



Anti-inflammatory Activity of Hexane and Ethyl Acetate Extracts of *Hura crepitans* L.

Opeyemi N. Avoseh^{1*}, Latifat O. Ogunbajo¹, Isiaka A. Ogunwande¹, Akintayo, L. Ogundajo¹ and Oladipupo A. Lawal¹

¹Natural Products Research Unit, Department of Chemistry, Faculty of Science, Lagos State University, Badagry Expressway, P.M.B. 0001, Ojo, Lagos, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors ALO and ONA designed the study and supervised author LOO in the extraction of crude extract from the plant. Author ONA supervised author LOO in the anti-inflammatory study and performed the statistical analysis. In addition, author OAL managed some aspect of the literature search. Author IAO managed the literature searches and wrote the first and final drafts of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2018/41439

Editor(s):

- (1) Paola Angelini, Department of Applied Biology, University of Perugia, Italy.
(2) Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.

Reviewers:

- (1) Roshan Keshari, Delhi Pharmaceutical Sciences and Research University, India.
(2) Thomas Heinbockel, Howard University College of Medicine, USA.
(3) Wagner Loyola, Brazil.

(4) Salud Pérez Gutiérrez, Universidad Autónoma Metropolitana-Xochimilco, Mexico.
Complete Peer review History: <http://www.sciencedomain.org/review-history/25038>

Original Research Article

Received 16th March 2018

Accepted 2nd June 2018

Published 7th June 2018

ABSTRACT

Aims: The present research aimed to evaluate the anti-inflammatory action of hexane and ethyl acetate extracts of *Hura crepitans* L. (Euphorbiaceae) grown in Nigeria

Study Design: The study involves the extraction of crude extracts from the leaf of *H. crepitans* and the evaluation of their anti-inflammatory potential.

Place and Duration of Study: Fresh leaves of *H. crepitans* were collected from Festac Town, Amuwo-Odofin in Lagos, Nigeria (6.4664°N, 3.2835°E). The sheets were air-dried in the laboratory of Lagos State University where the extraction of crude and anti-inflammatory studies took place. The study lasted between March and November 2017.

*Corresponding author: E-mail: opeyemi.avoseh@lasu.edu.ng;

Methodology: The dried and pulverised leaves (0.5 kg) of *H. crepitans* were separately macerated in hexane and ethyl acetate for five (5) days in an airtight bottle and shook periodically (agitation) to maximise full extraction of the phyto-constituents. The extracts were decanted, filtered and concentrated on a rotary evaporator to obtain dried samples.

The anti-inflammatory activity was determined on fresh egg albumins over 4 h by measurement of rat paw edema according to established procedure.

Results: The result of the extraction shows that more phytochemicals are present in the ethyl acetate extract (15.0%) as compared to the hexane extract (7.2%). The anti-inflammatory activities of the ethyl acetate and hexane extracts of *H. crepitans* on Wistar rats using egg-albumin as phlogistic agents shows a moderate inhibition with a significant value of $P < 0.05$ at a dose of 200 mg/kg orally. Percentage inhibition of the anti-inflammation decreases steadily from the 1st hr to the 4th hr for the hexane extract (11.7 to 1.5%) while there was an increment in the ethyl acetate extract from 12% to 32.5% for the 1st and the 4th hr respectively when compared with the control.

Conclusion: This study has shown that the extracts of *H. crepitans* leaves possessed a significant anti-oedematogenic effect on paw oedema induced by egg-albumin by inhibiting the release of mediators for the entire 4 h experimental period.

Keywords: *Hura crepitans*; extracts; anti-inflammatory; egg-albumin.

1. INTRODUCTION

Hura crepitans L. (Euphorbiaceae), is a large tree that grows up to about 40 m tall in its natural habitat. The bark is grey while the green leaves are ovate. The monoecious male flowers are red with no petals while the solitary female flowers are reddish brown. The fruit is a dehiscent pumpkin-shaped capsule about 3 - 5 cm long and 5 - 8 cm wide, turns to reddish-brown when ripe, contains flattened seeds about 2 cm wide. Extracts of *H. crepitans* exhibited antiviral activity [1], antioxidant [2] and antimicrobial [3,4] and Anti-hepatotoxicity [5] activities. Extract of *Hura crepitans* L. inhibited the DHT (dihydrotestosterone)-induced (Neutrophin)NT-4 transcriptional activation and ameliorated the retardation of hair regrowth by DHT -implanted mice [6]. The antidiabetic and hepatoprotective effects of *H. crepitans* seed extract have been published [7]. The seed oil of *H. crepitans* is an alternative feedstock for biodiesel production [8]. The seed contained oleic acid [4]. *H. crepitans* seed is very rich in glutamate, arginine and leucine [9]. Sandbox (*H. crepitans*) seed meal enhances the performance of broiler starter chicks at 10% (raw) and 5% (cooked) dietary levels [10]. The phytochemical investigation has led to the characterization of daphnane diterpenes, daphnetoxin acid apocynin and methylpentadecanoate [5], a piscicidal constituent, huratoxin [11,12]. Other compounds identified by GC/MS (Gas chromatography-Mass spectrometry) from the crude extracts of the bark of *H. crepitans* include 5-(hydroxymethyl)furan-2-carbaldehyde, 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, nitrocoumarin, methyl

14-methylpentadecanoate, hexadecanoic acid, methyl lino-elaidate, 9,12,15-octadecatrienoic-1-oil, stearic acid, methyl ester [13].

In continuation of our research on the biological activities of Nigerian medicinal plants and herbs [14,15], we report herein the anti-inflammatory action of hexane and ethyl acetate extracts of *H. crepitans*.

2. MATERIALS AND METHODS

2.1 Chemicals

Chemicals and reagents: Unless otherwise stated, all chemicals and reagents were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). All the chemicals used including the solvents were of analytical grade. Ibuprofen injection was purchased from Lagos State University Pharmacy manufactured by Shalina Laboratories Pvt. Ltd, Nigeria.

2.2 Animals

Eight weeks Wistar rats of the average weight of 150 to 200 g of either sex were bought and kept in the animal house of the Department of Biochemistry, Lagos State University, Nigeria. Standard range of temperature ($23 \pm 2^\circ\text{C}$), light accessibility (12 h light and darkness cycle) with free access to standard pellet feed, tidy environment and water ad libitum. All experimental procedures were approved under the Lagos State University Research Ethical Clearance Committee (RECC) of the University (Approval no: 012/2016/LASU/BCH).

2.3 Plant Material

Fresh leaves of *Hura crepitans* were collected from Festac Town, Amuwo-odofin in Lagos, Nigeria (6.4664°N, 3.2835°E) in March 2017. Botanical identification of the plant was carried out by Mr. Edewo of the University of Lagos Herbarium, where a specimen number LUH 6607(Lagos University Herbarium) was deposited. Sequential solvent extractions were done using Hexane and ethyl acetate to obtain the plant extracts.

2.4 Extraction of Crude Extracts

Prior to extraction, plant samples were air-dried and pulverised. The pulverised leaves (0.5 kg) of *H. crepitans* were macerated in hexane for five (5) days in an air tight bottle and shook periodically (agitation) to maximize full extraction of the phyto-constituents. The extract was decanted and filtered using Whatman No1 filter paper. The marc was further macerated with ethyl acetate. Both hexane and ethyl acetate extracts were concentrated on a rotary evaporator to obtain the dried crude samples which were stored under refrigeration at 4°C until analysis.

2.5 Anti-inflammatory Test: Egg-albumin Induced Rat Paw Edema

The method for the determination of anti-inflammatory activity by measuring rat paw edema reported previously [16] was used in this study. Rats were assigned to one of 3 groups consisting of 5 animals each as follows: the control group (treated with water), standard group 2- standard (treated with 1.0 mL of ibuprofen injection) while group 3 and 4 has the extract of hexane (HCOOHEX) and ethyl acetate (HCOOEA) respectively- treated with 1 mL of 200 mg/kg extract orally. This treatment was continued until the 3rd day of the experiment. However, animals were starved 12 h before the anti-inflammation study day (day 3). On day 3 of the trial, baseline paw measurements of all rats were taken with a pair of Venier callipers. Thirty minutes later, 1.0 mL of 50% (v/v) of fresh egg albumin was injected into their right hind limb, and baseline paw measurements were again taken for the 1, 2, 3, and four h. Oedema was assessed regarding the difference in the zero-time linear diameter of the injected hind paw and its long diameter at time t (i.e. 1st h, 2nd h, 3rd h and 4th h.) following egg-albumin administration. From the mean oedema size, the inhibition

percentage of the inflammatory reaction was determined for each animal by contrast with controls and calculated by the formula indicated earlier [14,15];

$$\% \text{ Inhibition of edema} = 100 * (Vc - Vt / Vc)$$

Where,

Vc = Mean paw edema size of control group

Vt = Mean paw oedema size of a treated group

2.6 Statistical Analysis

Repeated Measures One way ANOVA Analysis using Tukey's multiple comparisons Test was performed using GraphPad Prism (version 7.02), San Diego CA, USA, www.graphpad.com) to compare activity between treatment group, control and the standard. The P value was significant for P > 0.05 and above values. Results were expressed as a mean ± standard error of the mean [14,15].

3. RESULTS AND DISCUSSION

Sequential solvent extraction offers a reliable means of natural product extraction due to polarity variation for efficient phytochemicals extraction. The result of the mining shows that more phytochemicals are present in the ethyl acetate extract with a yield of 75.0 g (15.0%) as compared to the hexane extract 36.0 g equivalent to 7.2% of the total return. The anti-inflammatory activities of the leaves of *H. crepitans* reveal a minimal to moderate activity. The proteins nature and agglutination property of egg-albumin make it a good phlogistic agent for the study of inflammation in animal models. The present study shows that the rate of inhibition by the extracts of *H. crepitans* was partial as inhibition rate were meager which reduced exponentially for the *H. crepitans* hexane extract as shown in Table 1 below. An expected inhibition by the standard drug (ibuprofen) which shows a significant of P < 0.001 compared to the control (saline solution). The crude extracts of *H. crepitans* gave a non-significant inhibition P > 0.05 consistent throughout the 1st to the 4th hour of experimentation (Fig. 1).

Table 1 shows the percentage inhibition of the ethyl acetate and hexane extracts of *H. crepitans* with an incremental percentage inhibition of the

extracts investigated on Wistar rats using egg-albumin as the phlogistic agent. The observed inhibition progression is as a result of different levels of absorption of phytochemicals responsible for the anti-inflammatory effect in *H. crepitans* leaves.

3.1 Anti-inflammatory Activity of Hexane (HCOOHEX) and Ethyl Acetate (HCOOEA) Extracts

Acute and chronic inflammation caused by injury to specific body tissues and cells are thus classified based on the duration of healing and body response. Several white blood cells and anti-inflammation mediators (biomarkers) are released by the cells which help to monitor the

progression of healing and activity of such mediator. Irrespective of the activating factor, the usual signs of increased blood flow, elevated cellular metabolism, vasodilatation, the release of soluble mediators, extravasation of fluids and cellular influx mechanisms are common to all [17]. Acute inflammation, such as egg albumin-induced oedema, involves the synthesis of these mediators at the injured site occurs over a few periods of days, influenced by the release of some mediators in three different phases [18]. Histamine and serotonin are released in the first phase during the first 1.5 h [18]. The second phase involves the release of bradykinin from 1.5 h to 2.5 h, while the last step consists of the release of prostaglandins between 2.5 h to 6.0 h phlogistic administration [19].

Table 1. Percentage inhibition of the egg-albumin induced inflammation of *H. crepitans*

Extracts	Percentage (%) inhibition paw edema ^a			
	1 st hr	2 nd hr	3 rd hr	4 th hr
Hexane	11.7	9.3	6.98	0
Ethyl acetate	11.7	32.6	16.3	13.9
Standard (Ibuprofen)	85.7	97.4	98.9	90.7

^a means of triplicate

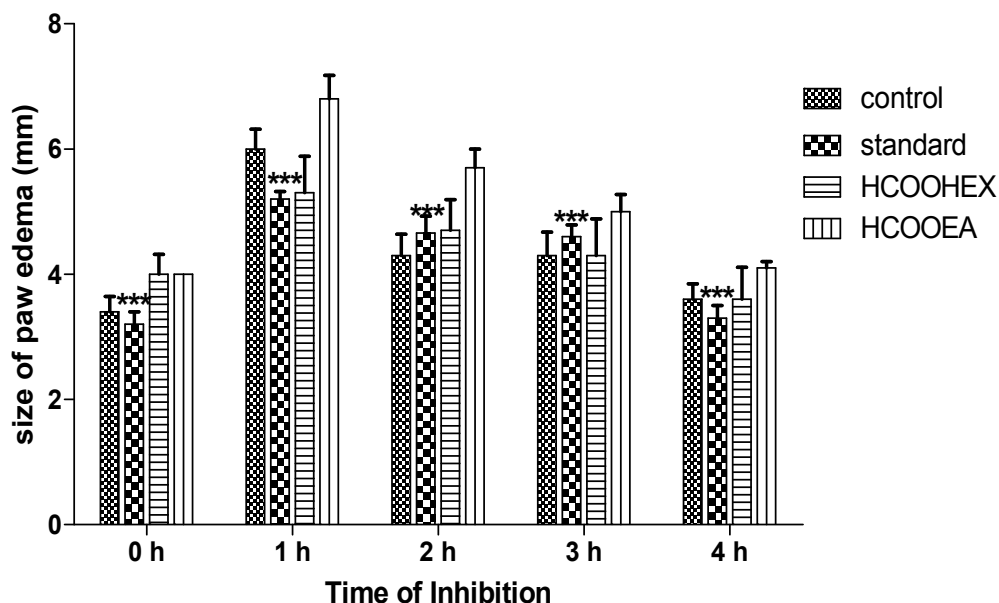


Fig. 1. Effect of HCOOHEX and HCOOEA on egg-albumin induced oedema. Control, Standard, and HCOOHEX and HCOOEA represent one mL saline solution, 100 mg/kg of Diclofenac injection and one mL HCOOHEX and HCOOEA respectively
^{*}*P*<0.5, ^{**}*P*<0.01, ^{***} *P*<0.001 (no asterisk represent zero significant) statistically compared to control

Natural products had proven to be an alternative source of cure to inflammation since ancient times and several medicinal plants and their isolated compounds are employed globally. Certain phytochemicals in these plants employed their active sites, lipophilicity, molecular geometry, binding ability [20] and another such mechanism for pain medication. NSAIDs (Non-steroidal anti-inflammatory drugs) can bind on the cyclooxygenase (COXs) enzymes inhibiting the synthesis of prostaglandins from the arachidonic acid metabolism which are indicators in the progression of inflammation, immunomodulation, and transmission of pain signals [21]. In this order, a drug is therefore classified as either an NSAID's or steroidal drug for inflammation treatment.

Although there is no report on the anti-inflammatory activity of *H. crepitans*, the plant exhibited other significant biological activities earlier mentioned. The ethanolic extract of the seed decreases the total serum protein and albumin significantly at a substantial level of $P < 0.05$ [7]. The different phytochemicals earlier observed may be responsible for the anti-inflammatory activities of *H. crepitans*.

4. CONCLUSION

This study has shown that the extracts of *H. crepitans* leave possessed a relatively low anti-oedematogenic effect on paw oedema induced by egg-albumin by inhibiting the release of mediators for the entire 4 h experimental period.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical clearance certificate was obtained from the Lagos State University Research Ethical Clearance Committee (RECC) of the University (Approval no: 12/2016/LASU/BCH).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Taborda NA, Acevedo LY, Patino CP, Forero JE, López-Herrera A. *In vitro* antiviral activity of extracts from

- Hura crepitans* and *Codiaeum variegatum* on bovine herpes virus type-1 and vesicular stomatitis virus. *Rev Colom Cienc Pecua.* 2007;20(3):241-249.
2. Oloyede G. *In vitro* antioxidant activity of extracts from the leaves of *Hura crepitans* (Euphorbiaceae)-a comparison of two assay methods cell membranes and free radical research. 2011;3(2):132-138.
3. David OM, Ojo OO, Olumekum VO, Famurewa O. Antimicrobial activities of essential oils from *Hura crepitans* (L.), *Monodora myristica* (Gaertn Dunal) and *Xylopi aethiopica* (Dunal A. Rich) seeds. *British J Appl Sci Technol.* 2014;4(23): 3332-3341.
4. Abdulkadir MN, Amoo IA, Adesina AO. Chemical composition of *Hura crepitans* seeds and antimicrobial activities of its oil. *Int J Sci Res.* 2013;2(3).
5. GK Oloyede OA Adaramoye MB Olatinwo. Chemical constituents of sandbox tree (*Hura crepitans* Linn.) and anti-hepatotoxic activity of the leaves and stem bark extracts.
6. Chiyoko U, Kazuhiro I, Takuya T, Atsushi N, Kei K, Hiroyuki H, et al. Effects of *Hura crepitans* and its active ingredient, daphne factor f3, on dihydrotestosterone-induced neurotrophin-4 activation and hair retardation. *Biol Pharm Bull.* 2012;35(1): 42-47.
7. Igwenyi IO, Agu EA, Awoke JN, Edwin N, Famurewa AC, Obasi NA, et al. Antidiabetic and hepatoprotective effect of *Hura crepitans* seed extract in alloxan-induced diabetic albino rats. *Int J Biol Pharm Allied Sci.* 2017;6(9):1771-1780.
8. Adewuyi A, Awolade PO, Oderinde RO. *Hura crepitans* seed oil: An alternative feedstock for biodiesel production. *J Fuels.* 2014;(2014):1-8. Article ID 464590. Available:<http://dx.doi.org/10.1155/2014/464590>
9. Fowomola MA, Akindahunsi AA. Nutritional quality of sandbox tree (*Hura crepitans* Linn.). *J Med Food.* 2007;10(1):159-164.
10. Ozeudu E, Esonu BO, Emenalom OO. Performance of starting broiler chicks on sandbox (*Hura crepitans*) seed meal Nigerian J Anim Prod. 2015;42(1):79-84.
11. Kanzo S, Kazuyoshi K, Tetsuo M. Studies on a piscicidal constituent of *Hura crepitans*. Part I. Isolation and characterization of huratoxin and its piscicidal activity. *Agric Biol Chem.* 1971; 35(7):1084-1091.

12. Kanzo S, Kazuyoshi K, Tetsuo M. Studies on a piscicidal constituent of *Hura crepitans*. Part II. Chemical structure of huratoxin. *Agric Biol Chem.* 1971;35(13): 2113-2126.
13. Igwe KK, Nwankudu ON, Ijioma SN, Madubuike AJ, Achi NK. Screening for secondary metabolites in *Hura crepitans* bark ethanol extract using GC-MS analysis: A preliminary study approach. *J Sci Technol Adv.* 2016;1(2):64-71.
14. Aboluwodi AS, Avoseh NO, Lawal AO, Ogunwande IA, Giwa AA. Chemical constituents and anti-inflammatory activity of essential oils of *Datura stramonium* L. *J Med Plant Stud.* 2017;5(1):21-25.
15. Oluwa AA, Avoseh NO, Omikorede O, Ogunwande IA, Lawal AO. Study on the chemical constituents and anti-inflammatory activity of essential oil of *Petiveria alliacea* L. *British J Pharm Res.* 2017;15(1):1-8.
16. Mujumdar AM, Misar AV. Anti-inflammatory activity of *Jatropha curcas* roots in mice and rats. *J Ethnopharm.* 2004;90(1):11-15.
17. Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. Chronic inflammation: Importance of NOD2 and NALP3 in interleukin-1beta generation. *Clin Exp Immunol.* 2007;147(3):227-235.
18. Lin CC, Lin W, Chang CH, Namba T. Anti-inflammatory and hepatoprotective effects of *Ventilago leiocarpa*. *Phytother Res.* 1995;9(1):11-15.
19. Suba V, Murugesan T, Kumaravelrajan R, Mandal SC, Saha BP. Anti-inflammatory, analgesic and antiperoxidative efficacy of *Barleria lupulina* Lindl extract. *Phytother Res.* 2005;19(8):695-699.
20. Lyß G, Knorre A, Schmidt TJ, Pahl HL, Merfort I. The anti-inflammatory sesquiterpene lactone helenalin inhibits the transcription factor NF-κB by directly targeting p65. *J Biol Chem.* 1998;273(50): 33508-33516.
21. Holgate ST, Burns GB, Robinson C, Church MK. Anaphylactic- and calcium-dependent generation of prostaglandin D2 (PGD2), thromboxane B2, and other cyclooxygenase products of arachidonic acid by dispersed human lung cells and relationship to histamine release. *J Immunol.* 1984;133(4):2138-2144.

© 2018 Avoseh et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sciencedomain.org/review-history/25038>