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Assessment of Anti-inflammatory and Anti-nociceptive Properties of Aqueous Seed Extract of *Carica papaya* in Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors URC, EJN, OK and AP designed the study, performed all the experimental work and collected the data. Authors OMC, AWCC and EUF performed the statistical analysis and wrote the manuscript. Authors EJN and URC wrote the manuscript and supervised all the research work. All authors read and approved the final manuscript.

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ABSTRACT

This study was undertaken to assess analgesic and anti-inflammatory properties of seed extract of *Carica papaya*. Aqueous extracts of the plant at doses of 100, 200, and 300 mg/kg body weight were used for the study on fifty adult white albino wistar rats weighing between 150 to 200 g. The extracts were evaluated for analgesic activity through tail immersion test using 25 rats while anti-inflammatory assay was performed by fresh egg albumin-induced paw oedema using 25 rats. For each model, the rats were divided into five groups of 5 rats each. Group 1 served as the negative control group, group 2 served as the positive control group while groups 3, 4 and 5 received 100, 200 and 300 mg/kg body weight of the plant extract respectively. Pethidine and aspirin were employed as a standard (positive control) for analgesic and anti-inflammatory studies respectively. Normal saline was used as negative control. Results showed statistically significant analgesic and anti-inflammatory properties in a dose and time dependent manner ($p<0.05$). The results thus validate the ethnomedicinal usage of *Carica papaya* seed extracts in the management of pain and inflammation.

Keywords: Seed extracts; Carica papaya; analgesia; inflammation; albino wistar rats.

1. INTRODUCTION

In many developing countries, about 80% of the population use traditional medicine for their primary health care needs [1], due to a number of reasons such as poverty and lack of access to modern medicines, high cost producing patentable chemicals and drugs [2]; high cost of antibiotics and antibiotic resistance [3] and unavailability of modern equipment [4]. It has been observed that plants are the cheapest and safer alternative sources to antimicrobials [5]. The current global economic recession, increasing unemployment, high cost of modern medicines and clear-cut enduring drug policy among others have made herbal medicine very attractive to the locals in many countries especially Nigeria.

Carica papaya, commonly called paw paw, is a widely grown perennial tropical tree in the family of C*aricaceae*. Among the *Caricaceae* family, paw paw is the most popular and economically important species. It was ranked third (15.4%), following production of mango (52.9%) and pineapple (26.6%) among total tropical fruit production in the world in 2012 [6]. The plant has its purified active constituents as proteolytic enzymes such as papain, which is found in most parts of the plant and chymopapain, which is found in the latex. Earlier works have demonstrated the clinical and pharmacological importance of these proteolytic enzymes [7]. Other phytochemical constituents of *Carica papaya* include alkaloids, flavonoids, glycosides, resin, and other phenolic compounds [8,9]. *Carica papaya* is a well known medicinal plant in many climes and different parts of the plant have been attributed with different medicinal values. Nsukka indigenes in Enugu State of Nigeria, chew the dry seeds to treat severe headache and swollen wounds [9]. The green leaves have also been used for the treatment of malaria, gonorrhea, syphilis and ameobic dysentery [10] and as sedative, muscle relaxant, antioxidant, and anticonvulsants [11]. The milky juice of the unripe fruit has been used in the management of helminthiasis, stomach disorders, enlarged spleen and liver and as a strong abortifacient [10]. Other attributed varied properties of *Carica papaya* include: anti-ulcerogenic, anti-ameobic, anti-fungal, anti-microbial, anti-tumour, hypolipidemic, anti-inflammatory, anti-nociceptive, immune modulator and in cases of kidney failure, low sperm count and uterine fibroids [8,12-20].

According to the American Pain Society (2000) [21], pain is the second leading cause of medically related work absenteeism, resulting in more than 50 million lost workdays each year. Many forms of therapies including medicinal herbs, have been used by man to relief pain. It has been observed that many of the current analgesia-inducing drugs such as opioids and Non-steroidal anti-inflammatory drugs (NSAIDs) have not been useful in all cases because of their side effects and low potency [22]. For example, morphine, a potent analgesic, causes acute morphine poisoning, hypotension, dependence, etc, while the NSAIDs such as aspirin, causes gastric irritation and ulceration, bleeding and perforation [23-25]. Therefore, there is the urgent need to continue to search for new therapeutics especially ones that are cheap, easily available and accessible, and with minimal

side effects if any. This work, assessment of anti-inflammatory and anti-nociceptive activities of the aqueous seed extracts of *Carica papaya* in rats, was one of such contributions in the quest for new drugs that possess good multiple properties.

2. MATERIALS AND METHODS

The reference drugs, pethidine and aspirin used in the study were bought from Pharmacy Department of Nnamdi Azikiwe University Teaching Hospital, Nnewi. Fresh eggs were bought from a dealer in Nnewi.

2.1 Plant Material and Extraction

The matured but unripe fruits of *Carica papaya* were harvested within the Nnewi Campus of Nnamdi Azikiwe University. The fruits were cut open with a clean knife, the black seeds were collected into a clean dry metal plate, thoroughly rinsed in tap water and then air dried at room temperature for two weeks. The dried seeds were pulverized into fine powder with a coronal manual grinder. 70 g of the paw paw seed powder was boiled in 500 ml of distilled water for 30 minutes and then filtered with clean white cotton gauze. The filtrate was poured into evaporating dish and evaporated to dryness at 30℃, producing a fine sweet smelling solid residue. The solid residue was weighed, stored in an air and water-proof container and kept in the refrigerator at 4℃ until required.

2.2 Experimental Animals

The research was conducted in accordance with the U.K. Animals (Scientific Procedures) Act (1986) and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC) [26,27] and the National Institutes of Health guide for the care and use of Laboratory animals [28]. Fifty adult white Albino wistar rats weighing between 150 and 200 g were purchased from the animal house of the Basic Medical Sciences of Nnamdi Azikiwe University, Nnewi Campus. The animals were divided into two of 25 rats each for the anti-inflammatory and anti-nociceptive studies. They were placed in cages and grouped into five (1-5) of 5 per group. The animals were then allowed to acclimatize to the laboratory environment under a 12-hour light/dark cycle for one week. They had free access to rat chow and portable water *ad libitum.* The animals were deprived of feed for 12 hours prior to the experiment but were allowed free access to portable drinking water. Furthermore, they were not allowed access to both water and feed during the experiment.

2.3 Assessment of Anti-inflammatory Activity of the Extract

2.3.1 Egg albumin-induced paw oedema

Acute inflammation was produced in the rats by injecting a fresh egg albumin into the plantar surface of the rat hind paw using a modified method of Winter et al. [29]. 0.1 ml of the fresh egg albumin was injected into the plantar surface of the right hind paw of the rats in groups 3-5, that received 100, 200 and 300 mg of the extract orally 30 minutes before the fresh egg albumin injection. Group 2 rats received 150 mg/kg of aspirin as positive control while group 1 that served as negative control received 2 ml/kg of normal saline orally. Veneer callipers were used to measure paw volume before administration of the extracts and at intervals of 1 hr for 3 hrs. All results were compared with negative control at time t and the percentage inhibition of oedema was calculated for each dose group using the formular [30].

% of oedema inhibition =

1- $\frac{\text{Paw oedema volume in treated group at time t}}{\text{Paw oedema volume in negative control group at time t}} \times 100$

2.4 Assessment of Anti-nociceptive Effect of the Extract

2.4.1 Tail immersion test

Analgesic activity of the reference drug and the extracts were assessed according to the modified method of Luiz et al. [31]. 25 rats were divided into five groups of five rats each. A suitable restrainer was used in holding them in position with the tail extending out. About 3-5 cm area of the tails were marked and immersed in the water bath thermostatically maintained at 50℃. The withdrawal time of the tail clearly from the hot water (in seconds) was recorded as the reaction time or tail immersion latency. Cut off period of 15 seconds was set to avoid tissue damage to the tail. 10 ml/kg of normal saline was given orally to control animals (Group 1); intraperitoneal pethidine 9.1 mg/kg body weight was administered as reference drug to Group 2 (positive control). The *Carica papaya* seed extract in doses of 100, 200 and 300 mg/kg were

given orally by intubation to animals in groups 3, 4 & 5 respectively. The pain threshold was noted immediately before administration of the test and reference drugs and then 60, 120, 180 and 240 minutes after the administration of the extracts.

2.5 Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, version 21). The results were presented as mean ± SEM. The data were analyzed using one-way Analysis of Variance (ANOVA) and LSD multiple comparison was used to test for significant differences between control and experimental groups. The differences were considered statistically significant at p= 0.05 or less.

3. RESULTS

3.1 Anti-nociceptive Activity

The results are presented in tables. Tables 1 and 2 show the results for the analgesic effects of the aqueous seed extracts of *Carica papaya* and pethidine treated rats with their corresponding percentage inhibition. The results showed that the aqueous extracts of *carica papaya* in the doses administered (100, 200 & 300 mg/kg b.w.) caused statistically significant suppression of nociceptive response in the rats ($p<0.05$). These analgesic responses were dose and time dependent. At all doses, the effect of the plant extract were more pronounced after 240 min at the dose of 300 mg/kg with a reaction time of 6.27±0.07 s (77.78% inhibition). The positive control drug pethidine (9.1 mg/kg) also produced a statistically significant anti-nociceptive activity at all observation times when compared with the control, with maximum tail immersion latency at the same 240 min (6.29±0.15, 79.20% inhibition). However, the test showed that the percentage inhibition for both the plant extract and pethidine was greatest at 180 min, with results of 90.03% and 95.33% respectively. Comparatively and marginally, pethidine was slightly more potent (95.33% inhibition) than *Carica papaya* extract (90.03% inhibition).

3.2 Anti-inflammatory Activity

The results obtained from the egg albumin - induced paw oedema (inflammation) test are presented in Tables in 3 and 4. Significant inhibition of the paw oedema (inflammatory activity) was noted at all doses of the plant extract ($p<0.05$) when compared with negative control and the inhibition was also dose and time dependent. Inhibition effects of aspirin was also significant at all doses ($p<0.05$). Percentage inhibition of inflammation by the aqueous extracts of *Carica papaya* ranged from 5.63% to 27.27% while that for aspirin ranged from 1.79% to 22.81%. Anti-inflammatory activity of the aqueous extracts of *Carica papaya* is therefore higher than that for aspirin.

4. DISCUSSION

Inflammation has varied definitions. It is a basic way in which the body reacts to infection,

Table 1. Effects of tail immersion latency in seconds (Mean±SEM) in *Carica papaya* and pethidine treated rats

	0 Mins	60 Mins	120 Mins	180 Mins	240 Mins	P value
Group 1	3.01±0.06	3.15±0.10	3.26±0.10	3.21±0.08	3.51±0.09	0.002
Group 2	3.23±0.05	5.30±0.30	5.84±0.23	6.27±0.28	6.29±0.15	0.001
Group 3	3.31±0.06	3.28±0.06	3.31±0.12	3.61±0.14	3.70±0.09	0.001
Group 4	3.14±0.07	3.53±0.14	4.83±0.24	5.40±0.28	5.75±0.18	0.001
Group 5	3.39±0.04	4.71±0.12	5.80±0.18	6.10±0.09	6.24±0.07	0.001

Each value represents mean±SEM, n=5, p<0.05 statistically significant compared to negative control

Table 2. Comparison of percentage inhibition following tail immersion latency (%)

	60 Mins	120 Mins	180 Min	240 Mins
Group 1	-	-	-	-
Group 2	68.25	79.14	95.33	79.20
Group 3	4.13	1.53	12.46	5.41
Group 4	12.06	48.16	68.22	63.82
Group 5	49.52	77.91	90.03	77.78

Table 3. Effects of *Carica papaya* seed extracts and aspirin on egg albumin-induced rat paw oedema volume (ml)

	0 Mins	60 Mins	120 Mins	180 Mins	240 Mins	P value
Group 1	2.13±0.07	5.70±0.27	5.51±0.32	5.60±0.24	5.50±0.28	0.378
Group 2	2.27±0.06	7.00±0.19	6.14±0.08	5.70±0.14	5.01±0.07	0.001
Group 3	2.22±0.03	5.70±0.25	5.20±0.09	4.90±0.12	4.21±0.07	0.001
Group 4	2.23±0.03	5.14±0.13	5.05±0.04	4.73±0.09	4.29±0.05	0.001
Group 5	2.25±0.04	4.70±0.16	4.65±0.17	4.37±0.08	4.00±0.07	0.001

Table 4. Comparison of percentage inhibition following egg albumin-induced paw oedema

	60 Mins	120 Mins	180 Mins	240 Mins
Group 1	-	-	-	-
Group 2	22.81	11.43	1.79	8.91
Group 3	0.00	5.63	12.50	23.45
Group 4	9.82	8.35	15.54	22.00
Group 5	17.54	15.61	21.96	27.27

irritation or other injury, the main characteristics being redness, warmth, swelling, pain and loss of function. It is a type of non-specific immune response [32]. All the signs are said to be secondary to one primary pathophysiological event – enhancement of vascular permeability as a direct consequence of tissue injury [33,34]. Of all the five classical signs, only one is said to be truly specific sign of inflammation, i.e. localized oedema. Oedema usually follows increased vascular permeability. Prostaglandins and leukotrienes are released by a host of mechanical, thermal, chemical, bacterial and other insults, and they contribute imperatively to the signs and symptoms of inflammation [35]. Mast cell, a very rich source of histamine has membrane receptors both for special class of antibody (IgE) and for complements components-$C3_a$ and $C5_a$. Mast cells can be activated to secrete inflammatory mediators through these receptors and also by direct physical damage. Furthermore, a transmembrane ptotein, CD40, expressed on platelets, neurtophils and monocytes also play roles in inflammation [36].

The paw oedema model has been accepted as a standard method used for the evaluation of anti-inflammatory activity of anti-inflammatory agents including several mediators such as histamine, serotonin, prostaglandins and bradykinin [29,37,38].

This study showed that the seed extract of *Carica papaya* exhibited statistically significant inhibition of the rat paw oedema ($p<0.05$) and the inhibition is both time and dose dependent. The plant extract also expressed higher level of oedema inhibition (5.63-27.27%) when compared with the standard anti-inflammatory agent, aspirin (1.79-22.81%). Furthermore, the anti-inflammatory activity (percentage inhibition) of *Carica papaya* extract was highest (27.27%) after 240 minutes at the dose of 300 mg/kg while that for aspirin was highest (22.81%) after 60 min. Thus, the plant extract seem to have delayed effects and may be useful in both acute and chronic inflammation. The possible mechanism(s) of action for the observed anti-inflammatory activity may include inhibition of the actions of inflammatory mediators such as histamine, prostaglandins, nitric oxide, cytokines, platelet activating factors and substance P [8,19,39-41]. *Carica papaya* extracts have also been demonstrated to have immune-modulatory property and this property can be attributed to its anti-inflammatory, wound healing, anti-hepatotoxic, diuretic and anti-hypertensive effects [40-42]. Extracts of *Carica papaya* has been shown to inhibit sub-chronic inflammation in which various types cellular migration are (e.g. fibroblasts) involved [40,43]. *Carica papaya* contains alkaloids, flavonoids, glycosides, resin, and other phenolic compounds [8,9]. Alkaloids, flavonoids and saponins have been found in other natural drugs with anti-inflammatory and anti-nociceptive effects [44]. Thus, the analgesic and anti-inflammatory effects of the aqueous seed extracts of *Carica papaya* may be due to the presence of alkaloids, flavonoids and other phenolics [9].

The anti-nociceptive activity of the seed extracts of *Carica papaya* was assessed using the rat tail

immersion test, one of the classical nociception models used in screening prospective anti-nociceptive compounds or plant extracts [45]. Our study showed that oral administration of the *Carica papaya* seed extract produced statistically significant inhibition of pain compared with the negative control ($p<0.05$). These inhibitory responses were both time and dose dependent. Both the plant extract (all doses) and the standard drug, pethidine showed maximum inhibition at 180 minutes with percentage inhibitions of 90.03% and 95.33% respectively. The anti-nociception property of the plant extract was therefore comparable with pethidine.

Our study showed that the plant extract was well tolerated and there was no obvious adverse motor deficits. It has been demonstrated that motor deficits can prolong reaction time in tail immersion and hot plate tests [46].

The brain and spinal cord play significant roles in central pain mechanism. The dorsal part of the spinal cord is rich with endogenous opioids, somatostatin, substance P, and other inhibitory hormones which are the targets of pain and inflammation. It has also been established that tail clip, tail flick, and tail immersion models are well documented methods for measuring central analgesic effects of drugs through opioid receptors [47]. Studies have shown that centrally acting analgesic drugs elevate the pain threshold of mice related to pressure and heat [48]. The tail immersion test is a thermal- induced nociception and indicates narcotic involvement [49]. Narcotic analgesics are known to be active against both peripheral and central pain, while nonsteroidal anti-inflammatory drugs inhibit peripheral pain [50]. Our findings in this study suggested that the seed extract of *Carica papaya* may act like narcotic drugs.

5. CONCLUSION

In conclusion, aqueous seed extract of *Carica papaya* has significant anti-inflammatory and anti-nociceptive properties on albino wistar rats and these positive/beneficial effects are comparable to standard anti-inflammatory drug (aspirin) and opioid analgesic (pethidine) respectively. This may justify the use of the extract by the natives in the management of inflammatory and nociceptive conditions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bodeker G, Bhat KKS, Burley J, Vantomme P, (Eds). Medicinal plants for forest conservation and health care. Food and Agriculture Organization of the United Nations, Rome. 1997;4.
2. Hack SK. Do not put too much value on conventional medicine. J. Ethnopharmacol. 2006;100:37-39.
3. Okeke IN, Lamikanra A, Edelman R. Socioeconomic and behavioural factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg. Infect. Dis. 1999;5:18-27.
4. Hamburger M, Hostettmann K. Bioactivity in plants: The link between phytochemistry and medicine. Phytochemistry. 1991; 30(12):3864-3874.
5. Doughari JH, El-Mahmood AM, Manzara S. Studies on the antibacterial activity of root extracts of *Carica papaya*. African Journal of Microbiology. 2007;037-041.
6. Edward AE, Fredy HB. An overview of global papaya production, trade, and consumption [Internet]. Gainesville, FL: Food and Resource Economics Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida; 2012.
   Available:http://edis.ifas.ufl.edu
   (Accessed on February 1, 2017)
7. Yarington CT, Bestler M. Double blind evaluation of enzymes properties in post-operative patients. Clin. Med. 1964;71:710-2.
8. Amazu LU, Azikiwe CCA, Njoku CJ, Osuala FN, Nwosu PJC, Ajugwo AO, Enye JC. Antiinflammatory activity of the methanolic extract of the seeds of *Carica papaya* in experimental animals. Asian

Pacific Journal of Tropical Medicine. 2010;884-886.

9. Anaga AO, Onehi EV. Antinociceptive and anti-inflammatory effects of the methanol seed extract of *Carica papaya* in mice and rats. African Journal of Pharmacy and Pharmacology. 2010;4(4):140-4.
10. Adeneye AA, Olagunju JA, Banjo AA. The aqueous seed extract of *Carica papaya* linn. Prevents carbon tetrachloride induced hepatotoxicity in rats. International Journal of Applied Research in Natural Products. 2009;2(2):19-32.
11. Akah PA, Akunyili DN, Eguatu CN. Investigations on the analgesics and antipyretic activities of aqueous extract of *Carica papaya* leaves. Nig J Neurosci. 2002;5:29-34.
12. Gupta A, Wambebe C, Parsons, DL. Central and cardiovascular effects of alcoholic extract of the leaves of *Carica papaya*. Pharmaceut Biol. 1990;28:257–266.
13. Nayak SB, Pinto Pereira L, Maharaj D. Wound healing activity of *Carica papaya* L. in experimentally induced diabetic rats. Indian J Exp Biol. 2007;45:739–743.
14. Goyal S, Manivannan B, Ansari AS, Jain SC, Lohiya NK. Safety evaluation of long term oral treatment of methanol sub-fraction of the seeds of *Carica papaya* as a male contraceptive in albino rats. J Ethnopharmacol. 2010;127:286–291.
15. Iyer D, Sharma BK, Patil UK. Effect of ether- and water-soluble fractions of *Carica papaya* ethanol extract in experimentally-induced hyperlipidemia in rats. Pharm Biol. 2011;49:1306–1310.
16. Kovendan K, Murugan K, Panneerselvam C, Aarthi N, Kumar P, Subramaniam J, Amerasan D, Kalimuthu K, Vincent S. Anti-malarial activity of *Carica papaya* (Family: *Carica-ceae*) leaf extract against *Plasmodium falciparum.* Asian Pacific J Trop Dis. 2012;2(S1):S306–S311.
17. Yasmeen M, Prabhu B. Anti-hyperglycemic and hypolipidemic activities of aqueous extract of *Carica papaya* Linn. leaves in alloxan-induced diabetic rats. J Ayurveda Integr Med. 2012;3:70–74.
18. Nunes NN, Santana LA, Sampaio MU, Lemos FJ, Oliva ML. The component of *Carica papaya* seed toxic to *A. aegypti* and the identification of tegupain, the enzyme that generates it. Chemosphere. 2013;92: 413–420.
19. Tamma NK, Ashraf TN, Nagakrishna L, Sudhakar L, Challa S. Evaluation of antinociceptive and anti-inflammatory effect of aqueous seed extract of *Carica papaya* Linn in albino rats*.* Int J Med Health Sci. 2013;2(3):2277-4505.
20. Pandey S, Cabot PJ, Nicholas Shaw P, Hewavitharana AK. Anti-inflammatory and immunomodulatory properties of *Carica papaya*. Journal of Immunotoxicology. 2016;13(4).
21. American Pain Society. Pain assessment and treatment in the managed care environment; 2000.
Available:http://www.ampainsoc.org/advocacy/assess_treat_mce.htm
(Accessed on 12/11/16)
22. Ahmadiani A, Fereidoni M, Semnanian S, Kamalinejad M, Sureni S. Anti-nociceptive and anti-inflammatory effect of *Sambucus ebulus* rhizome extract in rats. J. Ethnopharmacol. 1998;61:229-235.
23. Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG. The pharmacological basis of therapeutics. 9th ed. McGraw-Hill, San Francisco, USA. 1996;374–655.
24. Tripathi KD. Opiod analgesics and antagonists. In: Tripathi KD. Essentials of Medical Pharmacology 5th Ed. New Delhi. 2004;422-424.
25. Sharma HL, Sharma KK. Non steroidal anti-inflammatory agents, drugs for gout and antirheumatic drugs. In: Sharma HL, Sharma. Principles of Pharmacology 11th ed. Hyderabad. Paras Publishers. 2011;366-372.
26. Directive 86/609/EEC.
Available:ec.europa.eu/environment/chemicals/labanimals/nextstepsen.htm
(Accessed December 10th 2016)
27. Kilkenny C, Browne W, Cuthills IC, Emerson M, Athmans DG. Animal research: Reporting *in vivo* experiments. British Journal of Pharmacology. 2010;160(7):1577-1579.
28. Guide for the Care and Use of Laboratory Animals. 8th Edition, The National Academies Press, Washington DC; 2011.
Available:www.nap.edu
(Accessed December 12, 2012)
29. Winter C, Risley E, Nuss O. Carrageenin-induced inflammation in the hind limb of the rat. Fed. Proc. 1962;46:118-126.
30. Nemeth E, Ganz T. Regulation of iron metabolism by hepcidin. Annu Rev Nutr. 2006;26(1):323-42.

31. Luiz CDS, Mirtes C, Sigrid LJ, Mizuekirizawa M, Cecilia G, Jrotin G. Screening in mice of some medicinal plants used for analgesic purposes in the state of SãO Paulo. Journal of Ethno-pharmacology. 1988;24(2-3):205–211.
32. Rather LJ. Disturbance of function (functio laesa): the legendary fifth cardinal sign of inflammation, added by Galen to the four cardinal signs of Celsus. Bull NY Acad Med. 1971;47:303-22.
33. Ryan GB, Majno G. Acute inflammation. A review. Am J Pathol. 1977;86:183-276.
34. Majno G, Palade GE. Studies on inflammation. The effect of histamine and serotonin on vascular permeability: An electron microscopic study. J Biophys Biochem Cytol. 1961;11:571-605.
35. Jason DM, Robert LJ. Lipid-derived autacoids, eicosanoids and platelet activating factor. Goodman & Gilman`s: the pharmacological basis of therapeutics. New York. McGraw-Hill, Medical Publishing Division, USA. 2006;9-680.
36. Vanichakarn P, Blair P, Wu C, Freedman JE, Charkrabarti S. Neutrophil CD40 enhances platelet-mediated inflammation. Thrombosis Research. 2008;122(3):346-58.
37. Vinegar R, Truax JF, Selph JL, Johson PR, Venable AC, Makenzie KK. Pathway to carrageenan-induced inflammation in the hind limb of rat. Fed Proc. 1987;46:118-126.
38. Dananukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. Indian J Pharmacol. 2000;32:81-118.
39. Owoyele BV, Adebukola OM, Funmilayo AA. Anti-inflammatory activity of ethanolic extract of *Carica papaya*. Inflammo Pharmacology. 2008;16(4):168-73.
40. Otsuki N, Dang NH, Kumagai E, Kondo A, Iwata S, Morimoto C. Aqueous extract of *Carica papaya* leaves exhibits anti-tumor activity and immunomodulatory effects. Ethnopharmacol. 2010;127:760-767.
41. Tatyasaheb P, Snehal P, Anuprita P, Shreedevi P. *Carica Papaya* leaf extracts: An ethnomedicinal boon. International Journal of Pharmacognosy and Phytochemical Research. 2014;6:260-265.
42. Ghaisas MM, Desai BD, Ingale SP, Limaye RP. Immunomodulatory activity of aqueous extract of *Carica papaya* Linn leaves. Inventi Rapid: Ethnopharmacology; 2012. Article ID-Inventi: pep/620/12
43. Olajide OA, Makinde JM, Okpako DT. Evaluation of the anti-inflammatory of the extract of *Combretum micranthum*. G Don (Combretaceae), Inflammo Pharmacol. 2003;11:293-298.
44. Fernanda LB, Victor AK, Amelia TH, Elisabetsky E. Analgesic properties of Umbellatine from *Psychotria umbellate*. Pharm. Biol. 2002;44:54-56.
45. Sofodiya MO, Imeh E, Ezeani C, Aigbe FR, Akindemi AJ. Anti-nociceptive and anti-inflammatory activities of ethanolic extract of *Alafia barteri*. Revista Brasileira de Farmacognosia. 2014;24:348-354.
46. Ratnasooriya WD, Peiris LDC, Amerasekera AS. Analgesic activity of *Murraya koenigii* leaf extract in rats Med. Sci. Res. 1994;22:837-840.
47. Mc Curdy CR, Scully SS. Analgesic substances derived from natural products (natureceuticals). Life Sciences. 2005;78(5):476-484.
48. Singh S, Majumdar DK. Analgesic activity of *Ocimum sanctum* and its possible mechanism of action. Int. J. Pharmacogn. 1995;33:188-192.
49. Besra SE, Sharma RM, Gomes A. Anti-inflammatory effects of petroleum ether extract of leaves of *Litchi chinensis* Gaertu (Sapindaceae). J. Ethnopharmacol. 1996; 54:1-6.
50. Elisabetsky E, Ahmador TA, Albuquerque RR, Nunes DS, Caravalho ACT. Analgesic activity of *Pyschotria colorata* (Wild ex-R and S.) Muell. Arg. Alkaloids. J. Ethnopharmacol. 1995;48:77-83.

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