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Vitex trifolia's molecular anti-inflammatory effect: a review

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ABSTRACT

Inflammation is defined as a defensive response of the body against invading agents and harmful stimuli directly involved in the pathophysiology of various chronic diseases. Conventional anti-inflammatories have always been the first choice to treat and control inflammation and inflammation-related diseases. However, researchers have been searching for safer alternatives to conventional drugs with fewer possible side effects. Medicinal plants are considered potent alternatives to these drugs. Thus, understanding the molecular mechanisms of these plants and their biological targets can provide a new prospect for developing new anti-inflammatory agents with safer effects. *Vitex trifolia* belongs to the family Verbenaceae, a multipurpose medicinal plant, which can exhibit anti-inflammatory property. In recent literature, the plant was reported to have significant anti-inflammatory actions, including inhibitory effects on pro-inflammatory signaling pathways and mediators. Herein, the molecular anti-inflammatory effects of *Vitex trifolia* and its active components during inflammation have been reviewed.

Keywords: *Vitex trifolia*; inflammation; anti-inflammatory effect; mechanism of action

1. INTRODUCTION

Inflammation is a vital defensive response of the human body against harmful stimuli such as allergens, chemical irritants, injury, and infections. Inflammation can be characterized by its classic signs, including pain, heat, swelling, and loss of tissue function (Aoki and Narumiya, 2012; Conegundes et al., 2020). The inflammatory response is classified into acute, sub-acute, and chronic inflammation. Initially, the main aim of this vital defensive response is to eliminate the cause of injury and to restore tissue homeostasis (Chen et al., 2018a). Acute inflammation is a fast response of the immune system, which consists of two typical phases—vascular response and cellular response. Vasodilation and an increase in vascular permeability occur during the vascular response within a few seconds of the injury and last for several days. Meanwhile, the cellular response includes the recruitment of granulocytes and erythrocytes and occurs within a few hours after severe damage to the tissues (Placha and Jampilek, 2021). This initial phase of inflammation is mediated following the recognition of the stimuli.

Host immune cells have specific transmembrane receptors called the pattern-recognition receptors (PRRs), which are responsible for sensing and recognizing inflammatory stimuli through their recognition structure called pathogen-associated molecular pattern (PAMP). Endogenous stimuli, known as danger-associated molecular patterns (DAMPs) can also trigger PRRs (Ahmed, 2011; Mogensen, 2009). There are four families of PRRs namely, toll-like receptors (TLRs), C-type lectin receptors (CLRs), nucleotide-binding oligomerization

domain-like receptors (NLRs), and retinoic acid-inducible gene-1-like receptors (RLRs) (Chen et al., 2018a; Mogensen, 2009). Stimulated PRRs can trigger pro-inflammatory transcriptional factors through activation of intracellular inflammatory pathways such as nuclear factor kappa-B (NF-κB) and the mitogen-activated protein kinases (MAPKs) signaling pathways (Bao et al., 2018; Wang et al., 2019a). The transcription factors further activate some specific genes, leading to the expression of inflammatory mediators (Ahmed, 2011; Chen et al., 2018a). After recognizing the stimuli by leukocytes and mast cells, inflammatory mediators, which include cytokines, chemokines, vasoactive amines, and prostanoids are produced through specific inflammatory pathways.

Numerous mediators such as interleukins (IL) (IL-1, IL-6, IL-8, IL-11, IL-16, IL-17), tumor necrosis factor (TNF)-α, and histamine contribute to the acute phase of inflammation (Nurul Amin et al., 2020). Depending upon the severity of the injury and the type of stimuli, the initial phase of the inflammation process can be amplified by the activation of more mediators and the recruitment of leukocytes and macrophages. Immune cells attempt to eliminate the stimuli through enzymatic and non-enzymatic processes. This phase is followed by the release of anti-inflammatory mediators, such as IL-10, that facilitate the resolution phase. The inflammation process will typically end after the elimination of the stimuli and restoration of damaged tissues (Medzhitov, 2008; Palm and Medzhitov, 2009). Simply put, if the inflammation persists due to any failure in the elimination of stimuli, it will turn into chronic inflammation, which is characterized by an increase in the numbers of macrophages, lymphocytes, and plasma cells. Chronic inflammation can last for several weeks, months, or even years. All factors causing acute inflammation as well as lymphocytes and some specific mediators can be the main aetiology of the chronic inflammatory response (Placha and Jambilek, 2021). In addition, IL-4, IL-5, IL-6, IL-7, and IL-13 regulate the humoral response while IL-1, IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL-12, interferon (INF), TNF-α, and TNF-β coordinate the cellular response to the chronic inflammation. A few cytokines such as IL-1, IL-6, IL-11, IL-17, and TNF-α also play a significant role in both the initial and late phases of inflammatory responses (Nurul Amin et al., 2020).

Uncontrolled chronic inflammation, in contrast to acute, is destructive in nature. It is a hallmark of various chronic complications, such as diabetes, arthritis, cardiovascular diseases, and cancer. Multiple anti-inflammatory medicines are available to control and suppress the inflammation process, such as steroids, non-steroidal anti-inflammatory drugs, and immunosuppressants. Despite their side effects, these conventional drugs are used widely due to their excellent efficacy (Awaludin et al., 2017; Ghasemian et al., 2016). Herbal medicines are promising alternatives for developing safer and effective anti-inflammatory drugs. Moreover, to achieve effective treatment outcomes for complex diseases including inflammatory diseases, the classic concept of one drug for one target has been changed to one drug or drugs for multiple targets (Wu et al., 2108). Hence, therapeutic agents and their combinations acting on multiple targets are preferred for desirable pharmacotherapy. Thus, understanding the molecular mechanism of these agents can help in the discovery of new compounds with such effects. *Vitex trifolia* displays potent anti-inflammatory effects in various studies (Ankalikar and Viswanthswamy, 2017a; Laxmikant, 2011a; Wee et al., 2020). This review is meant to study the anti-inflammatory effect of *V. trifolia* and the underlying mechanism of action for its activity. It describes the effect of the plant's different extract types and some of its known isolated compounds on specific inflammation-related targets and pathways. For a better understanding, a brief description of the physiological and pathological roles of the latter targets during the inflammatory process is first provided.

1.1 *V. trifolia*

V. trifolia (Figure 1) is one of the species in the genus *Vitex* from the family Verbenaceae. The genus comprises approximately 270 known species, which widely grow in the tropic and sub-tropic regions (Dehsheikh et al., 2019; Saklani et al., 2017). The plant is a shrub and native to Asian countries, including Afghanistan, Malaysia, Micronesia, China, India, Myanmar, Pakistan, Sri Lanka, Vietnam and Indonesia (Chantaranothai, 2011; Orwa et al. 2009). It is widely distributed in places with sandy beaches (Chan et al., 2016). The shrub can grow up to 6 m with brown bark and trifoliate or simple leaves.

The upper surface of the leaf is green and shiny with a grey hairy lower surface (Chantaranothai, 2011; Dehsheikh et al., 2019). *V. trifolia*'s white and purple flowers are terminal (Chantaranothai, 2011; Laxmikant, 2011b) and its fruits are rounded, 4 to 5 mm in diameter, and contain four black seeds (Suchitra and Cheriyan, 2018).

1.2 *V. trifolia* chemical constituents

The chemical composition of *V. trifolia* has been widely reported. According to the reports, phenols, flavonoids, steroids, terpenoids, tannins, and glycosides are major chemical constituents of the plant (Ghafari et al., 2020; Jangwan et al., 2014; Wee et al., 2020). Several studies reported the isolation of several polyphenolic compounds from *V. trifolia*. Casticin or vitexicarpin is one of the main polyphenolic compounds of genus *Vitex* including *V. trifolia* has anti-inflammatory and anticancer activities. From *Vitex* species, casticin was isolated from the fruits and leaves and the content of casticin in the fruits and leaves was estimated to be 0.03% and 0.01%, respectively (Chan et al., 2018; Liou et al., 2014). Additionally, artemetin, chrysosplenol D, luteolin, penduletin, persicogenin, quercetin, ursolic acid, vitexin, and isovitexin are among polyphenolic compounds that have been isolated from the leaves, fruits, and aerial parts of *V. trifolia* (Aye et al., 2019; Matsui et al., 2009; Suchitra and Cheriyan, 2018; Wee et al., 2020). The total phenolic content and total flavonoid content of the plant leaf ethanol extract were reported to be 77.20±0.22 mg gallic acid equivalent (GAE)/g dry weight of extract and 57.41±0.37 mg quercetin equivalent (QE)/g dry weight of extract, respectively (Saklani et al., 2017).

A variety of diterpenes and triterpenes, including labdane-type diterpenes, abietane-type diterpenes, and halimane-type diterpenes, have been isolated from *V. trifolia*. Labdane-type diterpenes, viterotulin C, vitexilactone D, vitexilactone, rotundifuran, viterifolin B, and viterotulin B along with halimane-type diterpenes, and viterifolin D were isolated from the fruit ethanol extract of *V. trifolia* (Fang et al., 2018), while vitetrifolins D–G, vitetrifolin A, and an abietane-type diterpene were

found in acetone extract of the fruits (Aye et al., 2019). Moreover, *V. trifolia* leaf ethanol extract was also found to have vitextrifloxide A-I, vitexifolin B, viterifolin D–G, viterifolin I, viterotulin B, and viteagnusin I (Luo et al., 2017). In another study, vitrifolin C along with three diterpenes, including rotundifuran, dihydrosolidagenone, and abietatriene 3-β-ol have been isolated from *V. trifolia* (Laxmikant, 2011b). The fruits of the plant were reported to have vitexifolin A-C, vitexilactone, and previtexilactone (Chan et al., 2016). Moreover, vitextrifolin A-G have been identified in the dichloromethane extract of *V. trifolia* fruits (Zheng et al., 2013). Deacetylvitexilactone, vitexilactone, vitexilactone B, dysoxydensin G, 13-hydroxy-5, 14-halimadien-6-one, 9 α-hydroxy-13(14)-labden-16,15-amide, 9,13-epoxy-16-norlabda-13E-en-15-al, and negundoin C can also be found in *V. trifolia* leaf ethanol extract (Luo et al., 2017).

Ascorbic acid, β-carotene, benzoic acid, phthalic acid, and β-sitosterol have also been identified from the leaves and fruits of *V. trifolia* (Abdul Hakeem et al., 2016). Similarly, α –amyrin, β-amyrin, taraxcrol, taraxcrone, lupeol, and betulinic acid were isolated from the methanol extract of the plant's leaves (Huang et al., 2016). Agnuside, negundoside, and 6'-p-hydroxy benzoyl mussaenosidic acid were identified in the methanol extract of *V. trifolia*'s aerial parts (Tiwari et al., 2012). *V. trifolia* leaves were reported to have 0.45% of essential oil, which contains mainly monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, diterpenes, and oxygenated diterpenes. Moreover, *V. trifolia*'s leaf essential oil was reported to have β-caryophyllene, caryophyllene oxide, phytol, α-pinene, phytane, and manool oxide as their predominant compounds (Dehsheikh et al., 2019). The chemical composition of *V. trifolia* is summarized in Table 1.

Figure 1. The flowering branch of *V. trifolia* with trifoliate leaves
Adapted from "Verbenaceae-Vitex trifolia" by vanlaphoang, is licensed under CC BY- 2.0 (Van Lap, 2013)

1.3 *V. trifolia*'s pharmacological effects

V. trifolia was reported to possess several pharmacological effects that are summarized in Table 2. The plant is traditionally used against pain, allergy, fever, and inflammation (Rani and Sharma, 2013; Tsai et al., 2016). Its infusion and decoction are reported to be effective against tuberculosis and amenorrhea and the essential oil is often useful as a sedative (Dehsheikh et al., 2019). Its decoction is also used to treat skin allergies (Sujarwo et al., 2015). The fruits are useful to treat premenstrual symptoms (Bello et al., 2018; Rani and Sharma, 2013). In Chinese traditional medicine, *V. trifolia* is used to treat headaches, sore eyes, cold, amenorrhea, and migraine while in Unani medicine, it is used to treat decreased libido (Suchitra and Cheriyan, 2018). The bark of the plant is useful to treat hypertension and tuberculosis while the infusion of its flowers is used to treat fever and vomiting (Hernandez et al., 1999).

The antibacterial activity of *V. trifolia*'s extract was displayed against several bacterial strains, including *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa, Bacillus cerus,* and *Listeria monocytogenes* (Abd Aziz et al., 2011; Laxmikant, 2012; Luo et al., 2017). In a study, isolated 9-hydroxy-13(14)-labden-15,16-olide and isoambreinolide from *V. trifolia* leaf methanol extract significantly inhibited the growth of *Mycobacterium tuberculosis* with minimum inhibitor concentration (MIC) values of 100 and 25 μg/mL, respectively (Tiwari et al., 2012). Furthermore, *V. trifolia* leaf methanol extract was reported to inhibit the production of exopolysaccharide and the activity of protease in *Pseudomonas aeruginosa* by 96% and 69%, respectively, in a dose-dependent manner (50-100 μg/mL) (Mary and Banu, 2015). Some studies also reported the potential antiviral activity of the plant's extract against human immunodeficiency virus (HIV), mouse coronavirus, and herpes simplex virus (HSV). The aqueous extract of the plant's aerial parts was reported to have a significant HIV reverse transcriptase effect with an inhibitory rate of 90% (Chinsembu, 2019) while the plant's aerial parts methanol extract (0.5-2 μg/mL) showed a potent inhibitory effect on mouse coronavirus and HSV (Vimalanathan et al., 2009). The fungicidal effect of the hexane extract of *V. trifolia* leaves (500 μg/mL) was observed against *Penicillium*, *Trichoderma,* and *Fusarium* species with mycedial growth inhibition rates of 22%, 20%, and 100%, respectively (Hernandez et al., 1999). In a

similar study, the antifungal activity of plant leaf essential oil was reported against *Candida albicans, Candida parapsilosis, Candida krusei*, and *Candida kefyr* with MIC values of 62.5, 31.25, 1.95, and 3.9 µL/mL, respectively (Devi and Singh, 2014). Moreover, the antiparasitic effect of *V. trifolia* was seen against *Giardia lamblia* and *Entamoeba histolytica*. The methanol extract of the leaves at 1,000 ppm, significantly showed antigiardial and antiamoebic activities with maximum mortality rates of 75.25% and 61.64%, respectively (Garbi et al., 2015).

Table 1. The chemical composition of *V. trifolia*

Plant organ	Chemical constituents	References
Leaves	13-Hydroxy-5, 14-halimadien-6-one 9 α-Hydroxy-13(14)-labden-16,15-amide,9,13-epoxy-16-norlabda-13E-en-15-al Artemetin Ascorbic acid Benzoic acid Betulinic acid Caryophelene oxide Casticin Chrysosplenol D Deacetylvitexilactone Dysoxydensin G Gallic acid Isovitexin Lupeol Luteolin Manool oxide Maslinic acid Negundoin C Penduletin Persicogenin Phthalic acid Phytane Phytol Quercetin Taraxcrol Taraxcrone Ursolic acid Viteagnusin I Viterifolin D-G Viterifolin I Viterotulin B Vitexifolin B Vitexilactone B Vitexin Vitextrifloxide A-I α–Amyrin α-Pinene β-Amyrin β-Carotene β-Caryophellene β-Sitosterol	Aye et al., 2019; Dehsheikh et al., 2019; Huang et al., 2016; Luo et al., 2017; Matsui et al., 2009; Abdul Hakeem et al., 2016; Suchitra and Cheriyan, 2018; Wee et al., 2020
Fruits	Casticin Previtexilactone Pyronopyran-1,8-dione Rotundifuran Viterifolin B Viterifolin D Viterotulin B Viterotulin C Vitetrifolin A Vitetrifolins D–G Vitexifolin A-C Vitexilactone Vitexilactone Vitexilactone D Vitextrifolin A–G	Aye et al., 2019; Chan et al., 2016; Fang et al., 2018; Lee et al., 2017; Zheng et al., 2013
Aerial parts	6'-*P*-hydroxy benzoyl Agnuside, Mussaenosidic acid Negundoside	Tiwari et al., 2012

Table 2. Pharmacological effects of *V. trifolia*

Pharmacological effect	Plant organ	References
Anti-inflammatory	Leaves Fruits	Ankalikar and Viswanathswamy, 2017a; Ankalikar and Viswanathswamy, 2017b; Chan et al., 2018; Fang et al., 2018; Goverdhan and Bobbala, 2009; Laxmikant, 2011a; Lee et al., 2017; Liou et al., 2014; Matsui et al., 2009; Matsui et al., 2012
Antibacterial	Leaves	Abd Aziz et al., 2011; Luo et al., 2017; Mary and Banu, 2015; Tiwari et al., 2012
Antifungal	Leaves	Devi and Singh, 2014; Hernandez et al., 1999
Antiviral	Aerial parts	Chinsembu, 2019
Antiparasitic	Leaves	Garbi et al., 2015
Anthelminthic	Leaves	Thenmozhi et al., 2013
Anticancer	Fruits Leaves	Chan et al., 2016; Chan et al., 2018; Huang et al., 2016
Antihistaminic	Leaves	Alam, et al., 2002; Ikawati et al., 2001; Rani and Sharma, 2013; Suchitra and Cheriyan, 2018
Antioxidant	Aerial parts Leaves	Ankalikar and Viswanathswamy, 2017b; Dehsheikh et al, 2019; Saklani et al, 2017
Antidiabetic	Leaves	Nishina et al. 2017
Analgesic	Leaves	Laxmikant, 2014; Rani and Sharma, 2013;
Antituberculosis	Leaves Bark	Hernandez et al., 1999; Tiwari et al., 2012
Antiemetic	Flowers	Hernandez et al., 1999
Anti-premenstrual symptoms	Fruits	Bello et al., 2018; Rani and Sharma, 2013
Hepatoprotective	Flowers	Anandan et al., 2009

The anthelminthic effect of the plant was investigated. The aqueous and alcoholic extracts of *V. trifolia* leaves were reported to possess a significant anthelminthic effect against *Pheretima posthuma*; both types of extracts caused the death of the worms at 100 mg/mL after 20.71±0.77 and 31.76±1.05 min, respectively, compared to albendazole, which did the same after 21.32±1.9 min at 100 mg/mL (Thenmozhi et al., 2013).

The anticancer effect of *V. trifolia* is one of its most considered therapeutic properties, which has been reported by multiple studies. Reports from several studies indicated that the plant's extract could potentially inhibit the proliferation of some cancer cell lines, such as breast cancer cell line, colorectal cell line, liver cancer cell line, and gastric cancer cell line. The anticancer effect of *V. trifolia* is attributed to the presence of various secondary metabolites, such as polyphenols, labdane-type diterpenes, and ursane-like triterpenoids (Chan et al., 2016; Chan et al., 2018; Huang et al., 2016). The molecular studies showed that the anticancer activity of the plant and its active compounds is due to its ability to induce apoptotic signaling pathways such as caspase-3 and MAPKs signaling pathways (Chan et al., 2016). Moreover, the hepatoprotective effect of *V. trifolia* against carbon tetrachloride-induced hepatotoxicity in rats was reported by (Anandan et al., 2009). The effect was seen through the reduction of total levels of bilirubin and serum hepatic enzyme by the ethanol flower extract of the plant at 200 mg/kg body weight. The antidiabetic effect of *V. trifolia* was seen in 3T3-L1 pre-adipocytes. Vitexilactone, an isolated flavonoid of the plant leaf ethyl acetate extract, showed a thiazolidinedione-like effect and increased the expression of glucose transporter type 4 (GLUT4) in the cell membrane (Nishina et al., 2017). *V. trifolia* can possess a potential analgesic effect as it is used for the same purpose in Chinese traditional medicine (Suchitra and Cheriyan, 2018). The analgesic property of the plant was reported by (Laxmikant, 2014). The alcoholic leaf extract of the plant at 400 mg/kg body weight inhibited the acetic acid-induced writhing by 68.53% in mice compared to indomethacin (74.53%) at 10 mg/kg body weight (Laxmikant, 2014). Additionally, *V. trifolia* was reported to have anti-inflammatory and antioxidant effects, which are discussed in the following sections. Table 2 summarizes the pharmacological effects of different parts of *V. trifolia*.

1.4 Anti-inflammatory effect of *V. trifolia*

V. trifolia's anti-inflammatory property has been studied by several researchers using different methods. *In vivo* tests were conducted by Goverdhan and Bobbala (2009), Laxmikant (2011a), and Ankalikar and Viswanathswamy (2017a) to evaluate the anti-inflammatory effects of methanol, aqueous, and hydro-alcoholic extracts of the leaves of *V. trifolia*, respectively. All the researchers reported consensus findings on the potential anti-inflammatory effect of the plant. The plant's leaf aqueous extract and the fruit methanol extract were tested on RAW 264.7 cells in different studies, and both extracts showed anti-inflammatory properties (Bao et al., 2018; Matsui et al., 2009). Studies on isolated active compounds of *V. trifolia* also proved its anti-inflammatory property. Castisin, a flavonoid in *V. trifolia,* exhibited an anti-inflammatory effect when tested on murine macrophage (Chan et al., 2018; Lee et al., 2013). Pyronopyran-1,8-dione (PPY), isolated from *V. trifolia* fruit methanol extract, also displayed anti-inflammatory activity by reducing the expression of inflammatory mediators (Lee et al., 2017). The potential anti-inflammatory activity of *V. trifolia's*

terpenoids, including viterolutin C, vitexilacton D, vitexilactone, rotundifuran, vitetrifolin B, viterotulin B, and vitetrifolin D, have also been reported (Chan et al., 2018). A similar effect has been reported for 10-O-vanilloylaucubin, agnusoside, and 3-normal-butyl-nishindaside isolated from *V. trifolia* (Bao et al., 2018). Moreover, the plant's phenolic content can potentially contribute to its anti-inflammatory effects (Chan et al., 2018; Wee et al., 2020).

V. trifolia was observed to be effective in the inhibition of acute and chronic inflammation. The plant can potentially inhibit the mediators involved in both stages. As discussed earlier, the initial phase of inflammation is regulated by pro-inflammatory mediators such as IL-1, IL-6, TNF-α, prostaglandins (PGs), and histamine (Nurul Amin et al., 2020). These induce the production of other pro-inflammatory mediators and activate the immune cells. Moreover, *V. trifolia* was found to inhibit the later mediators (Alam et al., 2002; Liou et al., 2014; Matsui et al., 2012; Wee et al., 2020), hence, it has a potential inhibitory effect on the acute inflammatory response. In addition, the inhibitory effect of the plant on IL-1, IL-6, and TNF-α can contribute to its chronic inflammation inhibitory properties. IL-1, IL-6, and TNF-α play a significant role in the progression of the chronic inflammatory responses by the activation and differentiation of lymphocytes (Holbrook et al., 2019; Nurul Amin et al., 2020; Tasneem et al., 2019). *V. trifolia* was also reported to reduce the number of lymphocytes in the inflamed site (Ankalikar and Viswanathswamy, 2017a). Hence, there is evidence confirming the inhibitory effect of *V. trifolia* on both the acute and chronic phases of inflammation.

1.5 Anti-inflammatory mechanism of action of *V. trifolia*

The goal of inflammation pharmacotherapy is to reduce the inflammation process and tissue damage and accelerate the resolution process. Anti-inflammatory agents exhibit their anti-inflammatory effect by modulation of inflammatory pathway activity, inhibiting inflammatory mediators' production, inhibiting vasoactive agents' production, decreasing complements activity, stabilizing vascular endothelium, and decreasing leucocyte recruitment (Szollosi et al., 2018). Here, we discuss the possible molecular mechanisms of action associated with the plant's anti-inflammatory properties.

2. MODULATION OF NF-κB SIGNALING PATHWAY

NF-κB is one of the most important and well-studied transcription factors for an inflammatory response (Ahmed, 2011). At the physiological state, NF-κB proteins are kept inactive by binding to a specific protein called the inhibitor of kappa-B (IκB). Upon stimulation, IκB is phosphorylated by IκB kinase (IKK), which leads to the release and activation of NF-κB. The activated NF-κB is further translocated into the nucleus and causes specific gene expression, leading to pro-inflammatory cytokine production and inflammatory cell recruitment (Chen et al., 2018a; Serasanambati and Chilakapati, 2016). p50, p52, Re1A (p65), Re1B, and c-Re1 are NF-κB family transcription factors (Tasneem et al., 2019). *V. trifolia* leaf aqueous extract can inhibit the production of inflammatory mediators through a diminution of NF-κB translocation into the nucleus. This was observed with a decrease in the level of the p65 subunit of NF-κB by 57% in the nucleus after treatment with the leaf aqueous extract (250 or 5000 µg/mL) (Matsui et al., 2012). Casticin, a well-known flavonoid of genus *Vitex* and *V. trifolia,* has also been shown to act via blockade of NF-κB (Chan et al., 2018). In a study, vascular inflammation in the human umbilical vein was inhibited by casticin through blockade of the NF-κB transcription factor (Lee et al., 2012). Other active compounds of *V. trifolia* fruit ethanol extract, namely viterolutin C, vitexilacton D, vitexilactone, rotundifuran, vitetrifolin B, viterotulin B, and vitetrifolin D, were reported to block TNF-α-induced NF-κB activation in HEK 293 cell line. Among them, vitetrifolin B (68.86%) possessed the strongest inhibitory effect, followed by vitetrifolin D (66.46%), and viterotulin B (66.06%) at a concentration of 50 µmol (Fang et al., 2018). PPY, which is another active compound of *V. trifolia* fruit methanol extract also exhibited its anti-inflammatory effect by blocking the activation of NF-κB at a concentration of 10 µg/mL in a murine model of asthma (Lee et al., 2017).

3. MODULATION OF MAPK SIGNALING PATHWAY

MAPK is a group of serine/threonine-specific protein kinases that are involved in various cellular events, including pro- and anti-inflammatory responses (Chen et al., 2018a). The mammalian MAPKs family includes three major kinases: extracellular signal-regulated kinases (ERKs), c-jun N-terminal kinases (JNK), and p38 kinases (Thalhamer et al., 2008). Different stimuli, such as PAMPs and DAMPs, can trigger MAPKs pathways and activate a cascade of kinases. The activated ERK, JNK, and p38 further initiate the expression of specific inflammatory genes and induce inflammatory responses (Planas et al., 2006). ERK is involved in IL-6, and TNF-α synthesis, while p38 regulates IL-1 stimulated production of IL-6 and induction of COX-2 (Thalhamer et al., 2008). *V. trifolia* active compounds' inhibitory effect on the MAPKs pathway has been reported by several studies. Casticin (0.3-10 µmol) has been shown to have an inhibitory effect on p38 and ERK1/2 in LPS-induced murine macrophage by enhancement of their inhibitors. In contrast, casticin can enhance the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) that binds to the heme oxygenase-1 gene's promoter leading to anti-inflammatory and antioxidative responses (Liou et al., 2014). PPY isolated from *V. trifolia* fruit methanol extract can also block ERK1/2 phosphorylation at a concentration of 10 µg/mL (Lee et al., 2017).

4. MODULATION OF INFLAMMATORY CYTOKINE PRODUCTION AND ACTIVITY

Cytokines are soluble polypeptides and are released to the cell environment and blood circulation to induce cell responses against specific stimuli. At the physiological state, cytokine expression is low, but pathological conditions can highly upregulate their production. Cytokines mainly act on membrane receptors to activate

intracellular signaling pathways, resulting in changes in gene transcription (Planas et al., 2006; Roshene and Ramesh, 2017). The main functions of cytokines are to regulate and determine the nature of the immune response. Cytokines are produced primarily by antigen-presenting cells, including mononuclear phagocytic cells. These cytokines promote cellular infiltration and inflammation. An antigen is taken up by antigen-presenting cells and presented to the T-helper lymphocytes to produce cytokines through a specific intracellular pathway. Alternatively, monocytes can be triggered directly by pathogen components to produce cytokines through innate immune systems (Borish and Steinke, 2003; Ferreira et al., 2018). Immune cells' pro- and anti-inflammatory responses and their vital activities are mainly regulated by cytokines. Moreover, cytokines play a regulatory role in the production and activity of other pro-inflammatory and anti-inflammatory mediators (de Oliveira et al., 2011). Cytokines can be grouped into ILs, TNFs, chemokines, INF, and tumor growth factors (TGF). Among them, IL-1, IL-2, IL-6, IL-7, and TNF-α are pro-inflammatory cytokines, while TGF-β, IL-4, IL-10, and IL-13 act as anti-inflammatory mediators (Dinarello, 2007; Tasneem et al., 2019). TNF-α can induce the production of COX-2, IL-1, IL-6, chemokines, and adhesion of molecules (Holbrook et al., 2019; Liu, 2005; Tasneem et al., 2019). Interleukins are major contributors to the inflammatory responses. IL-1 and IL-6 are two main acute inflammatory response mediators that are induced by several stimuli. IL-1 is primarily produced by macrophages and monocytes, and some other cells including fibroblasts and endothelial cells (de Oliveira et al., 2011). IL-1α and IL-1β are two main isoforms of IL-1 (Feghali and Wright, 1997; Tasneem et al., 2019). The primary role of IL-1 is to activate T lymphocytes through activation of IL-2, augment B cell proliferation, and enhance immunoglobulin production (Borish and Steinke, 2003; Hegazi and Abdel-rahman, 2015). The cytokines can also stimulate fever and pain (Dinarello, 2007; Ferreira et al., 2018). IL-1β stimulates the production of COX-2 and PGE2 and induces systemic inflammation. It also influences the production of substrate-p, nitric oxide, and endothelial adhesion molecules (de Oliveira et al., 2011; Feghali and Wright, 1997; Zhang and An, 2009). IL-6 is primarily produced by monocytes, macrophages, and T cells. It is a major cytokine of acute inflammation (Tasneem et al., 2019) responsible for inducing the production of acute-phase proteins. IL-6 initiates T cell activation, growth, and differentiation (Hegazi and Abdel-rahman, 2015). IL-6 also promotes the activation and maturation of neutrophils and macrophages (de Oliveira et al., 2011)

The production of pro-inflammatory mediators such as TNF-α, IL-1β, and IL-6 can be inhibited by *V. trifolia* leaf alcoholic and aqueous extracts in a dose-dependent manner. In a study, the researchers displayed a reduction of TNF-α, IL-1β, and IL-6 mRNAs in LPS-induced murine macrophages when treated with 2500 μg/mL of *V. trifolia* leaf extract for 2 h. Conversely, the expression of IL-10 mRNA, an anti-inflammatory cytokine, was increased by 3.5 folds at 12 h post-incubation (Matsui et al., 2009). The ability of *V. trifolia* to impede the production of IL-1β, IL-6, and TNF-α may be due to the presence of casticin, PPY, and other phenolic compounds in the plant (Chan et al., 2018; Lee et al., 2017; Liou et al., 2014) reported a dose-dependent reduction of IL-1β in LPS-induced murine macrophages after incubation with casticin. The level of IL-1β decreased to 81.3±16.7 pg/mL in casticin-treated cells (10 μmol) compared to non-treated cells (182.5±21.3 pg/mL). According to (Lee et al., 2017), the activity of PPY (1, 2, 10 mg/kg of body weight) isolated from *V. trifolia* fruit methanol extract reduced the production of IL-6 and TNF-α in a dose-dependent manner in cigarette-smoke induced lung inflammation in mice. *V. trifolia's* inhibitory effect on the synthesis of other cytokines and chemokines was further corroborated in another study done much earlier by (Matsui et al., 2012) on LPS-induced macrophages. Compared to LPS alone, treatment with 5,000 μg/mL leaf aqueous extract of *V. trifolia* strongly downregulated the gene expression of IL-1α, IL-1β, and IL-18 by 5 folds. In a recent study on LPS-induced human U937 macrophage, the effects of various isolated compounds from *V. trifolia* were investigated (Wee et al., 2020). Artemetin, isolated from *V. trifolia* leaf dichloromethane extract, at 50 μg/mL significantly reduced the levels of TNF-α and IL-1β to 20% and 60%, respectively, in LPS-induced U973 human macrophages. A similar effect was displayed by casticin (2 μg/mL) on TNF-α synthesis, although the reduction of IL-1β was not as strong as seen with artemetin. Moreover, vitexilactone at 20 μg/mL significantly reduced TNF-α and IL-1β levels with IC_{50} values of 37.5±2.8 μg/mL and 80.6±9.2 μg/mL, respectively. Another compound, maslinic acid, also possessed the same effect on TNF-α expression with an IC_{50} value of 27.6±17 μg/mL. However, the latter compound upregulated the level of IL-1β by 1.7 times (Wee et al., 2020).

5. INHIBITION OF CYCLOOXYGENASE

COX is an essential enzyme of the arachidonic acid pathway, which produces PGs and thromboxanes from arachidonic acid after initiation of inflammation by any stimuli (Nworu and Akah, 2015 Wang et al., 2019b). COX-2 is mainly responsible for producing inflammatory PGs, including PGE2 and PGI2, upon induction by inflammatory stimuli. In contrast, COX-1, also known as the housekeeping enzyme, is involved in the production of PGs at a normal physiological state. PGE2 and PGI2 are both involved in all classic symptoms of inflammation. Both primarily cause redness and oedema by increasing arterial dilation and microvascular permeability alongside other inflammatory vasodilators (Ricciotti and Fitzgerald, 2011). It is believed that PGE2 and PGI2 also induce pain in the inflamed site by sensitizing the free ends of peripheral nervous system neurons (Khanapure et al., 2007). Many anti-inflammatory herbal extracts have been found to act on COX-2, which is closely associated with inflammatory disorders of acute and chronic states (Nworu and Akah, 2015). Studies showed that *V. trifolia* can reduce the level of COX-2 (Liou et al., 2018). This could be linked to the downregulation of COX-2 by castisin via the enhancement of ERK and p38 inhibitors. They also reported that casticin suppressed the level of PGE2 with an IC_{50} of 5.18±1.52 μmol, which is likely due to its inhibitory effect on COX-2 (Liou et al., 2014). In a similar study, casticin was found to significantly inhibit the production of PGE2 and the expression of COX-2 in a dose-dependent manner (6.25-25 μmol) in IL-1β-induced human articular chondrocytes (Mu et al., 2019). Another study corroborated these findings,

with inhibition of LPS-induced COX-2 overexpression upon treatment with 2,500 µg/mL *V. trifolia* leaf aqueous extract in murine macrophages (Matsui et al., 2012). The plant's inhibitory effect on COX-2 could be seen through the blockade of TNF-α, IL-1β, NF-κB, and MAPKs pathways.

6. INHIBITION OF NITRIC OXIDE SYNTHASE (NOS)

Nitric oxide (NO) is a free radical that participates in physiological functions, including the immune response, vasodilation, muscle relaxation, and neurotransmission (Nworu and Akah, 2015). NO is produced by three major isoforms of NOS—neural NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS)—through oxidization of L-arginine to L-citrulline. The nNOS and eNOS are physiologically produced and participate in normal physiological processes. In contrast, iNOS is only expressed upon cell stimulation by pro-inflammatory cytokines and bacterial lipopolysaccharides (Cinelli et al., 2020). During the inflammation process, iNOS produces a significant amount of NO, which helps to defend against pathogens and acts synergistically with other inflammatory mediators (Kapoor et al., 2011; Nworu and Akah, 2015; Salvemini et al., 2003). *V. trifolia* leaf aqueous extract has been reported to have an inhibitory effect on iNOS when tested on LPS-induced murine macrophage (Matsui et al., 2009). They reported that the extract inhibited the production of iNOS mRNA significantly in a dose-dependent manner with a maximum inhibition at 2500 µg/mL after 2 h of incubation. Moreover, *V. trifolia* fruit methanol extract can potentially inhibit the production of NO. Isolated compounds such as 10-*O*-vanilloylaucubin, agnusoside, and 3-normal-butyl-nishindaside were found to have a moderate inhibitory effect on the LPS-induced production of NO in macrophages with IC_{50} values of 88.51±1.01, 87.26±1.15, and 76.06±1.28 µmol, respectively (Bao et al., 2018). Castisin is also responsible for the inhibition of iNOS, and it is suggested that the inhibitory action of *V. trifolia* on iNOS is due to the activation of ERK and p38 inhibitors (Liou et al., 2014). Additionally, casticin and vitertrifolin F, isolated from *V. trifolia* fruits, reduced the LPS-induced production of NO in murine macrophages with an IC_{50} of 21.4 µmol and 16.4 µmol, respectively (Lee et al., 2013). In another study on LPS-induced murine macrophage, casticin suppressed the level of NO with an IC_{50} of 4.23±1.13 µmol (Liou et al., 2014).

7. INHIBITION OF REACTIVE OXYGEN SPECIES (ROS)

ROS are reactive and partially reduce oxygen metabolites with strong oxidization capabilities (Mittal et al., 2014). These molecules include superoxide anion, hydroxyl, hydrogen peroxide, nitric oxide, and hypochlorous acid (Chelombitko, 2018). At physiological concentration, ROS can impose their role as signaling molecules that play a regulatory role in some vital cellular responses, including cell adhesion, differentiation, survival, and apoptosis. However, in high concentrations, the molecules can cause cell damage by lipid oxidation and DNA damage (Adwas et al., 2019; Pizzino et al., 2017). These reactive molecules can trigger inflammation-related pathways and participate in the initiation, progression, and regulation of the inflammatory responses (Chen et al., 2018b; Forrester et al., 2018). Overproduction of ROS may cause oxidative stress and promote chronic inflammation and inflammation related to chronic diseases (Adwas et al., 2019; Hussain et al., 2016). Antioxidants play an important role in inhibiting ROS thus can prevent oxidative stress and inflammation-related diseases (Hussain et al., 2016). Antioxidants can interact with the excessive production of ROS and prevent any damage to the proteins, lipids, and DNA/RNA before cells are seriously affected. Any modifications of these macromolecules may increase the chances of mutagenesis. Many studies have shown that polyphenols, which are natural compounds in plants, act as antioxidants. Many pharmacological attributes of plants are related to these phytochemicals functioning as antioxidants (Kasote et al., 2015).

Studies showed that phenolic compounds present in *V. trifolia* leaf hydro-alcoholic extract can act as electron donors and inhibit the hydroxyl free radical production from H_2O_2 with IC_{50} of 3.22 µg/mL, compared to ascorbic acid with IC_{50} of 3.14 µg/mL and probably inhibit DNA damage. In contrast, terpenes in *V. trifolia* extract can inhibit the absorbance and production of NO. In the same study, the H_2O_2 scavenging activity of the extract has also been reported with an IC_{50} of 4.08 µg/mL (Ankalikar and Viswanthswamy, 2017b). In another study, the antioxidant activity of leaf methanol extract was evaluated by its ability to reduce free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), and the extract (100 µL) showed a strong antioxidant activity with a maximum antiradical potential of 91.02% (Dehsheikh et al., 2019). The plant leaf ethanol extract also exhibited chelating agent property, and the strongest chelating activity of the extract was 89.97% with an IC_{50} value of 40.0 µg/mL compared to ethylenediaminetetraacetic acid (EDTA) (98.78%), which had an IC_{50} value of 6.03 µg/mL (Saklani et al., 2017). In a similar study, agnuside, negundoside, and 6'-p-hydroxy benzoyl mussaenosidic acid, isolated from the methanol extract of *V. trifolia*'s aerial parts, showed significant DPPH scavenging activity with IC_{50} values of 9.81, 9.96 and 10.31 µg/mL, respectively (Tiwari et al., 2012). The 2(3,4-dihydroxyphenyl)ethyl-2-O-[6-deoxy-α-L-mannopyranosyl-4′-(3,4-dihydroxyphenyl, vitexcin, and isovitexcin, isolated from *V. trifolia* aerial part methanol extract, were reported to inhibit DPPH activity with IC_{50} values of 4.70±0.12, 15.36±0.07, and 30.48±0.47 µg/mL, respectively, compared to ascorbic acid with an IC_{50} value of 7.90±0.20 µg/mL (El-Kousy et al., 2012).

8. INHIBITION OF HISTAMINE PRODUCTION

Histamine is a potent inflammatory mediator involved in inflammatory conditions such as allergy, asthma, autoimmune diseases, and chronic pruritus (Branco et al., 2018; Stojković et al., 2015). Histamine exhibits its effect through histamine receptors (H1-H4). The H1-receptors are responsible for vasodilatation, bronchoconstriction, cellular migration, and nociception (Benly, 2015; Shahid et al., 2009), whereas the H2-receptors are mainly responsible for regulating gastric acid secretion, airway mucus production, smooth muscle relaxation, and vascular permeability. The H3-receptors act as an

autoreceptor that regulates the release of central nervous system neurotransmitters including histamine. The H4-receptors are directly involved in allergy and inflammation by regulating the inflammatory cascade and production of pro-inflammatory mediators. These mediators may further initiate immune cell recruitment and migration (Stojković et al., 2015; Thangam et al., 2018). *V. trifolia* can potentially exhibit antihistaminic effects since its decoction is used to treat skin allergy (Sujarwo et al., 2015). In the work of Ikawati et al. (2001), the antihistaminic effect of ethanol extract of *V. trifolia* leaves was observed. The extract (0.5 mg/mL) inhibited the release of histamine from RBL-2H3 cells by 80.13±3.95%. The antihistaminic effect of *V. trifolia* is potentially exhibited by its ability to stabilize mast cells and antagonize histamine receptors (Suchitra and Cheriyan, 2018). Vitetrifoline E (13 µmol), isolated from *V. trifolia* leaves, was reported to exhibit tracheospasmolytic effects and reduced histamine-induced contraction (Rani and Sharma, 2013). Meanwhile, viteosin-A (0.4 mmol) and vitexicarpin (0.4 mmol), isolated from *V. trifolia* leaf n-hexane extract, have been reported to inhibit the histamine-induced contraction of the isolated trachea of male guinea pigs by 92.2% and 42.9%, respectively (Alam et al., 2002).

Various extracts of *V. trifolia* plant parts have been shown to exhibit a remarkable anti-inflammatory effect on different inflammation-related signaling pathways, namely NF-κB and MAPKs signaling pathways, which are linked to the reduction of cytokine production and inhibition of COX, NOS and ROS (Table 3). Figure 2 illustrates the pharmacological modulation of *V. trifolia* against inflammation.

Table 3. Anti-inflammatory mechanism of action of *V. trifolia*

Mode of action	Plant organ	References
NF-κB signaling pathway modulation	Leaf aqueous extract Fruit alcoholic extracts	Chan et al., 2018; Lee et al., 2012; Lee et al., 2017; Matsui et al., 2012
MAPKs signaling pathway modulation	Fruit alcoholic extract	Lee et al., 2017; Liou et al., 2014
Cytokines production and activity modulation	Leaf aqueous extract Fruit alcoholic extract Leaf dichloromethane extract	Chan et al., 2018; Lee et al., 2017; Matsui et al., 2009; Matsui et al., 2012; Wee et al., 2020
COX inhibition	Leaf aqueous extract	Liou et al., 2014; Matsui et al., 2012
NOS inhibition	Leaf aqueous extract Fruit methanol extract	Bao et al., 2018; Lee et al., 2013; Matsui et al., 2009
ROS inhibition	Leaf hydro-alcoholic extract Leaf methanol extract Leaf ethanol extract Aerial part methanol extract	Ankalikar and Viswanthswamy, 2017b; Dehsheikh et al., 2019; El-Kousy et al., 2012; Saklani et al., 2017; Tiwari et al., 2012
Histamine inhibition	Leaf ethanol extract Leaf n-hexane extract	Alam et al., 2002; Ikawati et al., 2001

9. CONCLUSION

V. trifolia is traditionally used to treat inflammation, pain, and fever. The anti-inflammatory effect of this plant has been reported in several studies. *V. trifolia* can potentially act on six different targets of the inflammation process to exhibit its anti-inflammatory effect: (1) modulation of pro-inflammatory pathways, (2) modulation of pro-inflammatory cytokine production and activity, (3) inhibition of COX-2, (4) inhibition of iNOS, (5) scavenging and inhibition of ROS, and (6) inhibition of histamine. The known chemical constituents involved in its anti-inflammatory activity are polyphenolic compounds, diterpenes, and triterpenes, such as casticin, artemetin, vitexin, isovitexin, and PPY. However, there is a lack of data on the effect of the plant on systemic inflammation and specific immune cells. Further extensive studies are required to describe more specific targets and an in-depth description of the molecular mechanism in association with the isolated active compounds involved in the anti-inflammatory effect of *V. trifolia*.

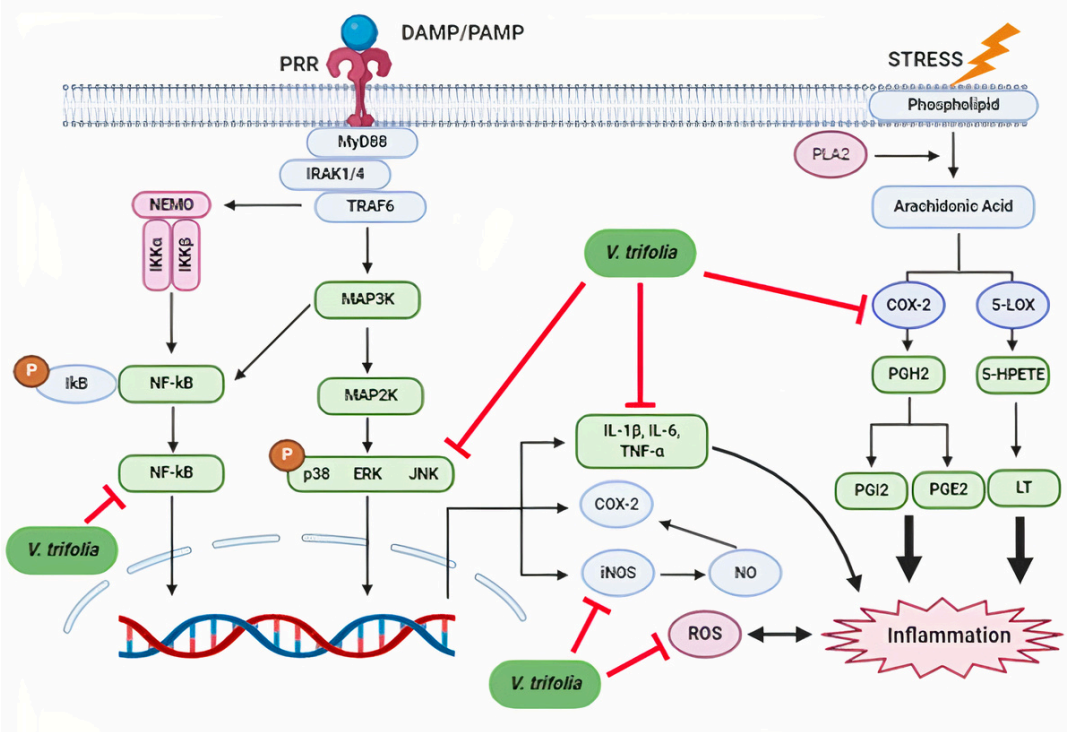

Figure 2. A schematic representation of the molecular anti-inflammatory effect of *V. trifolia* on different inflammation-related signaling pathways

Note: 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; 5-LOX, 5-lipoxygenase; COX-2, cyclooxygenase; DAMP, danger-associated molecular pattern; ERK, extracellular signal-regulated kinase; IκB, inhibitor of kappa-B; IKKα, inhibitor of kappa-B kinase-α; IKKβ, inhibitor of kappa-B kinase-β; IL-1β, interleukin-1β; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; IRAK, IL-1 RI-associated protein kinase; JNK, c-Jun N-terminal kinase; LT, leukotriene; MAP2K, mitogen-activated protein kinase kinase; MAP3K, mitogen-activated protein kinase kinase kinase; MyD88, myeloid differentiation primary-response gene 88; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-kappa B; NO, nitric oxide; PAMP, pathogen-associated molecular pattern; PGE2, prostaglandin E2; PGH2, prostaglandin H2; PGI2, prostaglandin I2; PLA2, phospholipase A2; PRR, pathogen recognition receptor; ROS, reactive oxygen species; TNF. Tumor necrosis factor; TRAF, TNF receptor-associated factor

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