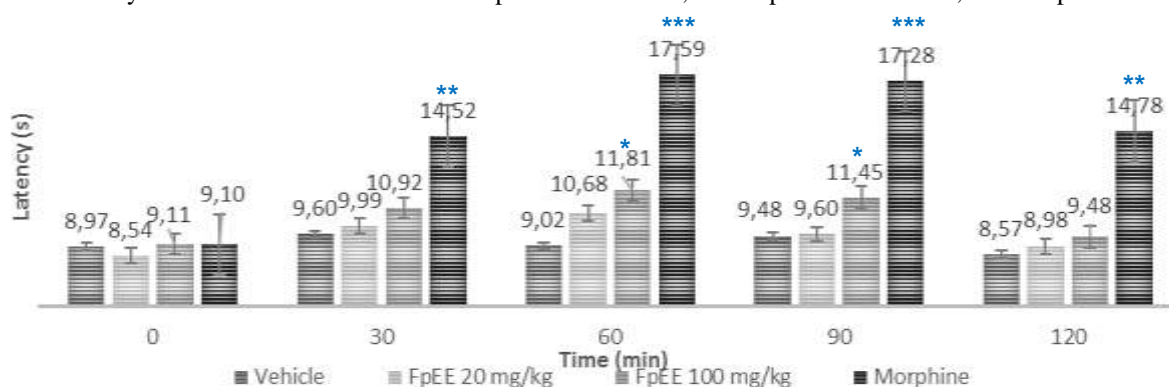
Source: Personal archive

Figure 02 – Effects of *F. platyphylla* (FpEE) administered orally in the hot plate test. Each point represents the mean ± S.E.M. The asterisks denote the significance levels when compared with the control group according to ANOVA followed by Bonferroni's test: *p < 0.05, **p < 0.01.
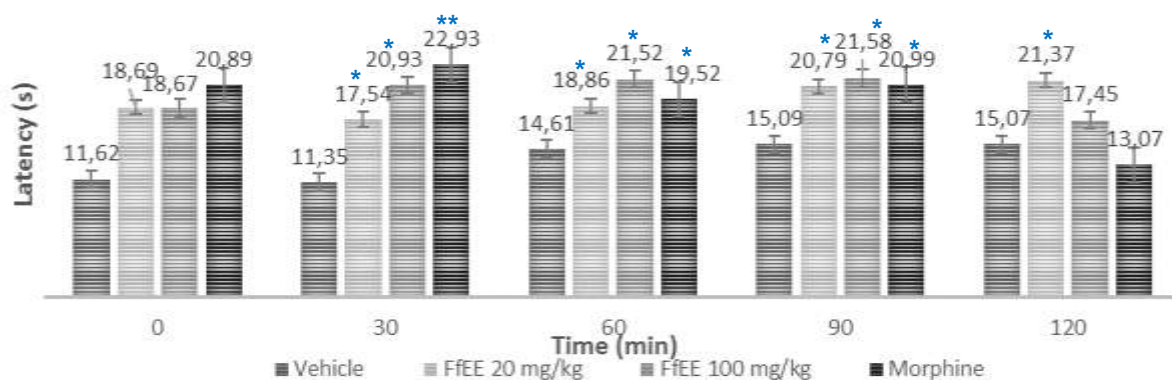
Source: Personal archive

The difference between the result from two analgesic assays may be explained because hot-plate model, the most commonly tests of analgesic measure of analgesic drugs, acts at the level of spine and higher brain centres and appears to be mediated through μ2, k1 and d2 receptor (Pasternak, 1993; Vogel, 2002). This assay involves higher brain functions being suitable for evaluation of centrally acting drugs but not of peripherally acting analgesics drugs (Karandikar et al., 2016). The acetic acid-induced writhing, on other hand, is a visceral pain model, so the nociceptors seem to be sensitized by sensory nerves.

The involvement of endogenous substances such as prostaglandins (PGs) is minimized in hot-plate model (Vogel, 2002).

Rocha et al. (2015), in otherwise, found that 3β-stearyloxy-olean-12-ene, a compound isolated from *A. brachypoda* roots at doses of 5 and 15 mg/kg increased the latency time.

The treatment with FfEE, in both doses taken in this study, presented very close

activity to positive control (morphine), increasing the latency time of animals (Figure 02). The effect was significantly observed at all times observation, and is considered more pronounced in times 60 and 90 minutes by both 20 and 100 mg/kg doses.

This allow infer higher efficiency and higher power to relieve termonociceptive injury of heated plate, therefore it is an indicative of analgesic activity with centrally mediated anti-nociceptive responses, focused mainly on changes above the spinal cord level.

3.4 Formalin test

The formalin test is a model which consists of two distinct phases. The first phase, or neurogenic pain phase, is caused by the direct effect of formalin on sensory C-fibers, and the second phase, or inflammatory pain phase, is associated with the development of an inflammatory response and the release of nociceptive mediators in the mice (Davidson and Carlton, 1998).

The phase 2, corresponding to inflammatory pain, can be inhibited by non-steroidal anti-inflammatory drugs (NSAIDs) (Tjølsen et al., 1992). In this test, the centrally acting drugs, such as narcotics, inhibit the two phases in the same way, while the peripheral acting drugs, such as NSAIDs and corticosteroids, inhibit only the second phase (Yaksh et al., 2001).

In the first phase, the treatment with FpEE and control group had no significant difference in the time that remained licking paw injured by formalin, suggesting that FpEE does not provide a response related to direct chemical stimulation of nociceptors of sensory C-fibers (Table 03).

During the initial phase of acute pain FpEE showed no analgesic effect, indicating that it is not composed by centrally acting drugs, which matches with hot plate test results and the second phase results of formalin test, whereupon the treatment with FpEE showed the highest significant difference ($p<0.001$) at both doses when compared with control group, inhibiting the licking time by 78.02% and 84.67%. This suggest that peripherally acting drugs are related with these results.

The treatment with FfEE, in otherwise, showed a significant difference ($p<0.01$) both in the first (neurogenic pain) and in the second phase (inflammatory pain) when compared with control group, inhibiting the licking time in 29.58% and 19.28% (phase 1) and 20.27% and 21.29% (phase 2) by both doses, respectively. This is a strong indication that FfEE presents centrally acting drugs, which matches with hot plate test results where was observed a difference between the treatment and control group (Figure 02).

Table 03 – Effects of *F. platyphylla* (FpEE) and *F. florida* (FfEE) given by oral administration in formalin test in mice. The total time spent licking the hind paw was measured in the first (0-5 min) and second (15-30 min) phases after intraplantar injection of formalin. Values are expressed as mean ± S.E.M. The asterisks denote the significance levels when compared with the control group: *p < 0.05, **p < 0.01.

Treatment	Licking time (Mean ± S.E.M)		% Inhibition	
	Neurogenic pain (0-5 min)	Inflammatory pain (15-30 min)	Neurogenic pain (0-5 min)	Inflammatory pain (15-30 min)
Vehicle (10 mL/kg)	92.3 ± 0.79	97.8 ± 0.88	-	-
FpEE (20 mg/kg)	96.1 ± 0.54	21.5 ± 0.64 ***	- 4.12	78.02
FpEE (100 mg/kg)	95.1 ± 0.89	15.0 ± 0.70 ***	-3.03	84.67
FfEE (20 mg/kg)	65.0 ± 0.90 **	78.0 ± 1.06 **	29.58	20.27
FfEE (100 mg/kg)	74.5 ± 1.10 **	77.0 ± 1.36 **	19.28	21.29

Source: Personal archive

The analgesic activity presented by FpEE may be related to the reduction of release of inflammatory mediators, or those for receptor blockade, resulting in a peripheral antinociceptive effect (Rocha et al., 2011).

The presence of compounds having anti-inflammatory activity in this genus has been reported by authors in the literature as Zorn et al. (2001) in which the lipophilic extract (200 µg/mL) from leaves of *A. chica* (*F. chica*) showed anti-inflammatory action *in vitro* by the method that assesses the capacity of inhibition of the nuclear factor kappa B (NF-κB), a central mediator of the immune response in humans, which regulates the transcription of several genes encoding proinflammatory cytokines, among others, and inflammatory enzymes.

Rocha et al. (2011), on other hand, found in *A. brachypoda* (*F. platyphylla*) phytochemical analysis the presence of saponins, phenolic compounds, tannins, flavonoids and steroids.

Flavonoids have been associated with various degrees of anti-inflammatory and analgesic activities because, in inflamed tissue, flavonoids inhibit cyclooxygenase, so they can prevent the formation of prostaglandins, which stimulate pain receptors in brain. Many plants containing flavonoids present these functions by inhibiting cyclooxygenase (Pilehvarian et al., 2010).

Flavonoids inhibit nitric oxide (NO) synthesis. Thus, it is leading to decrease analgesic activity. Other studies have shown that flavonoids can decrease intracellular calcium with inhibition of N-Methyl-D-aspartate receptor. So, synthesis of nitric oxide and phospholipase A and activity of NO decrease analgesic effect of NO and prostaglandins appear (Lopes et al., 2009).

Therefore, the observed anti-inflammatory and analgesic effects of FpEE and FfEE may probably be due to the activity of one or more of these classes of compounds identified, which are present in both extracts (unpublished data).

3.5 Acute toxicity

The FpEE and FfEE at a dose of 500–5000 mg/kg p.o. had no affect on the behavioral responses of the mice during the observation period of seven days after administration; no mortality was observed up to seven days of monitoring. The estimated LD_{50} value was therefore more than 5 g/kg p.o. Because we assume that the doses of 20 and 100 mg/kg p.o. given to mice in this study were safe.

4. Conclusion

This study demonstrated that the ethanolic extracts of *F. platyphylla* and *F. florida* plants induced anti-inflammatory and analgesic effects in different tests carried out with Swiss mice albine. Concluding, therefore, that treatments have compounds that act on pathways that cause inflammation and analgesia. Thus the study supports the use of these plants for the pharmacological control of

inflammation and pain process that has been made by empirical knowledge.

However we still need more studies to elucidate the mechanisms of action and chemical composition of these extracts, providing information to a rational and safe use, minimizing possible side effects and supplying an alternative to population in the treatment of diseases.

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