International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 02 Issue 10 October 2022

Page No: 400-406

DOI: https://doi.org/10.47191/ijpbms/v2-i10-03, Impact Factor: 5.542

Vitex trifolia as Cyclooxygenase-2 Inhibitors in Anti-Inflammatory Drug Discovery

Ifora Ifora[1], Aida Putri[2], Sri Oktavia[3]

[1,2,3] Department of Pharmacology and Clinical Pharmacy, School of Pharmaceutical Science Padang (STIFARM Padang), West Sumatera, Indonesia, 25147**.**

ABSTRACT

ABSTRACT: Inflammation is involved in several stages of diseases such as cardiovascular diseases, asthma, diabetes, neurodegenerative diseases, or cancer. Cyclooxygenase-2 (COX-2) is an important enzyme that catalyzes the production of prostaglandins from arachidonic acid and has an important role in various inflammatory-related pathologies. The selective suppression of COX-2 over COX-1 is thought to be a useful approach for treating inflammation. *Vitex trifolia* is traditionally used for various inflammatory ailments, thus giving scope for anti-inflammatory studies. Since the action of Ethanol extract of *Vitex trifolia* (EEVT) on COX-2 has not been previously described. In this study, we further investigated the effect of Cyclooxygenase-2 inhibitory effects spectrophotometrically in ELISA readers and evaluated anti-inflammatory activity in the carrageenan-induced rat paw edema model. The edema volume was measured using a plethysmometer. The results showed that the EEVt (200, 400, and 800 mg/kg BW) had significant anti-inflammatory activity and the EEVt demonstrated a COX-2 Inhibitory effect. The percentage of COX-2 inhibition was 8,04%, 20,74%, 21,92%, and the percentage of inhibition of paw edema was 39,56%, 45,60%, and 40,56%, respectively. These findings suggest that *Vitex trifolia* leaves extracts to possess promising and remarkable anti-inflammatory activity, which is directed against the enzymatic activity of COX-2.

KEYWORDS: Anti-inflammatory, *Vitex trifolia*, Cyclooxygenase-2, Carrageenan, Edema.

ARTICLE DETAILS

Published On:
04/10/2022

Available on:
https://ijpbms.com/

INTRODUCTION

Inflammation is the host's necessary protective response to harmful stimuli such as injury, infection, and/or irritation. Local and systemic inflammatory responses aim to remove the inciting stimulus, promote tissue repair and recovery, and establish immune memory in the case of infection so that the host can mount a more specific and faster response to future contact[1]. The important role of inflammation in various diseases such as diabetes, atherosclerosis, cencer, and Alzheimer's disease has driven major research efforts into processes that activate and control inflammatory responses[2,3].

Pro-inflammatory mediators play an important role in the regulation of physiological processes; however, Chronic inflammation can result from the uncontrolled production of pro-inflammatory mediators.. Cyclooxygenase exists in two isoforms: COX-1 and the inducible COX-2[4–6]. Inhibiting these mediators can have anti-inflammatory effects.

Nonsteroidal anti-inflammatory drugs (NSAIDs) or steroidal anti-inflammatory drugs (SAIDs) are used to block the pathway of inflammatory mediators[7]. Nowadays, NSAIDs are among the most commonly prescribed medications in the world, and their analgesic, anti-inflammatory, and antipyretic properties are well understood. NSAIDs, like many other drugs, have a variety of side effects, including cardiovascular (CV), renal toxicity, gastrointestinal (GI) events, high blood pressure, and worsening of congestive heart failure[8–11].

Ethnopharmacology-based research is regarded as a valuable strategy for the discovery of new agents with therapeutic potential[12–16]. In recent years, due to their milder action and lower adverse effects, ethnopharmacological remedies are becoming more popular as an alternative treatment for inflammation [17–21]. Consequently, there is a strong need for natural products with minimum side effects.

Data showed that plants have a wide range of therapeutic properties, including anti-inflammatory properties [22–24].

Vitex trifolia is a tropical shrub or a shrubby tree which grows up to 6m in height. *Vitex trifolia L.* (Verbenaceae) grows widely through-out Southeast Asia, Micronesia, Australia and East Africa. Traditionally the leaves are useful in inflammations, hair loss, cough bronchitis, fever, splenomegaly, tuberclosis and it is also mentioned to be having anti-cancerous activity [25]. Screenings for phytochemicals on the plant of *Vitex trifolis* have revealed the presence of various pharmacologically active principles of medical importance including flavonoids, alkaloids, terpenoids, and phenolic compounds[26,27]. Previous research also revealed that Vitex trifolia has a very broad pharmacological potential. However, Cox-2 inhibitory effect of *Vitex trifolia* ethanol extract in vivo have not been described previously. Thus, in the current study, we explored the effect of ethanol extract *Vitex trifolia* on anti-inflammation activity in carrageenan-induced rat paw edema and the COX-2 inhibitory effect in vivo.

MATERIALS AND METHOD

Materials

Rat PTGS2/COX-2 (Prostaglandin G/H synthase 2) ELISA Kit was purchased from Fine Biotech Co., Ltd. (Wuhan, China). Celecoxib was purchased from Pfizer Inc. (New York, NY, US). Carrageenan was obtained from Sigma-Aldrich (St. Louis, MO, USA) and other reagents were purchased from Bratachem (Indonesia).

The *Vitex trifolia* leaves were collected from Riau, Indonesia. The *Vitex trifolia* were identified by Dr. Nurainas, a botanist at Herbarium of Andalas University, West Sumatera, Indonesia.

Preparation of The Ethanol Extract of *Vitex trifolia* (EEVt)

The *Vitex trifolia* leaves was sun-dried. The dried *Vitex trifolia* was powdered using a conventional grinder. The powdered materials were then soaked in Ethanol (95%) for 24 hours by stirring at room temperature. The materials were filtered after 24 hours. The procedure was repeated three times. The filtrates were mixed and concentrated under vacuum using a rotary until a brownish semisolid extract was obtained, free of solvent. The extract was kept cold for further pharmacological testing.

Experimental Animal

18 adult male Wistar rats with body weights of 200–250 g and aged 2-3 months were obtained from West Sumatera animal houses were used for this study. Animals were housed and cared for in standard conditions with 12 h light/dark circle and were fed with a standard pellet diet and water ad libitum. All the animals were acclimatized for a minimum period of 1 week prior to the experiment. After 1 week, animals were randomly selected for different experimental groups (3 animal/ group) and used for the in vivo determination of anti-inflammatory activity. The rats were deprived of food, but not water, for 18–20 hours before an experiment.

Evaluation of Anti-Inflammatory activity

The anti-inflammatory activity was further examined by the carrageenan-induced rat paw edema method according to the method of Winter *et al* [28]. The experimental groups consisted of 18 rats split into six groups, Group I: Negative control (Na.CMC 0,5% p.o only), Group II: positive control (Carrageenan 1% s.c), Group III: Carrageenan 1% s.c + EEVt (200 mg/kg BW p.o),

Group IV: Carrageenan 1% s.c + EEVt (400 mg/kg BW p.o), Group V: Carrageenan 1% s.c + EEVt (800 mg/kg BW p.o), Group VI: comparative group (Carrageenan 1% s.c + Celecoxib 9 mg/kg p.o) were given 1 h before the injection of carrageenan.

By injecting 0.1 ml of 1% carrageenan in 0.9% saline into the right hind paw of the rat, edema was caused. After 1 h, 0.1 ml 1% carrageenan was injected subcutaneously into the subplantar area of the right hind paw of each rat except those in Group I. Edema volume was determined every 1 hour for up to 6 hours after carrageenan administration. The paw volumes were measured by a plethysmometer. The volume difference between before and after the right paw injection was measured to obtain the results. The inflammation degree of the paw and the rate of edema inhibition were calculated as follows:

% edema inhibition = (Vc −Vt) × 100/ Vc;

Vc and Vt are the average edema volumes of the control and test groups, respectively. The animal blood was collected in a heparin-coated tube at the end of the third hour. Groups I–VI was used to determine the activities of COX-2.

Evaluation of COX – 2 Inhibitory Activity

Rat serum was prepared at the 3rd hour after induction of Carrageenan 1%. EEVt (200, 400, and 800 mg/kg BW) was used for inhibition studies.The ability of the test compound to inhibit COX-2 was determined by using the Enzyme-Linked Immunosorbent Assay (ELISA) kit according to the manufacturer's instructions. The product of this enzymatic reaction produced a distinct yellow color, determined by spectrophotometrically (Microplate reader) at 450 nm.

Statistical Analysis

The statistical software SPSS version 25 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Data were analyzed using one-way ANOVA followed by Duncan's multiple range test. $p < 0.05$ was considered significant.

RESULT

Anti-Inflammatory activity

In the present study, the edema was measured for 6h after carrageenan injection. EEVt given at dose 200, 400, and 800 mg/kg BW were effective in inhibiting the induced paw edema (Table 1).

Tabel 1. Anti-inflammatory activity of *Vitex trifolia* extract

	Groups	Dose (mg/kg B.W)	**Percentage (%) of paw edema inhibition[a]**
I	Negative control (Na. CMC 0,5%)	-	
II	Positive control (0,1 mL Carrageenan **1%**)	-	-
III	EEVt	200	**39,56**
IV	EEVt	400	**45,60***
V	EEVt	800	**52,05***
VI	Celecoxib[b]	9	**40,56***
[a]Data are expressed as the mean of Three observations (n = 3), bUsed as comparative group *** Significant difference compared to the positive control (P < 0.05)**			

Table 1 shows the effect of EEVt and standard drug as compared to carrageenan control at different hours in the carrageenan-induced paw edema model. From Table 1, a significant anti-inflammatory activity of EEVt could be confirmed through paw edema inhibition by 45,60%, 52,05% and 40,56% after 6 h, at 200, 400, and 800 mg/kg dosage, respectively, while celecoxib diminished paw edema by 40,56% at the same time. These results demonstrate that the EEVt (200, 400, and 800 mg/kg) significantly inhibited the inflammatory processes induced by the injection of carrageenan ($p<0,005$). These findings indicate that the EEVt posses potent anti-inflammatory properties.

COX-2 Inhibitory Effect

The COX-2 Inhibitory effect of EEVt could be confirmed through COX-2 inhibition by 8,04 %, 20,74 % and 21,92% after 3 h, at 200, 400, and 800 mg/kg dosage, respectively, while celecoxib diminished paw edema by 26,51 % at the same time. These findings show that the EEVt (400, and 800 mg/kg BW) significantly inhibited COX-2 ($p<0,005$). it was obvious that the EEVt (400, and 800 mg/kg BW) ware the most potent COX 2 Inhibitory effect dose (Table 2).

Figure 1. clearly shows that a dose of 800mg/kg BW showed the most potency in inhibiting inflammation and inhibiting the enzyme cyclooxygenase-2.

Table 2. Cyclooxigenase-2 inhibitor Activity of *Vitex trifolia*

	Groups	Dose (mg/kg B.W)	**Percentage (%) of Cox-2 inhibition[a]**
I	Negative control (Na. CMC 0,5%)	-	-
II	Positive control (0,1 mL Carrageenan **1%**)	-	-
III	EEVt	200	**8,04**
IV	EEVt	400	**20,74***
V	EEVt	800	**21,92***
VI	Celecoxib[b]	9	**26,51***
aData are expressed as the mean of Three observations (n = 3), bUsed as comparative group *** Significant difference compared to the positive control (P < 0.05)**			

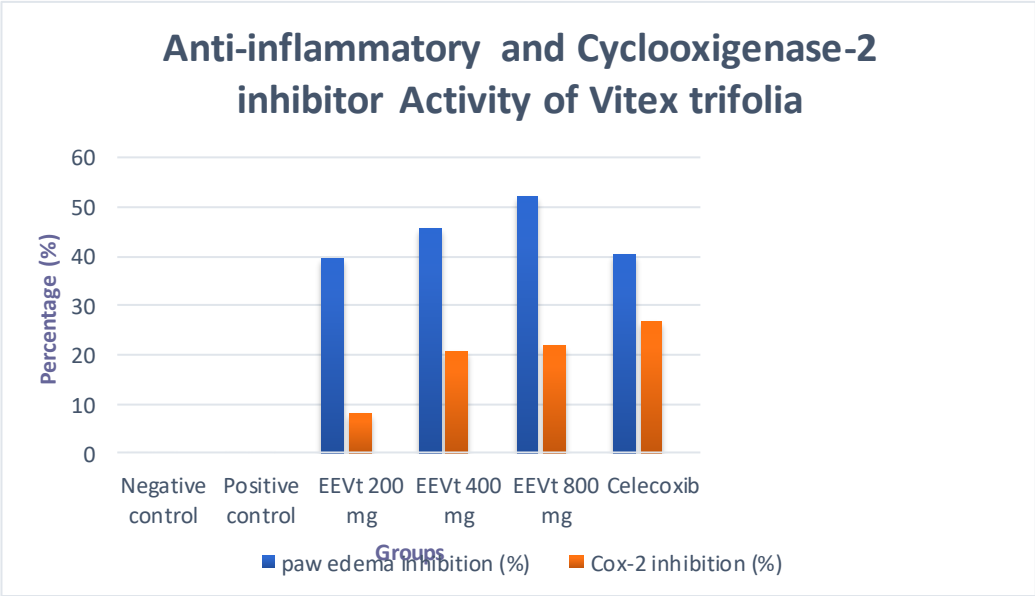

Figure 1. Comparison of percent inhibition of inflammation and percent inhibition of cyclooxygenase-2 enzyme at 3 hours after carrageenan induction

DISCUSSION

The paw edema caused by carrageenan is a useful phlogistic tool for studying systemic anti-inflammatory drugs. This test is divided into two phases and is sensitive to the majority of clinically effective anti-inflammatory drugs. The first phase, which occurs within 1-2 hours of carrageenan injection, is caused by serotonin release as well as an increase in bradykinin, histamine, and prostaglandins in the inflammatory location. The second phase occurs 3-5 hours after carrageenan injection and is associated with the production and release of kinin and prostaglandins in the inflamed area [29,30]. Throughout the second phase, The macrophages are known to produce more interleukin-1 (IL-1) which caused an increase in the accumulation of polymorphic nuclear cells (PMNs) in the inflammatory area. Activated PMNs then begin to release lysosomal enzymes and active oxygen species, resulting in connective tissue destruction and paw swelling[31].

This study reported the anti-inflammatory activity of the ethanolic extract of *Vitex trifolia* leaves and the possible anti-inflammatory mechanism in the experimental model. In the present study, the EEVt (200, 400, and 800 mg/kg) significantly inhibited the inflammatory processes. This is indicated by the ability of EEVt to significantly decrease rat paw swelling as shown in Tables 1. These results further corroborate previous studies that the hydroalcoholic extract *Vitex trifolia* leaves at doses 100 and 200 mg/kg BW showed anti-inflammatory activity in both acute and sub-acute stages of inflammation [25]. Other studies reported that Aqueous extract of *Vitex trifolia* leaves showed significant inhibitory activity on interleukin (IL)-1β, IL-6 and iNOS mRNA synthesis, all of which are involved in the inflammatory response. Moreover, the plant extract seemed to induce the LPS-dependant IL-10 anti-inflammatory cytokine[32]. Extract of *Vitex trifolia* leaves at doses 25-100 mg/kg BW showed analgesic activity. Pain receptors can be found all over the body and are sensitive to mechanical, thermal, toxic chemical, or inflammatory stimuli[33]. In most cases, regardless of etiology, the genesis of pain is inflammatory; thus, targeting inflammation and pain together is an important concept in pain management [34]. Because inflammation and pain are linked, a number of medications have both analgesic and anti-inflammatory properties.

COX and 5-LOX are two important enzymes that catalyze the formation of inflammatory mediators. Cox inhibitors are the main strays of current therapy aimed at modulating pain, inflammation, and fever control[35]. Many COX-2 or 5-LOX inhibitors have been developed as anti-inflammatory drugs; however, some have been withdrawn from the market, suggesting the need for inhibitors with minimal side effects [36]. Nonsteroidal anti-inflammatory drugs (NSAIDs) exert their analgesic effects by inhibiting cyclooxygenase activity, antipyretic, anti-inflammatory, and antithrombotic effects [37,38]. The anti-inflammatory potential of EEVt may be due to the presence of active phytoconstituents such as flavonoids. The previous report describes flavonoids can reduce the risk of atherosclerosis and atherothrombotic disease and several other inflammatory diseases [39,40]. The flavanol quercetin was found to suppress the expression of COX 2 mRNA in rat paw pouch exudates cells, indicating that quercetin's anti-inflammatory action may be due in part to suppressing COX-2 up-regulation[41]. Flavonoids can interfere with the oxidative synthesis of (Arachidonic Acid) AA from phospholipids and decrease the downstream production of inflammatory metabolites from AA metabolism, oxidative damage, and initiation of inducible pathways of Inflammation due to their powerful antioxidant capacity[42,43]. Other studies have demonstrated that flavonoids with antioxidant properties can reduce the cellular conversion of

AA to MDA (Malondialdehyde) in patients with chronic inflammation[44]. Radical scavenging activities of phenolic and polyphenolic compounds have been shown in previous studies [45,46]. There are also many studies on anti-inflammatory activities of plant extracts that contain flavonoids [47,48]. Furthermore, *Vitex trifolia* possessed enormous potential as a medicinal drug, particularly in cancer treatment and antioxidant[49,50]. The significant antioxidant capacity and flavonoid content of the extract used may have contributed to the anti-inflammatory effect.

This is the first report on the potent COX-2 inhibitory properties of ethanol extract of *Vitex trifolia* leaves. The obtained results suggest that the biological effects of this natural compound may be due to inhibition of prostaglandin synthesis via the arachidonic acid pathway.

CONCLUSION

From the results of the present investigation, it can be concluded that the ethanol extract of *Vitex trifolia* (EEVt) leaves possesses significant anti-inflammatory activity. The mechanism of anti-inflammatory action is thought to be mediated by COX-2 inhibition. The results presented also suggest the need for further research on the COX-1 inhibitory effect.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ACKNOWLEDGMENTS

The authors are grateful to all colleagues at The Departement of Pharmacology and Clinical Pharmacy, School of Pharmaceutical Science Padang, for helpful discussions.

REFERENCES

I. Fullerton JN, Gilroy DW. Resolution of inflammation : a new therapeutic frontier. Nat Publ Gr. 2016;15(8):551–67.

II. Granger DN, Senchenkova E. Inflammation and the Microcirculation. Vol. 2, Colloquium Series on Integrated Systems Physiology: From Molecule to Function. 2010. 1–87 p.

III. Jadhav RP, Kengar MD, Narule O V., Koli VW, Kumbhar SB. A Review on Alzheimer's Disease (AD) and its Herbal Treatment of Alzheimer's Disease. Asian J Res Pharm Sci. 2019;9(2):112–22.

IV. Kohno S, Murata T, Sugiura A, Ito C, Iranshahi M, Hikita K, et al. Methyl galbanate, a novel inhibitor of nitric oxide production in mouse macrophage RAW264.7 cells. J Nat Med. 2011;65(2):353–9.

V. Larsen BHV, Soelberg J, Jäger AK. COX-1 inhibitory effect of medicinal plants of Ghana. South African J Bot [Internet]. 2015;99:129–31. Available from: http://dx.doi.org/10.1016/j.sajb.2015.04.004

VI. Rumzhum NN, Ammit AJ. Cyclooxygenase 2: Its regulation, role and impact in airway inflammation. Clin Exp Allergy. 2016;46(3):397–410.

VII. Kumar S, Bajwa BS, Kuldeep S, Kalia A. Anti-Inflammatory Activity of Herbal Plants : A Review. Int J Adv pharmacy, Biol Chem. 2013;2(2):272–81.

VIII. Bally M, Dendukuri N, Rich B, Nadeau L, Helin-Salmivaara A, Garbe E, et al. Risk of acute myocardial infarction with NSAIDs in real world use: Bayesian meta-analysis of individual patient data. BMJ. 2017;357:1–13.

IX. Harirforoosh S, Asghar W, Jamali F. Adverse effects of nonsteroidal antiinflammatory drugs: An update of gastrointestinal, cardiovascular and renal complications. J Pharm Pharm Sci. 2013;16(5):821–47.

X. Sostres C, Gargallo CJ, Lanas A. Nonsteroidal anti-inflammatory drugs and upper and lower gastrointestinal mucosal damage. Arthritis Res Ther. 2013;15(SUPPL 3):1–8.

XI. Sostres C, Gargallo CJ, Arroyo MT, Lanas A. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. Best Pract Res Clin Gastroenterol. 2010;24(2):121–32.

XII. Meena A, Uttam S Niranjan, AK Yadav, Brijendra Singh, AK Nagaria, A Gaurav, et al. Vitex negundo Linn; a Review on Its Ethnobotany, Phytochemical and Pharmacological Profile. Res J Pharmacogn Phytochem. 2010;2(2):122–8.

XIII. Brusotti G, Cesari I, Dentamaro A, Caccialanza G, Massolini G. Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach. J Pharm Biomed Anal [Internet]. 2014;87:218–28. Available from: http://dx.doi.org/10.1016/j.jpba.2013.03.007

XIV. Ifora I, Hasyim N, Kardela W. Cyclooxygenase-2 Inhibitory Effect and Anti-Inflammatory Activity of Pomegranate (Punica granatum L .) Rind Extract. 2020;5:17–22.

XV. Ifora, Arifin H, Silvia R. Efek Antiinflamasi Krim Ekstrak Etanol Daun Kirinyuh (Chromolaena odorata (L) R.M. King & H. Rob) Secara Topikal dan Penentuan Jumlah Sel Leukosit Pada Mencit Putih Jantan. J Farm Higea. 2017;9(1):68–76.

XVI. Ifora I, Kardela W, Yora HYM. Uji Aktivitas Antikolesterol Ekstrak Etanol Buah Malur (Brucea Javanica (L .) Merr) terhadap Mencit Putih Jantan Hiperkolesterolemia. J Farm Higea. 2019;11(1):2–3.

XVII. Alamgeer, Uttra AM, Ahsan H, Hasan UH, Chaudhary MA. Traditional medicines of plant origin used for the treatment of inflammatory

disorders in Pakistan: A review. J Tradit Chinese Med. 2018;38(4):636–56.

XVIII. Elgorashi EE, McGaw LJ. African plants with in vitro anti-inflammatory activities: A review. South African J Bot. 2019;126:142–69.

XIX. Aravind R, Bindu A., Bindu K, Kanthlal S, AnilKumar B. Anti-Inflammatory Evaluation of the Cinnamomum malabatrum (Burm. F).Blume Leaves using Carrageenan Induced Rat Paw Oedema Method. Res J Pharm Technol. 2013;6(7):746–8.

XX. Ifora I, Sintia B, Srangenge Y. Pengaruh Penghambatan Enzim Siklooksigenase-2 dan Aktivitas Antiinflamasi dari Ekstrak Daun Ketumbar (Coriandrum sativum L.). J Kefarmasian Indones. 2021;11(1):17–24.

XXI. Ifora I, Fauziah F, Mayora SA. Aktivitas Anti-inflamasi dan Daya Hambat Siklooksigenase-2 Ekstrak Etanol Daun Tembelekan (Lantana camara L .). J Farm Higea. 2020;12(1):32–9.

XXII. Azab A, Nassar A, Azab AN. Anti-inflammatory activity of natural products. Molecules. 2016;21(10):1–19.

XXIII. Shah M, Parveen Z, Khan MR. Evaluation of antioxidant, anti-inflammatory, analgesic and antipyretic activities of the stem bark of Sapindus mukorossi. BMC Complement Altern Med. 2017;17(1):1–16.

XXIV. Ifora I, Dharma S, Maywidia Darma D. Pengaruh Pemberian Kombinasi Jahe Merah, Bawang Putih, Apel, Lemon Dan Madu Terhadap Kadar Kolesterol Total Dan Histopatologis Pembuluh Darah Aorta Jantung Tikus Putih Jantan. J Farm Higea. 2016;8(2):163.

XXV. Ankalikar A, Viswanathswamy AH. Effect of leaves of Vitex. trifolia linn on different stages of inflammation. Indian J Pharm Educ Res. 2017;51(3):461–71.

XXVI. Li WX, Cui C Bin, Cai B, Wang HY, Yao XS. Flavonoids from Vitex trifolia L. inhibit cell cycle progression at G 2/M phase and induce apoptosis in mammalian cancer cells. J Asian Nat Prod Res. 2005;7(4):615–26.

XXVII. Ono M, Sawamura H, Ito Y, Mizuki K, Nohara T. Diterpenoids from the fruits of Vitex trifolia. Phytochemistry. 2000;55(2):873–7.

XXVIII. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the Rat as an Assay for Antiinflammatory. Exp Biol Med. 1962;3(111):544–7.

XXIX. Crunkhorn P, Meacock SCR. Mediators of the inflammation induced in the rat paw by carrageenin. Br J Pharmacol. 1971;42(3):392–402.

XXX. Vinegar R, Scrheiber W, Hugo R. Biphasic Development of Carragenin Edema in Rats. J Pharmacol Exp Ther. 1969;166(1).

XXXI. Carl Nathan. Points of control in inflammation. Nature. 2002;420(19/26):846–52.

XXXII. Matsui M, Kumar-Roine S, Darius HT, Chinain M, Laurent D, Pauillac S. Characterisation of the anti-inflammatory potential of Vitex trifolia L. (Labiatae), a multipurpose plant of the Pacific traditional medicine. J Ethnopharmacol. 2009;126(3):427–33.

XXXIII. Yam MF, Loh YC, Tan CS, Adam SK, Manan NA, Basir R. General pathways of pain sensation and the major neurotransmitters involved in pain regulation. Int J Mol Sci. 2018;19(8).

XXXIV. Conaghan PG, Cook AD, Hamilton JA, Tak PP. Therapeutic options for targeting inflammatory osteoarthritis pain. Nat Rev Rheumatol [Internet]. 2019;15(6):355–63. Available from: http://dx.doi.org/10.1038/s41584-019-0221-y

XXXV. Ricciotti E, Fitzgerald GA, Ricciotti E, Fitzgerald GA. Prostaglandins and Inflammation. Arter Thromb Vasc Biol. 2011;31:986–1000.

XXXVI. Medzhitov R. Overview Essay Inflammation 2010 : New Adventures of an Old Flame. 2010;771–6.

XXXVII. Alessandri AL, Sousa LP, Lucas CD, Rossi AG, Pinho V, Teixeira MM. Resolution of inflammation: Mechanisms and opportunity for drug development. Pharmacol Ther [Internet]. 2013;139(2):189–212. Available from: http://dx.doi.org/10.1016/j.pharmthera.2013.04.006

XXXVIII. Kulwinder Singh, Monika, Neelam Verma. Gastrointestinal and Cardiovascular Risks of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs): Time to Translate Knowledge into Practice. Res J Pharm Technol. 2014;7(5):575–80.

XXXIX. Vazhappilly CG, Ansari SA, Al-Jaleeli R, Al-Azawi AM, Ramadan WS, Menon V, et al. Role of flavonoids in thrombotic, cardiovascular, and inflammatory diseases. Inflammopharmacology [Internet]. 2019;27(5):863–9. Available from: https://doi.org/10.1007/s10787-019-00612-6

XL. Lim T. Cajanus cajan. In: Edible Medicinal And Non-Medicinal Plants. Springer, Dordrecht. Springer, Dordrecht; 2012. 549–568 p.

XLI. Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, et al. Quercetin, inflammation and immunity. Nutrients. 2016;8(3):1–14.

XLII. Messina S, Bitto A, Aguennouz M, Mazzeo A, Migliorato A, Polito F, et al. Flavocoxid counteracts muscle necrosis and improves functional properties in mdx mice : A comparison study with methylprednisolone. Exp Neurol [Internet]. 2009;220(2):349–58. Available from: http://dx.doi.org/10.1016/j.expneurol.2009.09.015

XLIII. Sridharamurthy NB, Dhiman Vinay, R. Yogananda. Extraction and Screening of

Antioxidant, Anti-inflammatory and Analgesic Activity of Rhynchosia beddomel Leaves. Res J Pharmacol Pharmacodyn. 2012;4(5):319–27.

XLIV. Altavilla D, Squadrito F, Bitto A, Polito F, Burnett BP, Di Stefano V, et al. Flavocoxid, a dual inhibitor of cyclooxygenase and 5-lipoxygenase, blunts pro-inflammatory phenotype activation in endotoxin-stimulated macrophages. Br J Pharmacol. 2009;157(8):1410–8.

XLV. Jeremić SR, Šehović SF, Manojlović NT, Marković ZS. Antioxidant and free radical scavenging activity of purpurin. Monatshefte fur Chemie. 2012;143(3):427–35.

XLVI. Rita M C, Hemant J Dhongade, Manoj S Charde, Siddheshwar B Joshi. Evaluation of Wound Healing, Anti-Inflammatory and Antioxidant Activity of Rhizomes of Curcuma longa. Res J Pharmacol Pharmacodyn. 2010;2(1):42–7.

XLVII. Ravipati AS, Zhang L, Koyyalamudi SR, Jeong SC, Reddy N, Bartlett J, et al. Antioxidant and anti-inflammatory activities of selected Chinese medicinal plants and their relation with antioxidant content. BMC Complement Altern Med. 2012;12:5–10.

XLVIII. Parameswari P, Devika R. In silico Molecular Docking Studies of Quercetin Compound against Anti-inflammatory and Anticancer Proteins. Res J Pharm Technol. 2019 Nov 30;12(11):5305–9.

XLIX. Wu J, Zhou T, Zhang SW, Zhang XH, Xuan LJ. Cytotoxic terpenoids from the fruits of Vtex trifolia L. Planta Med. 2009;75(4):367–70.

L. Ojha DK, Jain AP. Phytochemical Screening, Estimation of Total Phenolic, Flavanoid Content and Estimation of Antioxidant Activity of Hydroalcoholic Extract of Vitex Negundo, Vitex Trifolia and Vitex Parviflora. J Adv Sci Res. 2021;12(04):268–71.