Review Article

Pharmacological Review of *Vitex trifolia*

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Abstract

Vitex triflolia is one of the *Vitex* species where known as *Lemuni*. It is widely used traditionally to reduce pain, fever and minor ailments. Every part of the plant had its advantages; for example, the leaves part can relieve pain, treat fever and can help to improve memory. It has potent pharmacological activities such as antioxidant, antinociceptive, anti-inflammatory and anticancer. Other than that, every part of the plant has unique constituents with different biological activities. A few isolated and identified flavonoids in *V. trifolia* include casticin, persinogenin, artemetin, luteolin, penduletin, vitexicarpin and chrysisplenol. These flavonoids can inhibit the cell cycle involved in carcinogenesis and have been reported to have anti-inflammatory properties. In putting together this review, PRISMA was adopted, which utilised two primary journal databases, Scopus and PubMed and a supporting database, Google Scholar. The search resulted in 17 journals for this review that covered the phytochemical constituents and pharmacological activities of *V. trifolia*. To conclude, this review provided insightful information on how the knowledge gap on *V. trifolia* can be exploited as possible treatments for COVID-19 as the extracts have shown potential anticancer, anti-inflammatory, antioxidant and other pharmacological properties due to the presence of the phytochemical constituents in the plant.

Keywords: *Vitex trifolia*, antioxidant, anti-inflammatory, anticancer, flavonoid

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1.0 Introduction

Currently, most of the global population is practising medicinal plants due to their folkloric claims. One of the species widely consumed is the *Vitex* species. There are almost 270 species of *Vitex* that have been reported in the traditional system of disease management and one of them is *Vitex trifolia*. *V. trifolia* is a plant locally known as *Lemuni* and its origin is unknown with several varieties have been identified in Malaysia, Indonesia, India and Mexico (1). A few studies have been done on this plant that showed medicinal properties that can alleviate pain especially in rheumatism and sprained joints when applied topically (1). It is traditionally used by the tribes and native medical practitioners to treat various ailments, including liver disorders, tumours, rheumatic pains, inflammation, sprains, and fever and is used in the treatment of tuberculosis (2). In India, the plant parts like leaves and flowers have significant therapeutic potential (3). The leaves are used to improve memory, relieving pain, removing bad taste in the mouth, cure fever, and treat hair loss. The aerial parts of this plant are useful in the treatment of diabetes. The aerial parts have friedelin, β-sitosterol, β-D-glucoside, long-chain hydrocarbon (4). Besides, the plant also possesses larvicidal, wound healing, anti-HIV, anticancer, trypanocidal, antimicrobial and antipyretic activities (2). The parts of fruits also can be used to treat headaches, colds, migraines, eye pain and many more (5). *V. trifolia* contains many phytochemicals with potent biological activities such as flavonoids, saponins, alkaloids and other active compounds (6). Different parts of plants have different constituents and give different biological activities for example fruits of *V. trifolia* consists of essential oil, diterpenes (7), alkaloid vitricine (4). The leaves, seeds and roots of *V. trifolia* were also found to have anti-cancer and anti-inflammatory properties due to the presence of flavonoids such as casticin, vitexin and luteolin (8, 9).

The anti-inflammatory activity of an aqueous extract of *V. trifolia* leaves has been assessed by measuring its effects on cytokine control, inflammation mediators, and the expression profiles of inducible nitric oxide synthase producing free radical nitric oxide (9)**.** The leaves of *V. trifolia* is also used to cure Ciguatera fish poisoning that is related to pruritus and it is also used as an antipyretic and anti-inflammatory agent. Other than that, leaves of *V. trifolia* acts as a nematicidal agent and cause an increase in body weight. Petroleum ether and ethanol extracts of *V. trifolia*'s leaves exhibited inhibition of both Gram-positive and Gram-negative bacteria, while hexane and dichloromethane extracts from stems and foliage demonstrated cytotoxic effects against several cancer cells. The hexane extract also inhibited the growth of fungal, *Fusarium sp*. The essential oils of *V. trifolia* showed insecticidal activity. Acetone extract of fruits of *V. trifolia* contained one abietane-type diterpene, which is vitetrifolin A, and two labdanetype diterpenes which are vitetrifolin B and vitetrifolin C. There were other diterpenes isolated from the leaves which are rotun-difuran, dihydrosolidagenone and abietatriene 3β-ol (9). Viteosin-A and vitexicarpin isolated from the leaves of *V. trifolia* had tracheospasmolytic activity (10).

This plant has been used traditionally for the treatment of various diseases based on the knowledge and experience of the community but failed to draw attention for further investigation. Some of the research done is unsatisfactory and needs extensive exploration of the scientific investigation to elucidate the exact mechanism of action. Hence, there should be a more concise and in-depth review of the current knowledge of the pharmacological activities of *V. trifolia* for a more focused

study of the plant of interest. Furthermore, *V. trifolia* has been reported to have significant anti-inflammatory and tracheospasmolytic properties (9,10). Different extracts from medicinal plants and purified molecules are suggested to exert their anti-SARS-CoV-2 actions by direct inhibition of the virus replication or entry that may inhibit the ACE-2 receptor or the serine protease TMPRRS2 required by SARS-CoV-2 to infect human cells. Hence, these plants including *V. trifolia* were shown to inhibit the SARS-CoV-2 life-cycle related proteins such as papain-like or chymotrypsin-like proteases (11).

Therefore, this work was carried out to concisely review recent progress in the pharmacological activities of *V. trifolia* and its phytoconstituents to facilitate the future direction of the study on this plant, especially in the current COVID-19 pandemic.

2.0 Method

This method explains five main sub-sections; PRISMA, resources, inclusion and exclusion criteria, systematic review process and data abstraction and analysis employed in this current study.

2.1 PRISMA

PRISMA or Preferred Reporting Items for Systematic Reviews and Meta-Analysis is a published standard to conduct a systematic literature review. Generally, publication standards are required to guide authors with the related and necessary information that will enable them to evaluate and examine the quality and rigour of a review. Besides, PRISMA emphasises the report of the review that evaluates randomised trials which can also be utilised as the fundamental in reporting systematic reviews for other types of research. PRISMA clearly defines the research questions towards the need for systematic review even though PRISMA is often utilised within medical studies. At the same time, it can identify the inclusion and exclusion criteria for a particular journal.

2.2 Resources

The review methods of the present study were conducted using two primary databases, Scopus and PubMed. Specifically, Scopus indexes 1063 journals related to medicine, while PubMed indexes about 8660 journals related to complementary medicine. Therefore, the present study conducted a manual searching effort using Google Scholar. Google Scholar has published around 4 million journals related to medicine.

2.3 *The systematic review process for selecting the articles*

2.3.1 Identification

The systematic review process in selecting some relevant articles for the present study consisted of three main stages. The first stage is identifying keywords, followed by the process of searching for related and similar terms based on the dictionaries, encyclopaedia and past research. The keyword was found using the search string on Scopus and PubMed (Table 1) where the current research work successfully retrieved a total of 15977 articles from both databases. As previously stated, manual searching based on similar keywords was conducted on Google Scholar resulting in an additional number of 45 articles where the total article retrieved in the first stage of the systematic review was 16902 articles.

2.3.2 Screening

At this stage, 16902 articles were screened based on the determining inclusion and exclusion criteria. The first criterion was the literature type, where the focus is only on the journal (research article) since the journal acts as the primary source that offers empirical data. Hence, this further implies that publication in the form of systematic review, review, meta-analysis, meta-synthesis, book series, book, chapter in the book and conference proceedings were excluded in the current research. Also, the review only focused on articles that were published in English between 2015 and 2020. The review included articles related to medicine, biological science, as well as biochemistry and 907 articles were included based on these criteria.

2.3.3 Eligibility

For the third stage, known as eligibility, the titles, abstracts and the main contents of all articles were examined thoroughly to ensure that they fulfilled the inclusion criteria and were fit to be employed in the present study to achieve the objectives of the current research (Table 2). Consequently, 890 articles were excluded due to their irrelevance (i.e. research were not based on the *Vitex* species. Finally, the total remaining articles that met the inclusion criteria were 17 journals. The overall process of the literature review is summarised in Figure 1.

Table 1: The search string

Database	Search string
PubMed	('*V. trifolia'* OR 'Medicinal plants' OR 'antioxidant' OR 'anti-inflammatory' OR 'anticancer' OR 'flavonoids') AND ('*V. trifolia'* AND 'Medicinal plants' AND 'antioxidant' AND 'anti-inflammatory' AND 'anticancer' AND 'flavonoids')
Scopus	('*V .trifolia'* OR 'Medicinal plants' OR 'antioxidant' OR 'anti-inflammatory' OR 'anticancer' OR 'flavonoids') AND ('*V. trifolia'* AND 'Medicinal plants' AND 'antioxidant' AND 'anti-inflammatory' AND 'anticancer' AND 'flavonoids')

Table 2 : The inclusion and exclusion criteria

Criterion	Eligibility	Exclusion
Literature type	Journal (research article)	Journal (review), book series, book, chapter in a book, conference proceeding
Language	English	Non-English
Timeline	2015 - 2020	<2015
Subject area	Medicine, Biological Science and Biochemistry	Other than Medicine, Biological Science and Biochemistry.

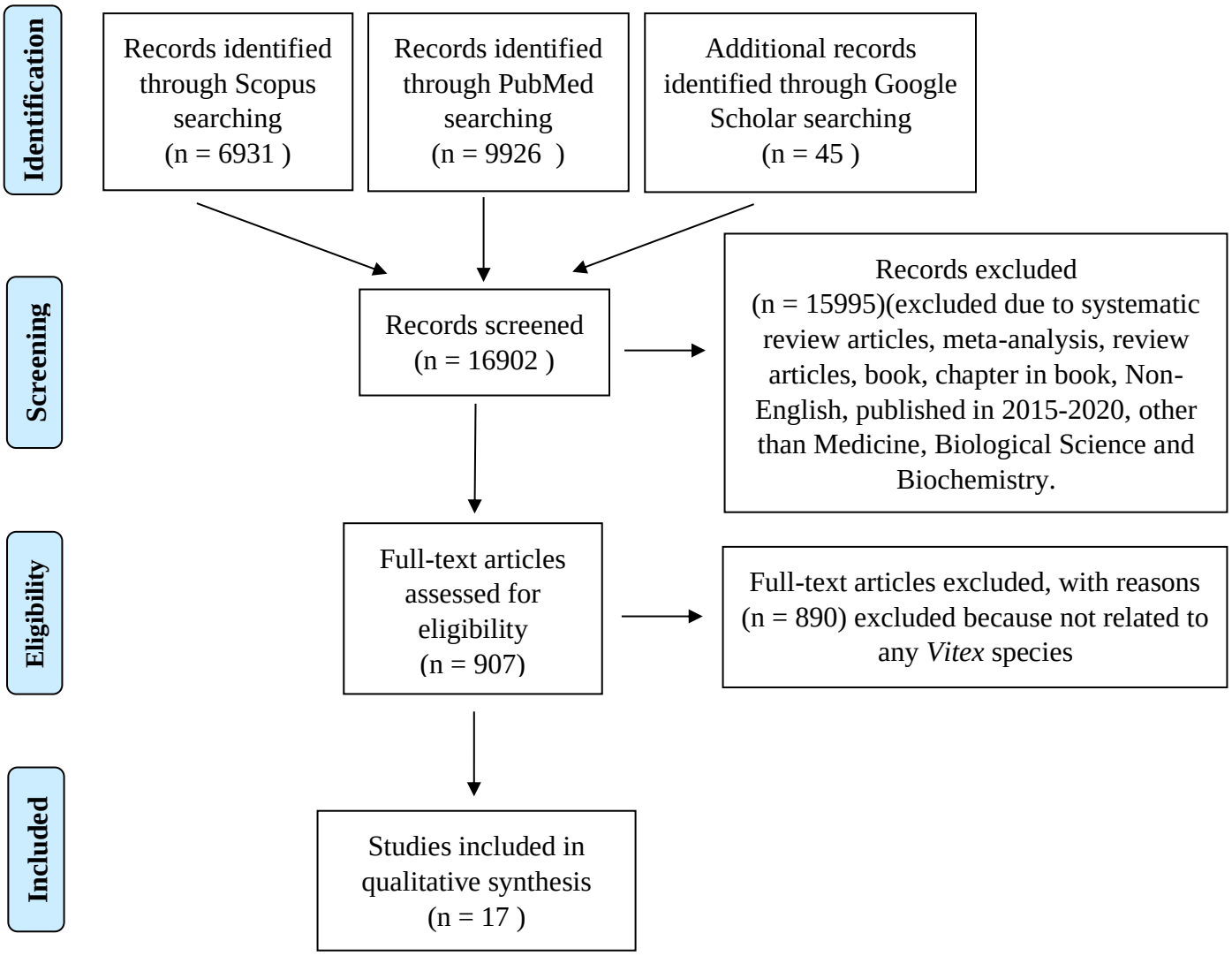

Figure 1: Study flow diagram: Literature review process of pharmacological activities of *Vitex trifolia*

3.0 Results

3. 1 Chemical constituent

World Health Organization (WHO) recognises medicinal plants as one of the sources for alternative treatments. Medicinal plants are referred to as when one or more of their plant parts contain substances - for therapeutic purposes or precursors for the synthesis of useful drugs. Chemical constituents in the plant will give different pharmacological actions to humans. Hence, screening of the plant constituents using the chromatographic technique is employed. Materials and methods used to conduct the study are plant material such as leaves of *V. trifolia,* chemical reagents, defatting of plant material, extraction by maceration process, qualitative phytochemical analysis of plant extract including the determination of the total phenol and total flavonoids content. The crude extracts obtained after successive maceration extraction processes were concentrated in a water bath by evaporation of the solvents completely to obtain the actual yield of extraction. In that study, the yields obtained from the extraction of the leaves using chloroform, ethyl acetate, ethanol and water as solvents were 3.2%, 4.9%, 8.9% and 6.5%, respectively (8). The results of qualitative phytochemical analysis of the crude powder of leaf of *V. trifolia* were shown in table 3. From the result, ethanolic and aqueous extracts of *V. trifolia* showed the presence of alkaloids, glycosides, flavonoids, saponins, phenols, proteins and amino acids and carbohydrates (Table 4).

Table 3: Result of phytochemical screening of extracts of *V. trifolia* (8).

Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
Alkaloids	-ve	-ve	+ve	+ve
Glycosides	-ve	-ve	+ve	+ve
Flavonoids	-ve	+ve	+ve	+ve
Saponins	-ve	+ve	+ve	+ve
Phenolics	-ve	+ve	+ve	+ve
Protein and Amino acid	-ve	+ve	+ve	+ve
Carbohydrate	-ve	-ve	+ve	+ve
Diterpenes	-ve	+ve	-ve	+ve

Table 4: Estimation of total phenolic and flavonoids content of *V. trifolia* (8).

Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
Chloroform	-	-
Ethyl acetate	0.178	0.098
Ethanol	0.214	0.125
Aqueous	0.174	0.085

Hai et al. (2020) had conducted a study on the effects of *V. trifolia* L. leaf extracts and phytoconstituents on cytokine production in human U937 macrophages. This study focused on comparing the effects of different extraction methods and solvents of *V.trifolia* leaves on cytotoxicity and cytokine production in human U937 macrophages. It was found that ultrasonic dichloromethane *V.trifolia* leaf extract is the most cytotoxic, while Soxhlet water leaf extract is the least cytotoxic against U937 macrophages (12). Further investigation on the most active leaf extract such as macerated ethanol and ultrasonic dichloromethane leaf extracts had led to the isolation and identification of artemetin, casticin, vitexilactone and maslinic acid (12). Twelve compounds were identified in the *V. trifolia* leaf extracts. Both artemetin and casticin are reported to have potent lipoxygenase inhibition, with casticin is more potent than artemetin by two-fold (12). Artemetin was shown to have anti-inflammatory activity using various experimental models in rats including inhibiting carrageenan-induced paw oedema, reducing granuloma formation and reducing vascular permeability to intracutaneous histamine. Artermetin can protect endothelial function by acting as an antioxidant and an anti-apoptotic agent. This compound managed to decrease the growth of human leukemia HL-60 cells in a dose-dependent manner. Casticin can alleviate airway inflammation by suppressing pro-inflammatory cytokine production such as TNF-α in the lungs and bronchoalveolar lavage fluid in an inflammatory murine model of asthma. It inhibited TNF-α and IL-1β cytokine production in LPS-stimulated mouse macrophages at the range of 3 μM to 10 μM of casticin.

Vitexilactone, a labdane-type diterpenoid was reported to induce adipogenesis in 3T3-L1 preadipocytes. Vitexilactone D managed to inhibit TNF-α induced NF-κB activation. β-sitosterol, campesterol, stigmasterol and phytol were also identified and have shown anti-inflammatory properties amongst other biological activities, such as antioxidant and anti-angiogenic. Maslinic acid is reported to reduce neuroinflammation in cultured rat cortical astrocytes by inhibiting nitric oxide and TNF-α mRNA and protein levels through NF-κB signalling pathway. The phenolic compound, 2,4-di-tert-butylphenol that was also identified in the leaf extract of *V. trifolia*, is typically used as an intermediate in preparing UV stabilizers and antioxidants, and in the manufacture of pharmaceuticals and fragrances. It possesses antifungal, anti-tumour activities and anti-inflammatory activities. Another phenolic compound is BHT where it is a synthetic antioxidant and widely used in the food industry, petroleum products and rubber. BHT was able to increase TNF-α and IL-1β cytokine production in human U937 macrophages.

Ikawati, Hertiani and Izzati (2019) conducted a study on the combination of

five medicinal plants; *Curcuma xanthorrhiza* rhizome, *Vitex trifolia* leaves and *Zingiber officinale* rhizome, *Citrus* fruit, and *Echinacea purpurea* herb for the treatment of respiratory disorders. *V. trifolia* leaves and C. *xanthorrhiza* rhizome extracts exerted tracheospasmolytic effects and anti-allergy activity (13). The addition of the other three medicinal plants is based on the effect of the individual plants as an antitussive, expectorant and immune stimulant, which can produce synergistic beneficial effects in relieving the symptoms of respiratory disorders when used together. *Citrus* fruits that are rich in flavonoids have pharmacological activities such as antioxidant, anti-allergic, anti-inflammatory and anticancer properties. They also contain flavanone glycosides such as hesperidin, naringin and naringenin, where hesperidin has anti-asthmatic activity by inhibiting the production of cytokine involved in asthma (13). *Z. officinale* exhibits pharmacological activities in the respiratory tract as antitussive and anti-inflammatory. *Echinacea purpurea is* used as an immune system booster by triggering the complement pathway and increase the number of leukocytes, stimulating phagocytosis and T-cell lymphocyte production, and increasing the activity of lymphocyte and cellular respiration (13).

The combination of the five medicinal plants showed synergistic effects as immunomodulatory on enhancing macrophage phagocytic activity and the production of antibody IgG but the combination is less effective in increasing lymphocyte proliferation where it also part of the immune response (13). This is because certain components from another medicinal plant can inhibit the pharmacological activities of another component. Studies have revealed that curcumin can inhibit cell proliferation, cell-mediated cytotoxicity (CMC), and cytokine production by inhibiting NFκB target genes involved in the induction of this immune response. Vitexicarpin, which is another identified compound in *V. trifolia* can inhibit the effect on lymphocyte proliferation. Several compounds from *Z. officinale* such as (6)-gingerol, (8)-gingerol, and (10)-gingerol also can inhibit lymphocyte proliferation with slightly different potencies. Furthermore, vitexicarpin and viteosin-A were reported to have inhibitory effects on histamine released from sensitized mast cells possibly by stabilizing the mast cells membrane function (9). This suggests that the extracts from *V. trifolia* may pose a possibility in the therapeutic against SARS-CoV-2.

Another study was conducted on antioxidant activity and phenolic compound of *V. trifolia var. simplicifolia* associated with antiproliferative activities against cancerous cell lines (14). The method used was Total Phenolic Content (TPC) and cytotoxicity assays. The TPC was assayed using the Folin-Ciocalteu method. The cytotoxicity activities of test samples were performed against MCF-7 (breast cancer cell line), HT-29 (colorectal cancer cell line) and WRL-68 (normal liver cell line). The result expressed as the dose that inhibited 50% control growth after the incubation period (IC_{50}). The values were estimated by plotting drug concentration (μg/ml) against the percentage of viable cells compared to control. The IC_{50} values of the extract against MCF-7, HT-29 and WRL-68 cell lines were 78.81, 77.50 and 78.29 μg/ml, respectively (14). These values suggested that the extract of *V. trifolia var. simplicifolia* was moderately cytotoxic against those cell lines with no selectivity towards normal cells.

Analysis of the extract shows high antioxidant activities with a phenol content of 44.66 μg of GAE/mg (44.66 mg GAE/g) of fresh weight. The high-level activities of antioxidant in *V. trifolia*

leaves give a major advantage. Antioxidant supplementation can block NF-kB activation and inhibit NF-kB activity through a mechanism distinct from redox regulation. NF-kB is a protein complex where it controls the transcription of DNA. From the cytotoxicity test, it is suggested that *V. trifolia* leaves can inhibit cancer cells.

Another study on 15 flavonoids from the *V. trifolia* showed inhibition of *Mycobacterium tuberculosis* H37Rv strain radiometrically by BACTEC 460 assay (15). The result was calculated and expressed as minimum inhibitory concentration (MIC). Luteolin, quercetin, baicalein, myricetin and hispidulin were active against *M. tuberculosis* in the MIC in the range of 25-100 µg/ml where the rest were found inactive at 100 µg/ml. Luteolin with 3', 4' di-hydroxy groups inhibited the growth of *M. tuberculosis* at MIC 25 µg/ml. However, with the addition of an OH group at position 3, which is quercetin, the activity decreased at MIC value of 50 µg/ml. Glycosylation of quercetin at position 3 (rutin) diminished the tubercular activity. The addition of an extra hydroxyl group at position 5 in quercetin (myricetin) showed no activity. Flavonoid baicalein also showed the antitubercular activity at MIC 50 µg/ml and methylation of the hydroxyl group in baicalein at position 6 (oroxylin) completely inactivated the bacteria. Oroxylin was inactive as antitubercular at 100 µg/ml, and the addition of an extra hydroxyl group at position 4 (hispidulin) induced the antitubercular activity at MIC 100 µg/ml (15).

4.0 Discussion

4.1. Anti-inflammatory

Inflammation occurs when the body produced white blood cells and other substances known as inflammation mediators to protect the body from infection (16). These mediators protect the body from harmful organisms such as bacteria and viruses. In general, inflammation is the process of the body's immune response to the irritant when the body is fighting against the virus or bacteria that attacks the body. The function of inflammation is to eliminate the initial cause of cell injury and initiate tissue repair (17). Inflammation can be an acute incident and may lead to a chronic condition if assault persists. The acute phase of inflammation is characterized by the rapid influx of blood granulocytes, typically neutrophils, followed swiftly by monocytes that mature into inflammatory macrophages that subsequently proliferate and thereby affect the functions of resident tissue macrophages (17). This process can give general signs during the inflammation; redness, heat, swelling, pain and loss of function. Fever and exhaustion are also signs of inflammation that happen when the immune system is very active and needs a lot of energy, which may lack other activities. The antibodies and cells of the immune system will be produced if the rate of the metabolism is higher due to fever. Chemical mediators released during inflammation are vasoactive amines such as histamine and serotonin, peptides such as bradykinin and eicosanoid, which are leukotrienes and prostaglandins, too (17).

A study conducted by Ankalikar & Viswanathswamy (2017) showed that leaves of *V. trifolia* could inhibit inflammation. Ethanolic leaves extract of *V. trifolia* (100 mg/kg and 200 mg/kg) has been evaluated for its activity on carrageenan-induced paw oedema (18). This study investigated carrageenan-induced inflammation which involved three distinct phases of the release of mediators including serotonin and histamine in the first phase (0-2 h), also called the exudative stages of

inflammation. In the second phase (3 h), the mediators such as kinins, leukotrienes and PMN cells are released. Prostaglandins will be released in the third phase, where oedema reaches its peak volume. Carrageenan is responsible for causing the formation of paw oedema due to the production of protein-rich exudates to contain a large number of neutrophils. This study stated that the inhibitory effect of the plant extract may be due to the presence of flavonoids such as persicogenin, artemetin, luteolin, penduletin and vitexicarpin (18).

Another study also showed that *V. trifolia* showed an anti-inflammatory effect. The alcoholic extract showed more effectiveness than aqueous extract and in a dose-dependent manner (19). This study also suggested that the anti-inflammatory effects may be due to the presence of flavonoids and tannins in the leaves of *V. trifolia.* Flavonoids inhibit both inflammatory and allergic reactions as well as offer some protection in ulcer development by increasing capillary resistance and improving microcirculation which renders the cell less injurious to precipitating factors (19). Some studies have shown that both ethanol extract and the water extract of *V. trifolia* leaves produce anti-inflammatory activity against carrageenan without significant adverse effects (20). According to Liou et al. (2018), casticin isolated from *V. trifolia* has anti-inflammatory and anti-tumour properties. Casticin reduced pro-inflammatory cytokine and ICAM-1expression in inflammatory pulmonary epithelial cells (21). This study has been conducted on female BALB/c mice with asthma. Casticin reduced airway hyper-responsiveness (AHR), goblet cell hyperplasia, and oxidative responses in the lungs of mice with asthma. This study revealed casticin attenuated the level of Th2 cytokine in bronchoalveolar lavage fluids and regulated the expression of Th2 cytokine and chemokine genes in the lung. Casticin significantly inhibited the levels of pro-inflammatory cytokine and eotaxin, and decreased THP-1 monocyte cell adherence to BEAS-2B cells via suppressed ICAM-1 expression in the inflammatory tracheal epithelial BEAS-2B cells. Hence, casticin is suggested to be a strong immunomodulator that can suppress Th2 cytokine expression in mice with had asthma (22). Also, casticin inhibited the levels of nitric oxide and $PGE_{2,}$ and decreased pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6 and tumour necrosis factor-α (TNF-α) that eventually blocks the NF-κB, Akt, and MAPK signalling pathways (22, 23). This is represented in Figure 2.

4.2. Anti-asthmatic

Asthma is a common allergic and inflammatory disease of the respiratory system. The prevalence of asthma increased worldwide due to worsening air pollution and immune system dysfunction (24). Activated Th2 cells will release excess cytokine to stimulate AHR. It will also induce eosinophil infiltration that leads to exacerbation of inflammation and allergic reaction in the lungs. Cytokine induces goblet cell hyperplasia and mucus secretion, which is causing severe respiratory obstruction. Improper activation of Th2 cells is essential for the amelioration of asthma (25). Production of cytokine not only induces inflammation but also stimulates airway epithelial cells to secrete more cytokine and chemokine. This leads to exacerbation of the inflammatory reaction and also cell damage in the airways and lungs. The pulmonary function will also reduce.

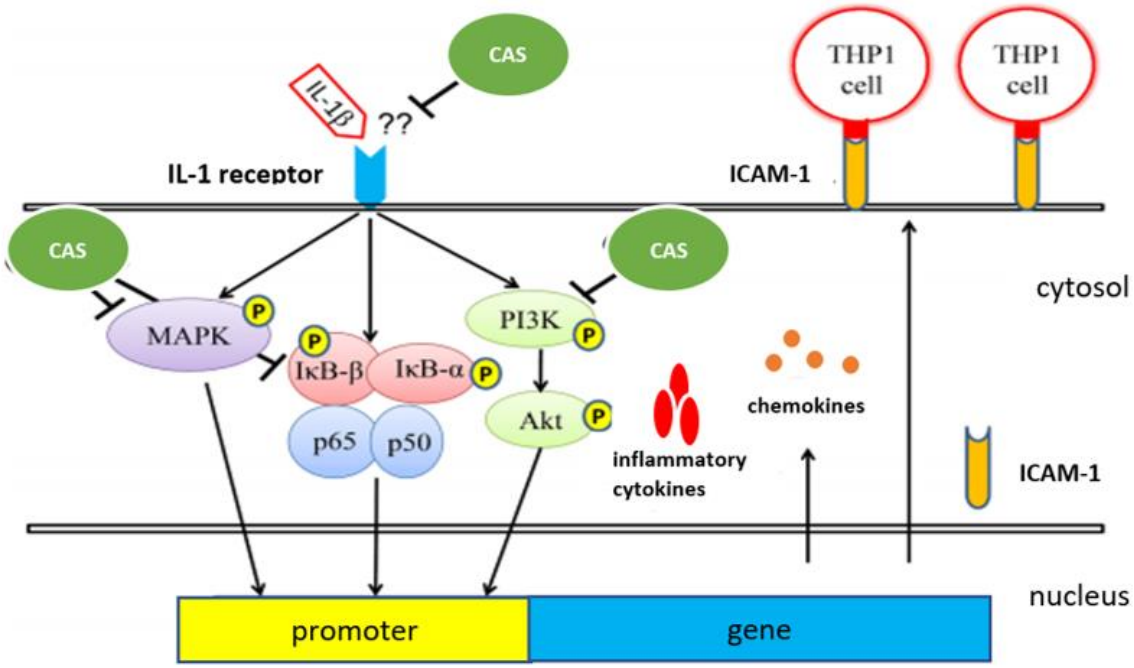

Figure 2: Schematic representation of the mechanism for the anti-inflammatory effects of casticin (CAS) in IL-1β–induced A549 cells. Adapted from Liou and Huang (2017) (23).

Next, excessive reactive oxygen species (ROS) that deteriorate AHR will be induced due to the inflamed respiratory epithelial cells. Smooth muscle thickness also increased, and narrowing the airway will happen (26). Furthermore, more eosinophils will release due to activated eosinophils, which could catalyse hydrogen peroxide and chloride ions to form hypochlorous acid causing oxidative stress and damaging the lung tissue. Suppression of Th2 cells can reduce the inflammation and oxidative damage of the airways and improve asthma symptoms.

In Indonesia, leaves of *V. trifolia* are widely used to treat asthma (27). This was proven by Lisprayatna et al. (2012) who produced syrup from the extract of *V. trifolia* leaves to evaluate the relative level change of vitexicarpin in syrup after formulation. The syrup was made from 4 formulations of variations of propylene glycol (PG) level. PG at 11% was selected for the optimum formulation because of the good flavour and appearance, less viscosity, the least precipitation and the highest relative level of vitexicarpin. Vitexicarpin is a flavonoid found in the leaves of *V. trifolia* and suitable as an anti-asthma (27).

In another study, three compounds were isolated from leaves of *V. trifolia* which were viteosin–A, vitexicarpin and vitetrifolin-E. However, only viteosin–A had anti-tracheospasmolytic activity, which is related to the suppression of the trachea contraction in asthma (28). These compounds are non-competitive antagonistic towards histamine and can stabilize mast cells membrane function. Vitexicarpin showed the strongest effect to inhibit the histamine release as compared to viteosin–A and vitetrifolin-E after testing against Rat Basophilic Leukemia cells represent mucosal mast cells (RBL-2H3) induced by A Dinitrophenylated Bovine Serum Albumin (DNA-BS) (29).

Casticin was reported to inhibit inflammation in LPS-induced acute lung injury in mice and improve the cigarette smoke-induced acute lung inflammatory response in mice (30,31). Furthermore, casticin can reduce pro-inflammatory cytokines and ICAM-1 expression by blocking the NF-kB, MAPK, and PI3K/Akt pathways in IL-1β-activated A549 human lung epithelial cells (21). Casticin can diminish AHR, airway inflammation, and oxidative stress in the lungs of a murine asthma model and alleviate inflammatory and oxidative responses in tracheal epithelial cells (21). The study used female OVA-induced asthma BALB/c mice treated with an intraperitoneal injection of casticin (5 or 10 mg/kg) and prednisolone (5 mg/kg), as positive controls.

4.3 Anticancer

Cancer is one of the major causes of death, globally, with breast cancer as one of the most common occurrences in females. Casticin is a flavonoid where it known as vitexicarpin or casticine (Figure 3). Casticin can be found in fruits, leaves and also reported in fruits, aerial parts and seeds. In general, casticin targets cancer cells by mitochondria-mediated apoptosis, reactive oxygen species (ROS)-mediated apoptosis, caspase-mediated apoptosis, tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis and apoptosis mediated by proteins (32).

Casticin induces apoptosis in many cancer cell lines via various molecular mechanisms. The mechanisms that have been reported are caspase-3 activation. G_2/M phase arrest, induction of death receptors, activation of c-Jun N-terminal kinase (JNK) and activation of apoptosis signal-regulating kinase1 (ASK 1), to name a few (33).

Flavonoids isolated from *V. trifolia* other than casticin, which are compounds artemetin, luteolin, penduletin and chrysosplenol-D, are also bioactive constituents that can inhibit cell cycle G_2/M phase and have partial apoptosis-inducing activities **(**Figure 4**)** (33). Most of the compounds are methoxylated flavonoids called anthocyanins and there has not been any report on either cell cycle inhibitory effect or the anticancer effects for compounds 1 and 4. Anthocyanin is an antioxidant flavonoid that can block NF-κB, preventing carcinogenesis (34).

4.4 Antioxidant

Medicinal properties of plants are due to the secondary metabolites such as alkaloids, phenols and tannins present in different parts of the plants. Phenols have redox properties and thus impart antioxidant properties to the plants. Phenols act as reducing agents, hydrogen donors, singlet oxygen quenches and metal chelators. Flavonoids and tannins are major groups of compounds that act as primary antioxidants or free radical scavengers. Anthocyanin is known as an antioxidant, flavonoid where it will be expressed when the chlorophyll in a plant is destroyed due to high temperature (34). The colour of anthocyanin can be influenced by pH, where it can turn into bright pink in acidic medium, reddish-purple in neutral medium and green in alkaline.

Anthocyanin can block NF-kB activation as well as inhibit NF-kB activity (35). NF-kB is involved with carcinogenesis thus, indirectly, anthocyanin can inhibit cancer (34). The human body may produce oxygen-centred free radicals and other reactive oxygen species as byproducts. Overproduction of free radicals can cause oxidative damage to biomolecules, eventually leading to many chronic diseases, including cancer (14).

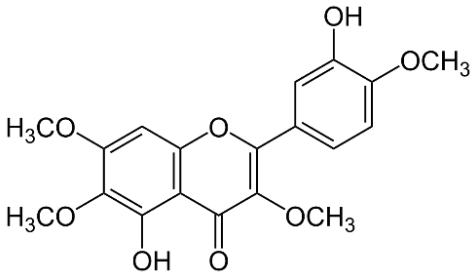

Figure 3: Molecular structure of casticin (vitexicarpin). Retrieved from http://www.chemfaces.com/natural.

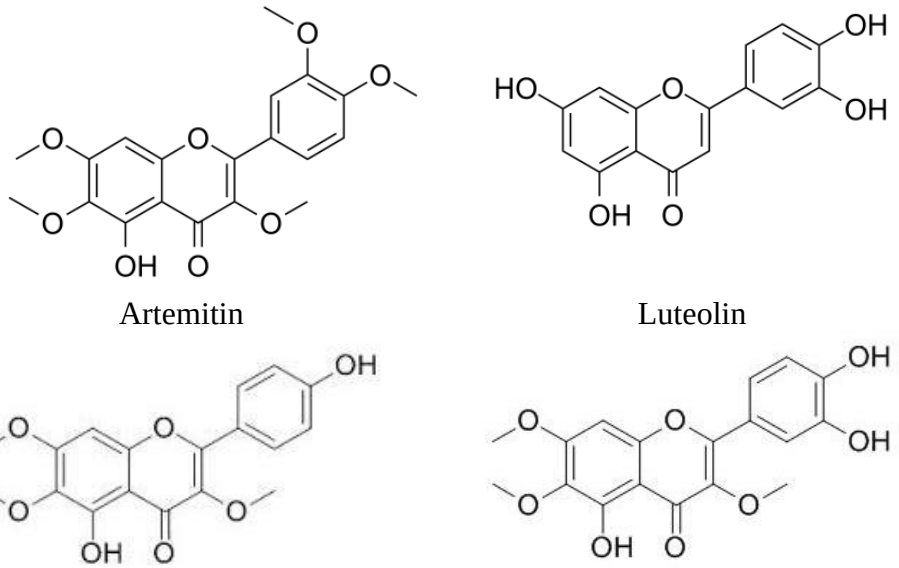

Figure 4: Structure of flavonoid compounds; artemetin, luteolin, penduletin and chrysosplenol-D; other flavonoid compounds than casticin, isolated from *Vitex trifolia* (33). Retrieved from http://www.chemfaces.com/natural.

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity of the plant. The compounds having reducing power ability act as electron donors and reduce the oxidized intermediates of lipid peroxidation processes.

Tannic acid has high reducing power than ascorbic acid and Trolox (water analogue of vitamin E) (36). It is reported that there is a positive correlation between reducing capacities and individual antioxidant phytochemicals in order of carotene > flavonoids > ascorbic acid > total anthocyanins. *Adhatoda vasica* and *Vitex negundo* contain high antioxidants such as tannins, saponins and a sufficient amount of alkaloids, phenols and flavonoids. This suggests that these plants have strong total antioxidant activity and reducing power due to their phytochemical constituents (37). *V. trifolia* also contains high amounts of

saponins, flavonoids, alkaloids and phenols where they can act as antioxidants (36).

Besides, antioxidant compounds, significantly flavonoids, display antiviral action in models of coronavirus infections that may be attributed to the inhibitory effect on the enzymatic activity of targets involved in coronavirus replication (9,38). These ezymes include SARS-CoV 3CLpro, SARS-CoV papain-like protease (PLpro), SARS-CoV helicase protein, and MERS-CoV 3CLpro. A few studies demonstrated that the decline of ROS accumulation delays the coronavirus-activated apoptotic signalling (38). Therefore, the mechanisms of oxidative stress could be the key element to be studied in coronavirus infections, including those related to inflammatory processes arising from the action of this virus. Thus, indicating the beneficial effect of antioxidants.

4.5 Antibacterial

Infectious disease is the number one cause of death and many medicinal plants are used to treat infectious disease. There have been several reports on the chemical constituents of Vitex species that are anti-infective (39). These include iridoid glycosides (agnuside, aucubin), flavonoids (vitexin, kaempferol, casticin, quercetagetin), progestins (progesterone, hydroxyl progesterone, androstenedione), alkaloids (viticin), volatile oil (1,8-cineol, limes, α-pinenes, β-pinenes) and essential fatty acids (palmitic acid, oleic acid, stearic acid). According to Arokirayaj et al. (2009), flavonoids are effective as antibacterial due to their ability to form a complex with extracellular, soluble protein and bacterial cell wall (39). Besides, more lipophilic flavonoids may also disrupt the microbial membrane. The antibacterial activity may also be due to secondary metabolites because they can control these antibiotic-resistant human pathogens (40).

Another study was conducted where *V. trifolia* inhibited most of the bacterial isolates (41). The methanol extract was more potent compared to ethanol and ethyl acetate extracts due to the presence of some active constituents in the extract. The active components of the plant parts are better extracted with methanol than other solvents because of the polarity of the solvent. The extracts are also more active against Gram-positive bacteria than Gram-negative bacteria because the gram-positive bacteria are more permeable to a series of antibiotics than is the gram-negative cell wall which is built like other biological membranes (41,42). Furthermore, the active compounds may be good permeating compounds that diffuse through the cell wall to enter the periplasmic space (42).

5.0 Conclusion

V. trifolia is widely used in traditional medicine for various inflammation-related diseases. The phytochemical constituents found in leaves, roots, flowers and fruits that have been identified in *V. trifolia* especially casticin have anti-inflammatory, anti-cancer, anti-bacterial and many other pharmacological activities including antioxidants. Hence, this review further confirms the importance of this plant for future development in the pharmaceutical and nutraceutical industries especially in therapy for inflammatory-related diseases and the possibility of *V. trifolia* to be exploited as a possible treatment for COVID-19.

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