EUROPEAN ACADEMIC RESEARCH
Vol. IX, Issue 8/ November 2021

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

# Hydroethanolic extract from barks of the Amazonian species *Dacryodes kukachkana*: potential usage in painful conditions

ROBERTA DE FREITAS LOPES
Laboratório de Fisiofarmacologia,Universidade Federal do Acre
ANA PAULA AZEVEDO BARROS DA SILVA
Laboratório de Fisiofarmacologia,Universidade Federal do Acre
RENATA MORAIS FERREIRA AMORIM
Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará
DIEGO FREITAS DE ARAUJO
Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará
FELIPE MOURA ARAÚJO DA SILVA,
Departamento de Química, Universidade Federal do Amazonas
HECTOR HENRIQUE FERREIRA KOOLEN
Metabolômica e Espectrometria de Massas
Universidade Estadual do Amazonas
EMERSON SILVA LIMA,
Lab. de Atividade Biológica, Universidade Federal do Amazonas
QUINTINO MOURA DIAS
Fundação Oswaldo Cruz – FIOCRUZ RO
ANA MARIA SAMPAIO ASSREUY[1]
Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará
RENILDO MOURA DA CUNHA
Laboratório de Fisiofarmacologia, Universidade Federal do Acre

**Abstract**

*Barks of Dacryodes kukachkana or "breu" are used to treat inflammatory diseases. This study aimed to identify the chemical composition of the hydroethanolic extract of D. kukachkana stem barks (HEDk) and investigate its antinociceptive effect. The nociception was induced by acetic acid (0.8%), formalin (2.5%), hot plate (55 ºC). HEDk chemical analysis (HPLC-MS) revealed the presence of polyphenols. HEDk presented antinociceptive activity, via inhibition (38 – 44%) of the abdominal writhes induced with acetic acid; increase (1.4 – 1.5 fold) of the animal's permanence time in the hot plate; and reduction (37% – 39%) in the behavioral responses*

[1] Corresponding author: Ana Maria Sampaio Assreuy; E-mail address: anassreuy@gmail.com

*observed in the formalin test. The polyphenol hydroethanolic extract of D. kukachkana barks possesses antinociceptive effect in mice models.*

**Keywords**: Burseraceae, breu, Polyphenols, nociception; ellagic acid.

## INTRODUCTION

Over many years the increasing incidence of painful conditions has been quite evident, being influenced by the growth of the elderly population and much more present in chronic inflammatory diseases (Lunenfeld and Stratton 2013). Despite of the effectiveness of current analgesic drugs, such as opioids and nonsteroidal anti-inflammatory drugs (NSAIDs), several disadvantages may be exhibited (vertigo, sedation, addiction, gastrointestinal disorders) during treatment (Atkinson and Fudin 2020). Unquestionably, the study of natural substances that may interfere in the pathophysiology of pain is increasingly relevant.

Medicinal plants have historically been a rich source of natural products of therapeutic potential, producing numerous secondary metabolites of diverse chemical aspects and pharmacological activities (Atanasov et al. 2015). The Burseraceae family comprises more than 750 species distributed worldwide among 19 genera (Daly et al. 2012) and represents one of the largest botanical taxa in the Amazon Forest possessing representatives scattered throughout Brazil (Daly 2018). The Burseraceae family is well known as source of frankincense (Daly et al. 2010), family of the "mirra" and "the tree that lights up", and includes the genera *Dacryodes*, the most dispersed in Amazon, in addition to *Hemicrepidospermum*, *Paraprotium*, *Protium*, *Tetragastris* and *Trattinickia*. The common resin produced by many Burseraceae generais known as "pitch" being used as insect repellents and incense, in boats caulking, religious rituals, varnishes preparation and also in popular medicine (Marques and Ribeiro 1994). *Dacryodes* is derived from the Greek word 'Dakrun', which means 'tear', referring to the resin or exudate formed on the surface of the plant stem barks after

injury (Onana 2008), being rich in volatile substances used in the folk medicine to treat tonsillitis, diabetes, cancer (Omonhinmin 2012), headache, fever, malaria (Ndah et al. 2013), anemia, expectorant and skin diseases (Ajibesin 2011).

Experimental studies demonstrated antinociceptive activities of various parts of plants belonging to the Burseraceae family, being these activities accounted for the presence of organic molecules such as tannins, flavonoids (Marques and Ribeiro 1994) and terpenes (Okwu and Nnamdi 2008). Experimental studies performed with the Dacryodes genus have demonstrated efficient hypoglycemic and antioxidant effects without apparent toxicity in rats (Ononamadu et al. 2019),and antioxidant and bactericidal effects for the essential oil obtained from the resins of *D. rostrata* (Kong et al. 2011) and *D. buettneri* (Obame et al. 2007), respectively.

*Dacryodes kukachkana* L.O. Williams is a tall tree, popularly known as "breu", "breu-mescla" (Brazil), widely found in the Amazonian forest of Brazil and Peru (Daly 2018). Up to date, there are no scientific studies in the literature describing the pharmacological activities or characterization of the chemical components present in extract preparations from this species.

This study aimed to perform the phytochemical characterization and to investigate in mice the antinociceptive activity of the hydroethanolic extract obtained from *Dacryodes kukachkana* stem barks.

## MATERIAL AND METHODS

### Hydroalcoholic Extract Preparation

Stem barks of *Dacryodes kukachkana* were collected between the months August and September, in Mâncio Lima, Acre, Brazil, location 18 UTM 723548 7439155643 471 and a voucher specimen (n◦ 45912-1) was deposited at the herbarium of the Federal University of Acre, authorized by Sisbio/ICMBio number 45912-1. The access activity was registered in the National System for the Management of the Genetic Heritage and the Associated Traditional Knowledge (SISGEN/MMA,

code A76DD68), in accordance to the Brazilian Federal Law No. 13123/2015. Barks were dried (40 °C) duringfour days, grounded into fine particles and stored at room temperature (r.t.) until use. Samples were subjected to percolation (72 h, r. t.) in 70% ethanol, filtered and concentrated in rotary evaporator (45 °C) (Silva et al. 2018). The extract was frozen and lyophilized, before being tested, and named hydroethanolic extract of *D. kukachkana* (HEDk).

**Phytochemical Characterization**

Phytochemical prospection was performed for qualitative detection of organic acids, alkaloids, anthraquinones, coumarins, phenols, flavonoids, saponins, terpenes, steroids and tannins (Matos 1997).

Chemical analysis was performed by ultra-high pressure liquid chromatography tandem mass spectrometry (HPLC-MS), consisting of an Accela 600 liquid chromatography coupled to a LCQ Fleet mass spectrometer bearing a 3D ion trap mass analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Electrospray ionization in negative mode ($ESI^-$) was applied to access the HEDk composition. Mass spectra (MS) was set to scan the range between $m/z$ 150-1000 and tandem mass spectra (MS/MS) performed by collision-induced dissociation (CID) of previously isolated precursor ions using Helium as the collisional gas.

Chromatographic separations were performedin Kinetex C18 column (5 µm, 150 × 4.6 mm, 100 Å pore size) (Phenomenex, Torrance, CA, USA)by the use of binary mobile phase (solvent A: ultrapure water; solvent B: methanol). The gradient elution (0-15 min, 20-80% B; 15-25 min 80-100% B; 22-25 min 100% B) was performed at 28 °C, flow rate of 1.0 mL/minand injection volume of 10 Ml. The optimized ionization parameters were as follows:spray voltage 5 kV; sheath gas 30 arbitrary units; auxiliary gas 10 arbitrary units; sweep gas 0 arbitrary units; capillary temperature 250 °C; capillary voltage of -40 V; collision energy from 20 to 30 %.

## Animals

Male Swiss adult mice aging from 5-7 weeks (25 – 35 g) were maintained under controlled temperature (22 ± 1 °C) in light-dark cycle of 12 hours, receiving free access to food and water. The protocols were conducted in accordance to NIH guidelines (publication nº 85-23, revised 2011) and approved by the Animal Care and Use Committee of the Federal University of Acre-Brazil (UFAC - N° 23107.014897/2014-70).

## Drugs and Reagents

Formaldehyde and glacial acetic acid were purchased from Sigma (St. Louis, MO, USA). Drugs were solubilized directly insterile saline (0.15 M NaCl).

## Behavioral Nociception Tests

For evaluation of HEDk antinociceptive activity, it was administered in mice (50 – 1000 mg/kg; p.o.) 30-60 min before the nociceptive stimuli. Control animals received saline (0,1 mL/g; p.o.) in substitution of HEDk.

## Writing Test

Acetic acid (0.8%; v/v; 0.1 mL/10 g body mass) was injected in mice by intraperitoneal (i.p.) route. The number of writhes, typical contractions of the abdominal musculature followed by hind limb (stretching), was recorded from 10 to 30 min after injection (Koster et al., 1959).

## Hot Plate

For evaluation of sensorial response to thermal stimuli, animals were placed at hot plate (53 C° ± 1 °C) up to 25 s (Carter 1991). The reaction (jumping, licking or shaking hind paws) time to thermal stimulus was registered before (basal value) and up to 3 h after saline or HEDk administration.

### Formalin

Formalin (2.5%; 25 □l/paw) was injected subcutaneously (s.c.) in the animals right hind paws, and the number of lifting, shaking or paw licking was evaluated for 60 min (Shibata et al., 1989).

### Statistical Analysis

Results were analyzed by One-way and Two-way ANOVA followed by Bonferroni multiple comparison post hoc test when appropriate. All the results are presented as mean ± SEM and the level of significance was set as $P < 0.05$. The analyses were performe dusing the GraphPadPrism ® version 6.0 (GraphPad Software, San Diego, USA).

## RESULTS

### Phytochemical Characterization

The phytochemical prospection of HEDk, performed by chromogenic or precipitation chemical reactions, revealed the presence of coumarins, flavonoids, saponins, taninns, terpenes and steroids. Seven antioxidant compounds were identified by de replication throught the analysis of their corresponding fragments arising from product ion scan experiments (Figure 1A). The identification of the compounds found in HEDk was performed in comparison with data previously described for other fruits by HPLC-MS analysis (Hofmann et al. 2006, García et al. 2012, Silva et al. 2019), being detected organic acids, simple phenols, flavonoids and tannins as main constituents designated as: quinic acid (**1**, rt 6,10 min); a castalagin derivative (**2**, rt 8,07 min); ethyl gallate (**3**, rt 9,12 min); quercetin glucoside (**4**, rt 10,16 min); isorhamnetin-3-O-rhamnoside (**5**, rt 10,71 min); ellagic acid (**6**, rt 10,93 min) and epiafzelechin gallate (**7**, rt 12,16 min) (Figure. 1B).

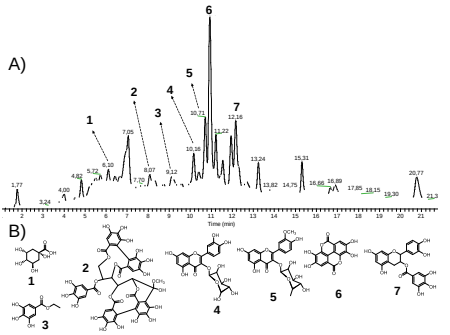


**Figure 1. Phytochemical analysis of HEDk. (**A) HPLC-UV-MS/MS chromatogram; (B) Identified compounds: quinic acid (1), castalagin derivative (2), ethyl gallate(3), quercetin glycoside (4), isorhamnetim-3-O-rhamnoside (5), ellagic acid (6), epiafzelechin gallate (7).

## HEDK Inhibits Chemical and Thermal Nociception

The number of acetic acid-induced abdominal writhes was decreased by HEDk at 100 (26.0 ± 3.05) and 500 mg/kg (23.7 ± 2.72), by 38% and 44% respectively, but not at 1000 mg/kg, compared to control (42.3 ± 4.33) (Fig. 2A).

The permanence time in the hot plate was increased by HEDk at the first hour of evaluation at 500 mg/kg in 1.5 fold (14 ± 0.20 s) and 1000 mg/kg in 1.4 fold (13.17 ± 0.17s) compared to control (9.1 ± 0.19 s). However, at the second hour, HEDk increased this behavior only at 500 mg/kg in 1.3 fold (14.5 ± 0.29 *vs.* control: 10.8 ± 0.31 s) (Fig. 2B).

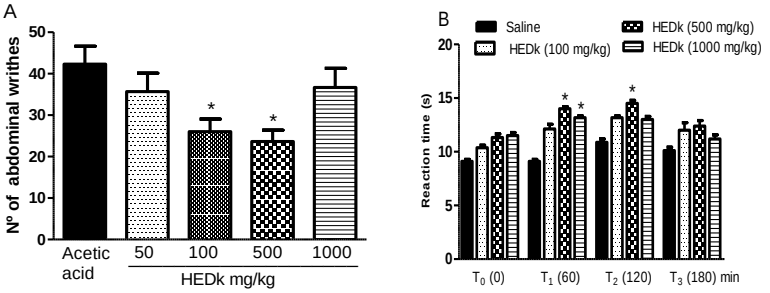


**Figure 2. HEDk inhibits nociception induced by chemical and thermal stimuli.** Mice received saline or HEDk (50-1000 mg/kg; *p.o.*) 30 min before stimuli. (A) Writhes induced by 0.8% acetic acid (v/v; *i.p.*) were counted from 10-30 min after injection (B) The reaction time (s) (jumping, licking or shaking) evaluated in the Hot plate test (55 ± 5 °C) was registered from 0-180 min after treatment. Mean ± S.E.M. (n = 8). One-way ANOVA/ Bonferroni. *p<0.05 *vs.* nociceptive stimuli.

In the formalin test, HEDk (100 – 500 mg/kg) did not alter the behavioral responses (lifting, shaking or paw licking) in the neurogenic phase (0- 5 min), but inhibited the inflammatory phase at 100 and 500 mg/kg.At 100 mg/kg the inhibitory effect occurred from 20 to 35 min by 37%, and at 500 from 25 to 35 min by 39% (Fig. 3A/B). However, at 1000 mg/kg HEDk did not alter the behavioral responses (Fig. 3C).

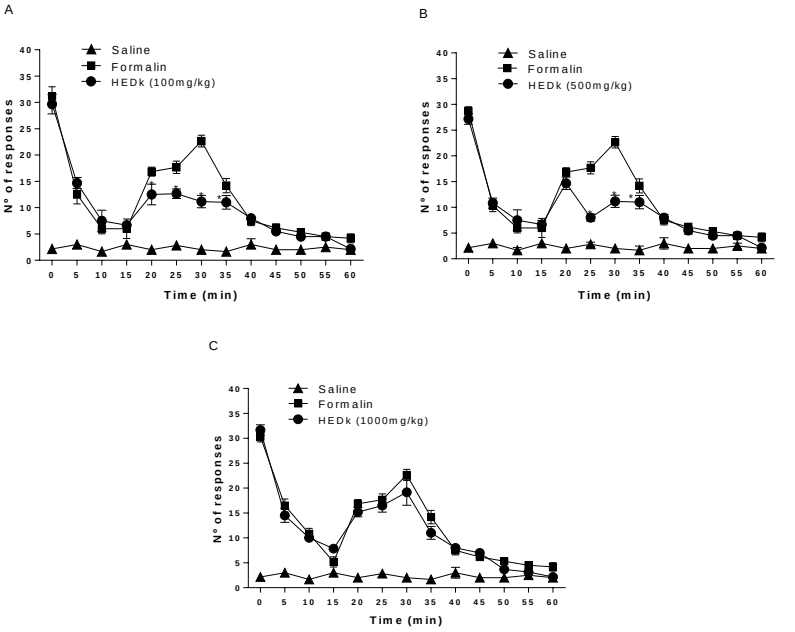


**Figure 3. HEDk inhibits the inflammatory phase of the formalin-induced nociception.** Mice received *p.o.* saline or HEDk at (A) 100 mg/kg, (B) 500 mg/kg or (C) 1000 mg/kg) 30 min before 2.5% formalin (*s.c.*). N° of responses (lifting, shaking or paw licking) was counted at 5 min-interval during 1 h. Mean ± S.E.M. (n = 8). Two-way ANOVA /Bonferroni. *p<0.05 *vs*. Formalin.

## DISCUSSION

In the present study, it was demonstrated in mice experimental models the antinociceptive activity of the hydroalcoholic extract obtained from the stem barks of *Dacryodes kukachkana* (HEDk). It was also demonstrated that HEDk contains phenolic compounds in its composition.

Several studies performed with medicinal plants had been established close relation between antinociceptive/anti-inflammatory activity and phenol/flavonoid content (Saeed et al. 2010, Sofidiya et al. 2014). In alignment with these studies, the phytochemical prospection of HEDk by HPLC-UV-MS/MS revealed the presence of different polyphenols, such as phenolic acids (ellagic acid) (De Souza et al. 2016), tanins (castalagin derivate) and flavonoids (isorhamnetim-3-O-rhamnoside, ethyl gallate and epiafzelechin gallate), as major constituents (Atawodi et al. 2009, Ononamadu et al. 2019).The antinociceptive and anti-inflammatory activities had been demonstrated for these molecules: isorhamnetin, a quercetin derivate (Jamali-Raeufy et al. 2019), ellagic acid (Murphy et al. 2020) and epiafzelechin gallate (Min et al.1999). Thus, it is possible to assume, that the HEDk's antinociceptive activity could be partially accounted to its phenolic compounds.

This hypothesis is reinforced by the study performed with the hydromethanolic extract of *Pimenta racemosa* leaves, rich in phenol compounds (gallic acid, methyl gallate, quercetin, ellagic acid) (UPLC-ESI-MS), that reduced the chemical nociception induced by acetic acid(writing test), as well as that induced by thermic stimulus (hot plate test)in rats (Moharram et al. 2018).

In addition, the antinociceptive activity of HEDk was evaluated in a broad set of rodent models (formalin test, acetic acid-induced writhing abdominal, hot plate), classical nociception models used to screen antinociceptive activity of novel substances (Sofidiya et al. 2014).

In the formalin test, there are two clearly distinct nociceptive phases (neurogenic and inflammatory). The initial phase is of short duration (0– 5 min) and is caused mainly by C fibers activation, reflecting neurogenic pain. The second phase (15– 30 min) is associated to the inflammatory response and characterized by the release of mediators such as prostaglandins, cytokines, bradykinin, serotonins and NO (Rosland; Hunskaar; Role, 1988). HEDk inhibited only the inflammatory phase, suggesting its involvement in peripheral components and late phase inflammatory mediators. Besides, the

abdominal contortion test in mice is widely used to evaluate inhibitory activity of substances on inflammatory pain (Simões et al. 2018). HEDk inhibited the writhes induced by intraperitoneal injection of acetic acid that produces irritation on the visceral surfaces by the release of high levels of inflammatory mediators such as prostaglandins to the peritoneal fluid, reducing the nociception threshold and stimulating nociceptive fibers (Ikeda et al. 2001).This data is highly suggestive of HEDk peripheral action.

The above data, altogether, points that HEDk utilize inflammatory mediators as targets to exert its antinociceptive action, since it was able to inhibit the nociceptive responses in the late phase, but not in the initial phase, of the formalin test, and also inhibited the abdominal contortions induced by acetic acid.

In addition to the involvement of peripheral pain components in the HEDk antinociceptive effect, the extract seems to play a role in the central mechanisms of nociception. In fact, HEDk was effective to inhibit nociception in the hot plate test, used to evaluate drugs that act via direct activation of central nociceptors (Carter 1991). The response to the thermal stimulus results from the direct activation of nociceptors and depends on the supraspinal integration (Le Bars; Gozariu; Cadden, 2001) and predominantly centrally acting drugs increase the length of stay of animals on the hot plate (Rosland; Hunskaar; Hole, 1988).

Considering that the majority of the studies are focused on the specie *D. edulis*, the main contribution of the present investigation is the proposition of the potential biotechnological applicability of HEDk as analgesic. However, additional research is necessary to be carried out with its components, using other approaches in order to elucidate its mechanisms of action.

In conclusion, the hydroethanolic extract of *Dacryodes kukachkana* (HEDk) stem barks, containing polyphenols, presents significant antinociceptive effect in mice models nociception induced by chemical and thermal stimulus.

**ACKNOWLEDGMENTS**

This work was supported by Brazilian grants from National Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES) and Fundação de Amparo à Pesquisa in the state of Acre - FAPAC (No. 7616180077/2015). Dr. A.M.S. Assreuy is senior investigator of CNPq (Process No. 308433/2017-3).

## REFERENCES

1. Ajibesin KK. 2011. *Dacryodes edulis* (G. Don) H. J. Lam: a review of its medical, phytochemicals and economical properties. Res. J. Med. Plant. 5, 32-41.
2. Atanasov AG, Waltenberger B. Pferschy-wenzig EM, Linder T, Wawrosc HC ,Uhrin P,Temml V. Wang L, Schwaiger S, Heiss E H, Rollinger JM, Schuster D, Breuss JM, Bochko VV, Mihovilovic MD, Kopp B,Bauer R,Dirsch VM and StuppneR H. 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol. Adv. 33, 1582-1614.
3. Atawodi SE, Atawodi JC, Idakwo P, Pfundstein B, Haubner R, Wurtele G, Spiegelhalder B, Bartsch H and Owen R W. 2009. Evaluation of the Polyphenol Composition and Antioxidant Activity of African Variety of *Dacryodes edulis* (G. Don) H. J. Lam Fruit. J. Med. Food. 12, 1321-1325.
4. Atkinson TJ and Fudin J. 2020. Non steroidal Antiinflammatory Drugs for Acute and Chronic Pain. Phys. Med. Rehabil. Clin. N. Am. 31, 219-231.
5. Carter RB. 1991. Differentiating analgesic and non-analgesic drug activities on rat hot plate: effect of behavioral endpoint. Pain. 47, 211-220.
6. Carter RJ, MORTAN J AND DUNNETT SB. 2001. Motor coordination and balance in rodents. Curr. Protoc. Neurosci. 15, 812.1-812.14.
7. Daly DC. Harley MM, Martínez-habibe M and Weeks A. 2010. Burseraceae. *In*: Kubitzki, K. (eds.), Flowering plants. Eudicots. Sapindales, Cucurbitales, Myrtaceae. The families and genera of vascular plants. Springer-Verlag, Berlin. 10, 76-104.
8. Daly DC, Fine PVA and Martínez-habibe MC. 2012. Burseraceae: a model for studying the Amazon flora.Rodriguésia. 63, 21-30.
9. Daly DC. 2018. Notes on the Burseraceae in central Amazonia, including four new taxa. Studies in neotropical Burseraceae XXVI. Brittonia 70, 427-444.
10. García BA, Lobato SG, Berrueta LA, Gallo B and Vicente F. 2012. On line characterization of 58 phenolic compounds in *Citrus* fruit juices from Spanish cultivars by high-performance liquid chromatography with photodiode-array detection coupled to electrospray ionization triple quadrupole mass spectrometry. J. Talanta. 99, 213-224.
11. Hofmanntglabasnia A, Schwarz B, Wisman KN, Gangwer, KA and Hagerman AN. 2006. Protein Binding and Astringent Taste of a Polymeric Procyanidin, 1,2,3,4,6-

Penta-*O*-galloyl-ß-d-glucopyranose, Castalagin, and Grandinin. J. Agric. Food Chem.54, 25, 9503–9509.

12. Ikeda Y, Ueno A and Naraba OS. 2001. Involvement of vaniloid receptor VR1 and prostanoids in the acid-induced writhing response of mice. Life Sci. 69, 2911-2919.
13. Jamali-raeufy N, Baluchnejadmojar AD T, Roghani M, Keimasi S and GoudarzI M. 2019. Isorhamnetin exerts neuroprotective effects in STZ-induced diabetic rats via attenuation of oxidative stress, inflammation and apoptosis. J Chem. Neuroanat.
14. Koster R, Anderson M and De-beer EJ. 1959. Acetic acid for analgesic screening. Federation Proceedings. 18, 412-418.
15. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. 2001. Pharmacol. 53, 597-652.
16. Lunenfeld B and Stratton P. 2013. The clinical consequences of an ageing world and preventive strategies. Best. Pract. Res. Clin. Obstet. Gynaecol. 27, 643-659.
17. Marques MFS and Ribeiro MNS. 1994. Estudo dos constituintes químicos das cascas da madeira de *Trattinickia peruviana* Swartex Loes. Acta Amazônica. 24, 49-52.
18. Matos FJA. 1997. Introdução à Fitoquímica Experimental. 2. ed. Fortaleza: Edições UFC, 141p.
19. Moharram FA, Al-gendy AA, El-shenawy SM, Ibrahim BM and Zarka MA. 2018. Phenolic profile, anti-inflammatory, antinociceptive, anti-ulcerogenic and hepatoprotective activities of *Pimenta racemosa* leaves. BMC Complement. Altern. Med. 18, 208.
20. Min KR, Hwang BY, Lim HS, Kang BS, Oh GJ, Lee J, Kang SH, Lee KS, Ro JS and Kim Y. 1999 (-)-Epiafzelechin: cyclooxygenase-1 inhibitor and antiinflammatory agent from aerial parts of *Celastrus orbiculatus*. Planta Med. 65, 460-462.
21. Murphy MT, Qin X, Kaul S, Barrientos G, Zou Z, Mathias CB, Thomas D and Bose DD. 2020. The polyphenol ellagic acid exerts anti-inflammatory actions via disruption of store-operated calcium entry (SOCE) pathway activators and coupling mediators.Eur. J. Pharmacol. 15, 173036.
22. Ndah NR, Egbe AE, Bechem E, Asaha S, Yengo T, Chia EL, Eyenieh NM. 2013. Ethnobotanical study of commonly used medicinal plants of the Takamanda Rainforest South West, Cameroon. Afr. J. Plant Sci. 7, 21-34.
23. Obame LC, Koudou J, Chalchat JC, Bassolé I, Edou P, Aboubakar S. Ouattara AS and Traore AS. 2007. Volatile components, antioxidant and antibacterial activities of *Dacryodes buettneri* H. J. Lam. essential oil from Gabon. Scien. Reser. and Essay. 2, 491-495.
24. Okwu DE and Nnamdi FU. 2008. Evaluation of the Chemical Composition of *Dacryodes edulis* and *Raphia Hookeri* Mann and Wendl Exudates used in Herbal Medicine in South Eastern Nigeria. Afr. J. Tradit. Complement. Altern. Med. 5, 194-200.
25. Omonhinmin CA. 2012. Ethnobotany of *Dacryodes edulis* (G Don) HJ Lam in Southern Nigeria 1: practices and applications among the Yoruba speaking people. Ethnobotany Res. Appl. 10, 175-184.

26. Onana JM. 2008. A synoptic revision of *Dacryodes* (Burseraceae) in Africa, with a new species from Central Africa. Kew Bull. 63, 385-400.
27. Ononamadu CJ, Alhassan AJ, Ibrahim A, Imam AA, Ihegboro GO, Owolarafe TA and Sule MS. 2019. Methanol Extract/Fractions *of Dacryodes edulis* Leaves Ameliorate Hyperglycemia and Associated Oxidative Stress in Streptozotocin-Induced Diabetic Wistar Rats. J. Evid. Based Integr. Medi. 24, 1-12.
28. Rosland JH, Hunskaar S, Hole, K. 1988. Modification of the antinociceptive effect of morphine by acute and chronic administration of clomipramine in mice. Pain. 33, p. 349-555.
29. Saeed MK, Deng Y, Dai R, Li W, Yu Y and Iqbal Z. 2010. Appraisal of antinociceptive and anti-inflammatory potential of extract and fractions from the leaves of *Torreya grandis* Fort Ex Lindl. J. Ethnopharmacol. 127, 414-418.
30. Shibata M, Ohkubo T, Takahashi H and Inoki R. 1989. Modified formalin test; characteristic biphasic pain response. Pain. 38, 347-352.
31. Silva APAB, Amorim RMF, Lopes RF, Mota MRL, DA Silva FMA, Koolen HHF, Lima ES, Assreuy AMS and Cunha RM. 2018. *Calycophyllum spruceanum* BENTH ameliorates acute inflammation in mice. J. Ethnopharmacol. 12, 103-109.
32. Silva NL, Saldanha AA, Vieira L, Silva DB, Carollo CA, Sartori ALB, Ribeiro RIMA, Thomé RG, Santos HB, Soares AC and Siqueira JM. 2020. Chemical composition, anti-inflammatory and antinociceptive effects of the butanolic fraction of *Annona nutans* (Annonaceae) leaves. Nat Prod Res, 4, 1-6.
33. Silva FMA, Hann, ACS, Souza AA, Silva Filho FA, Canhoto OMF, Magalhães A, Benevides PJC., Azevedo MB, Siani AC, Pohlit AM, Souza, ADL and Koolen HHF. 2019. Integrative Analysis Based on HPLC-DAD-MS/MS and NMR of *Bertholletia excelsa* Bark Biomass Residues: Determination of Ellagic Acid Derivatives. J. of the Braz. Chem. Society, 30, 830-836.
34. Simões RR, Kraus SI, Coelho IS, Dal-secco D, Siebert DA, Micke GA, Alberton MD, Santos ARS. 2018. *Eugenia brasiliensis* leaves extract attenuates visceral and somatic inflammatory pain in mice. J. Ethnopharmacol, 10, 178-186.
35. Sofidiya MO, Imeh E, EZeani C, Aigbe FR and Akindeleb AJ. 2014. Antinociceptive and anti-inflammatory activities of ethanolic extract of *Alafia barteri*. Rev. Bras. Farmacogn. 24, 348-354.