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VEGF and Macrophage Expression in Pulp Inflammation Following Administration of a Combination of Tricalcium Silicate Cement and Galam (*Melaleuca cajuputi* var. *cumingiana*) Leaf Extract: *In Vivo* and *In Silico* Studies

Sherli Diana[1,2], Irene E. Rieuwpassa[3]*, Juni J. Nugroho[2], Rasmidar Samad[4], Risfah Yulianty[5], Nurhayaty Natsir[2], Nurlindah Hamrun[3], Muthi'ah N. Putri[2], Agung S. Wardhana[6], Maharani L. Apriasari[7], Tri Nurrahman[8], Juliyatin P. Utami[9]

[1]*Doctoral Study Program Dentistry, Faculty of Dentistry, Hasanuddin University, Indonesia*
[2]*Department of Conservative Dentistry, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia*
[3]*Department of Oral Biology, Faculty of Dentistry, Hasanuddin University, Indonesia*
[4]*Department of Dental Public Health and Preventive Dentistry, Faculty of Dentistry, Hasanuddin University, Indonesia*
[5]*Department of Pharmaceutical Science and Technology, Faculty of Pharmacy, Hasanuddin University, Indonesia*
[6]*Department of Dental Materials, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia*
[7]*Department of Oral Medicine, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia*
[8]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia*
[9]*Department of Biomedicine, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia*

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ABSTRACT

The use of natural compounds with anti-inflammatory and regenerative effects for wound healing has been found beneficial. Galam (*Melaleuca cajuputi* var. *cumingiana*) is a typical Indonesian medicinal plant with anti-inflammatory activity. This study aimed to evaluate the combined effect of galam leaf extract and tricalcium silicate cement on vascular endothelial growth factor (VEGF) and macrophage expression in pulpitis rat model. Twenty-four male Wistar rats were divided into six groups: Groups 1 and 2 - received a combination of galam leaf extract and tricalcium silicate cement for 3 and 7 days, respectively; Groups 3 and 4 - received tricalcium silicate cement alone for 3 and 7 days, respectively; Groups 5 and 6 - received 40% propylene glycol for 3 and 7 days, respectively. After the treatment periods, macrophage and VEGF expressions were assessed by histological and immunohistochemical analyses. The binding interactions of phytoconstituents of galam leaf with VEGF protein was predicted through molecular docking simulations. Results showed a significant increase in VEGF expression and a reduction in pulp inflammation in the combination groups compared to the controls. Macrophage numbers increased on day 3, indicating immune activation, and decreased by the 7th day, suggesting inflammation resolution. The compound dammarane-3,12,25-triol present in galam leaf demonstrated strong binding affinity to VEGF *in silico*, suggesting potential angiogenic effect. These findings indicate that the combination of galam leaf extract and tricalcium silicate cement enhanced pulp healing by reducing inflammation, regulating macrophage activity, and increasing VEGF expression in pulpitis rat model.

Keywords: Direct Pulp Capping, Tricalcium Silicate Cement, Galam Leaf, Macrophage Cells, Vascular Endothelial Growth Factor, Dammarane-3,12,25-triol.

Introduction

Dental caries is a chronic infection transmitted by bacteria and caused by multiple factors. Untreated caries can progress to pulp and periapical inflammation.[1] Pulp inflammation is a response to several factors such as bacterial infection, periodontal disease, operative procedures, and dental trauma.[1] Shah *et al*. (2020) reported that the inflammatory response is a prerequisite for and can stimulate tissue repair in dental structures. When the dental pulp is exposed to caries, bacteria, or dental filling materials, the pulp-dentin interface undergoes a range of inflammatory responses, from mild to severe.[2]

*Corresponding author. Email: ireneedith@unhas.ac.id
Tel: +62 813-5510-7432

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Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), Interferon-gamma (IFN-γ), Interleukin-1 beta (IL-1*β*), and IL-6 work to enhance the host immune response. In contrast, anti-inflammatory cytokines, including steroids, transforming growth factor-beta 1 (TGF-*β*1), IL-10, and nitric oxide (NO), are released to limit tissue damage. Molecules like TGF-*β* and nitric oxide (NO) can act as anti-inflammatory in some situations but may become pro-inflammatory under certain conditions, such as high levels or chronic disease.[3,4] The interactive balance between pro- and anti-inflammatory signals allows the pulp-dentin tissue to respond to cellular necrosis, bone resorption, pulp calcification, or revascularization. Low-level of inflammatory cytokine signals can direct cellular responses that promote differentiation and mineralization to support healing. For instance, chemokine receptor 4 (CXCR-4) is involved in both pro-inflammatory processes and tissue repair.[2]

The inflammatory phase of pulp-dentin tissue, along with the proliferative and cellular differentiation phases, are interconnected and essential for the wound healing process. Vascularization plays a critical role in supplying nutrients and oxygen and removing metabolic waste. Additionally, vascularization guides the migration of mesenchymal stem cells (MSCs) from perivascular areas to the site of inflammation. Mesenchymal stem cells (MSCs), including dental pulp stem cells (DPSCs), have the capacity to differentiate into various cell types such

as odontoblasts, fibroblasts, endothelial cells, and neural cells.[2] This vascularization process is supported by angiogenic signaling molecules such as vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), and transforming growth factor-β1 (TGF-β1), which are released by injured pulp cells, endothelial cells, and the extracellular matrix (ECM). Thus, the inflammatory, proliferative, and differentiation phases synergistically collaborate to support the natural healing process of pulp-dentin tissue.[2]

Among these factors, VEGF plays a particularly important role in the formation of reparative dentin. The presence of VEGF in dentin and the response of pulp cells to VEGF promote the recruitment of endothelial progenitor cells to the pulp, along with odontoblast progenitors and neural cells. The involvement of endothelial progenitor cells in vascularization during tissue regeneration, along with VEGF and vascular endothelial cells, is critical for dentin regeneration.[5]

Pulp inflammation can be managed through pulp capping treatment, which involves the application of bioactive materials over the pulp tissue to stimulate the formation of reparative dentin.[6] Materials used for pulp capping include calcium hydroxide, which is considered the gold standard, mineral trioxide aggregate (MTA), and tricalcium silicate cement (Biodentine®).[7] Tricalcium silicate cement offers several advantages, including superior biocompatibility, long-term impermeability, low solubility, and ease of manipulation.[8] Direct pulp capping treatment using tricalcium silicate cement (Biodentine®) results in lower VEGF secretion compared to TheraCal and Xeno III.[9] Vascular endothelial growth factor (VEGF), expression in human bone marrow mesenchymal stem cell (hBMSC) has been shown to be lower when tricalcium silicate cement such as Biodentine® is used compared to that observed with MTA and TotalFill.[10] However, its disadvantage lies in its sensitivity, which may lead to ongoing inflammation for 1 - 2 weeks following application. Contact between tricalcium silicate cement and exposed pulp stimulates the release of proinflammatory cytokines such as IL-6, IL-10, and TNF-α, which activate macrophages.[11] Direct contact between tricalcium silicate cement and macrophages stimulates the inflammatory process by increasing the production of proinflammatory cytokines such as IL-1, IL-6, IL-1β, Monocyte Chemoattractant Protein (MCP), and Macrophage Inflammatory Protein (MIP1), thereby enhancing the inflammatory response.

Galam **(***Melaleuca cajuputi* var. *cumingiana*) is a typical Indonesian medicinal plant with anti-inflammatory properties. Galam leaf extract has been shown to exhibit broad-spectrum antimicrobial, anticancer, and anti-inflammatory activities.[12] Galam leaves contain bioactive compounds such as terpenoids and flavonoids, which have been shown to promote tissue regeneration. A study by Musta *et al*. (2022) demonstrated that a 100% concentration of galam (*Melaleuca cajuputi* var. *cumingiana*) leaf extract exhibited optimal antibacterial activity against *Streptococcus aureus*.[13] Antibacterial activity was also observed at concentrations of 25%, 50%, 75%, and 100% of galam leaf extract against *Lactobacillus plantarum*, with efficacy increasing in a concentration-dependent manner.[14] In addition, a mixture of 50% galam leaf gel extract and tricalcium silicate cement used as a direct pulp capping material have shown potential in enhancing the number of odontoblast-like cells.[15] Toxicity testing using the MTT assay showed that 100% ethanol extract of galam leaves does not have cytotoxic effect.[16]

Currently, there is no study on the use of galam (*Melaleuca cajuputi* var. *cumingiana*) leaf extract as an alternative material for direct pulp capping or on its potential as an anti-inflammatory agent that could enhance the effectiveness of tricalcium silicate cement. Therefore, this study aims to investigate the effect of the combination of galam (*Melaleuca cajuputi* var. *cumingiana*) leaf extract and tricalcium silicate cement on macrophage activity and VEGF expression in inflamed dental pulp tissue of Wistar rats.

Materials and Methods

Chemicals and Equipment

The chemicals used in the study include 70% ethanol (Merck KGaA, Darmstadt, Germany), potassium dichromate ($K_2Cr_2O_7$) (Merck KGaA, Darmstadt, Germany), tricalcium silicate cement (Biodentine®) (Septodont, Saint Maur des Fosses, France), type II Fuji IX glass ionomer cement (Fuji IX®, GC Corporation, Tokyo, Japan), cocoa butter (GC Corporation, Tokyo, Japan), ketamine (Ketalar®, Warner Lambert, Ireland), xylazine HCl (Rompun®, Bayer, Leverkusen, Germany), Phosphate Buffered Saline (PBS) (Gibco™, Thermo Fisher Scientific, USA). The materials used for histological preparation and staining included 10% formalin buffer, 2% nitric acid decalcifying solution, xylene, paraffin, and haematoxylin–eosin (HE) staining kit (Sigma-Aldrich, St. Louis, MO, USA). The equipment used included rotary evaporator (IKA® RV OS-ST IP-B, IKA-Werke GmbH, Staufen, Germany), vacuum oven (Memmert GmbH, Schwabach, Germany), water bath (Memmert GmbH, Germany), analytical balance (Shimadzu AY220, Kyoto, Japan), micromotor and handpiece (NSK, Tokyo, Japan), round dental bur 0.84 mm (Edenta®, Switzerland), dental mixer (3M ESPE, Seefeld, Germany), microscope slides and coverslips (Leica RM2125 RTS, Leica Biosystems, Germany), microtome (Leica RM2125 RTS, Leica Biosystems, Germany), water bath for histology (Thermo Fisher Scientific, USA), hot plate (Stuart, UK), and a light microscope (Olympus CX23, Tokyo, Japan). All glassware used were products of Pyrex®, Corning Inc., USA.

Plant collection and identification

Fresh galam (*Melaleuca cajuputi* var. *cumingiana*) leaves were collected from Gambut District, South Kalimantan, Indonesia in January 2023. The plant material was identified by Dr. Totok Wianto, S.Si., M.Si, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjarbaru, Indonesia. A voucher specimen with reference number Galam 1-23-034 was deposited at the Herbarium of Lambung Mangkurat University (ULM Herbarium) for future reference.

Preparation of plant extract

The leaves were washed under running water and then dried in an oven at 50°C. The dried samples were ground into a fine powder using a blender. The powdered leaves (60 g) was macerated in 70% ethanol (300 mL) at room temperature for 7 days. The maceration process was repeated three times. The resulting extract was evaporated over a water bath at 70°C until a viscous liquid was formed, which was further dried by exposure to air on a porcelain dish. Finally, the dried extract was tested for ethanol-free content using potassium dichromate ($K_2Cr_2O_7$).

Animals

Twenty-four (24) male healthy Wistar rats (*Rattus norvegicus*) weighing between 270 – 350 g were obtained from the Veterinary Center of Banjarbaru, South Kalimantan, Indonesia, and maintained at the Biochemistry Laboratory, Faculty of Medicine, Lambung Mangkurat University Indonesia under standard conditions (temperature 22 ± 2°C, 12-h light/dark cycle). The rats were kept in well-ventilated cages and acclimatized to the laboratory conditions for 7 days. During the acclimatization and treatment periods, the rats were fed with standard rodent pellets (Comfeed BR-2) at 20 g/day and allowed access to drinking water *ad libitum*.

Ethical approval

The study was approved by the Animal Research Ethics Committee, Faculty of Dentistry, Hasanuddin University, Indonesia (Ethical Approval No. 0101/PL.09/KEPK FKG-RSGM UNHAS/2023). The experiment was conducted in accordance with the OECD Guidelines for animal welfare.

Induction of pulp inflammation

The rats were anesthesized by intramuscular injection of ketamine (Ketalar®, Warner Lambert, Ireland) at a dose of 65 mg/kg body weight and xylazine HCl (Rompun®, Bayer, Leverkusen, Germany) at a dose of 7 mg/kg body weight. A Class I cavity preparation was made on the occlusal surface of the left maxillary molar using a low-speed contra-angle handpiece with a diamond bur (diameter 0.84 mm), extending to a depth of 1 mm or the diameter of the bur head, until the pulp chamber was exposed, indicated by visible redness. Another clinical sign indicating pulp exposure was the occurrence of bleeding. The

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perforated tooth was irrigated with sterile saline and dried using a cotton pellet. Bleeding was stopped using the tip of a sterile paper point.

Animal grouping and treatment

The rats were divided into six groups of four animals per group. Treatments were then applied according to the respective group. Groups 1 and 2 received a combination treatment of galam leaf extract and tricalcium silicate cement (0.5 mm pasta application) for 3 and 7 days, respectively. Groups 3 and 4 received tricalcium silicate cement for 3 and 7 days, respectively. Groups 5 and 6 received 40% propylene glycol for 3 and 7 days, respectively. After each treatment period, the rats were sacrificed by cervical dislocation under ketamine (25 mg/kg body weight) and xylazine (3 mg/kg body weight) anesthesia. Following sacrifice, the maxillary bone was harvested in the interdental area of the left maxillary molars.

Histological examination

The tissue was immersed in 10% EDTA for 3 days to decalcify the left maxillary molar bone. The dehydration process involved the gradual removal of water from the tissue using a graded series of ethanol concentrations: 70%, 80%, 95%, and 100%. Clearing was performed using xylene to render the tissue transparent. Embedding was carried out using paraffin wax at a melting point of 56°–60°C. The paraffin block was mounted on a microtome head, and sections were cut at a thickness of 4 µm. The obtained tissue sections were floated in a water bath to allow proper expansion and then drained. The deparaffinization step involved the removal of paraffin with xylene (three changes), followed by rehydration through a descending series of alcohol concentrations. Staining was performed using Mayer's hematoxylin for 15 minutes, followed by differentiation under running water. The slides were then immersed in 1% eosin solution for 30 seconds as a counterstain. Subsequent steps included dehydration, clearing, and mounting by applying a coverslip using a mounting medium.[17]

Immunohistochemical analysis

The slides were subjected to deparaffinization using xylene, performed three times for 3 minutes each. Rehydration was carried out using 100% ethanol for 2 minutes, 95% ethanol for 1 minute, 70% ethanol for 1 minute, and finally rinsed in distilled water for 1 minute. The slides were then incubated with peroxidase blocking solution at room temperature for 10 minutes. This was followed by incubation in prediluted blocking serum at 25ºC for 10 minutes. The sections were then immersed in polyclonal antibody solution at 25ºC for 10 minutes. After incubation, the slides were washed with phosphate-buffered saline (PBS) for 5 minutes. The slides were then incubated with secondary antibody at 25ºC for 10 minutes, followed by another PBS wash for 5 minutes. Subsequently, the sections were incubated with peroxidase at 25ºC for 10 minutes and washed again with PBS for 5 minutes. The slides were then incubated with diaminobenzidine (DAB) chromogen at 25ºC for 10 minutes. Counterstaining was performed using hematoxylin for 3 minutes, followed by rinsing under running water. Finally, the sections were cleared, mounted with mounting medium, and covered with a cover glass. Vascular endothelial growth factor (VEGF) protein expression was observed under a light microscope at 400x magnification. The expression was assessed based on the brown staining produced by DAB in the immunohistochemistry preparations, with the intensity and distribution of the staining used to determine the level of protein expression in the examined tissue. Macrophages were observed and counted under a light microscope at 400x magnification from the preparations of each sample. The counts were performed in four different fields of view by three observers, then averaged.[18]

In silico study

Ligand and protein preparation

The ligands used were phytochemicals of galam (*Melaleuca cajuputi* var. *cumingiana*) leaves. The 3D and Canonical SMILE structures of the compounds was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The target protein used in this study was VEGF. The 3D structure of VEGF (PDB ID:1BJ1) was obtained from the RCSB PDB database (https://www.rcsb.org/).[15]

Virtual screening

The binding interactions of the test ligands with the target protein was predicted through molecular docking simulations using PyRx 0.9.9 version software. Docking outcomes were reported in terms of binding affinity and types of bonding interactions.[16-19]

Visualization of bonding interactions

The positions and types of molecular interactions such as Van der Waals, hydrogen, hydrophobic, electrostatic, and pi-pi stacking were visualized using the Discovery Studio software 2016 version.[16,20]

Results and Discussion

Effect of Galam leaf extract on macrophages in dental pulp

The mean macrophage counts in the different groups are presented in Table 1. For the group treated with a combination of galam leaf extract and tricalcium silicate cement, the highest mean macrophage count of 10.17 cells was observed on day 3. The negative control group exhibited the lowest average number of macrophages on day 3, with 4.98 cells (Figure 1). On day 7, a decrease in the number of macrophages was observed across all groups, with the most significant reduction occurring in the combination treatment group, while the lowest reduction was observed in the negative control group (Figure 2).

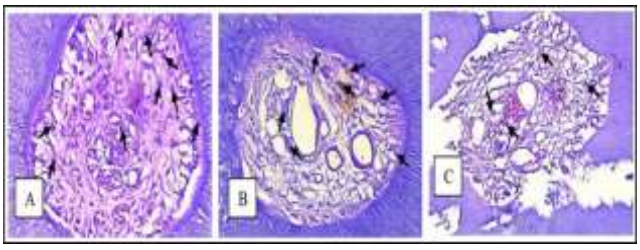

Figure 1: Photomicrograph of rat dental pulp on day 3 showing macrophage cells (400x magnification) **(A)** Combination of galam (*Melaleuca cajuputi*) leaf extract and tricalcium silicate cement, **(B)** Tricalcium silicate cement only as a positive control, **(C)** 40% propylene glycol as a negative control

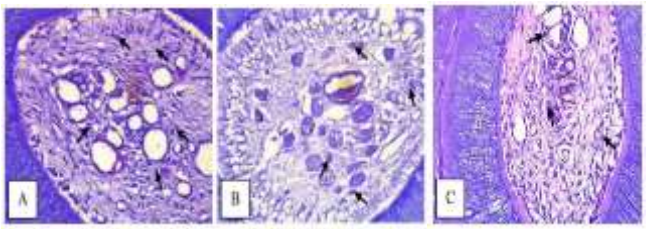

Figure 2: Photomicrograph of rat dental pulp on day 7 showing macrophage cells (400x magnification) **(A)** Combination of galam (*Melaleuca cajuputi*) leaf extract and tricalcium silicate cement, **(B)** Tricalcium silicate cement only as a positive control, **(C)** 40% propylene glycol as a negative control

Table 1: Number of Macrophage cells in dental pulp of Wistar rats exposed tp the different treatments

Treatment	Number of Macrophage cells	
	Day 3	Day 7
Combination of galam leaf extract and Tricalcium silicate cement	10.17 ± 1.32	5.81 ± 0.42
Tricalcium silicate cement	7.87 ± 0.52	4.62 ± 0.84
Propylene glycol (40%)	4.98 ± 0.45	3.21 ± 0.42

Values are mean ± standard deviation (SD), n = 4.

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The Shapiro-Wilk normality test yielded a p-value of 0.334 (>0.05), indicating that the data were normally distributed. A subsequent homogeneity test using Levene's Test showed a p-value of 0.906 (>0.05), indicating that the data were homogeneously distributed. The results of the Two-Way Anova revealed a statistically significant difference in the number of macrophage cells between day 3 and day 7 of observation. These results indicated that the combination of galam leaf extract with tricalcium silicate cement caused a significant increase in the number of macrophages on day 3 and a decrease on day 7, compared to the groups treated with tricalcium silicate cement and propylene glycol alone. This effect can be attributed to the anti-inflammatory properties of the bioactive compounds in galam leaf extract, including flavonoids, tannins, phenols, alkaloids, and terpenoids.[12,22]

On day 3 of inflammation, flavonoids in galam leaves are thought to play a role in increasing lymphokine production by T cells, which stimulates macrophages' phagocytic function. Flavonoids accelerate the inflammatory response by inhibiting cyclooxygenase and lipoxygenase enzymes, thereby reducing prostaglandin and leukotriene production through the arachidonic acid pathway.[21] Phenolic metabolites in galam leaves have been shown to inhibit cyclooxygenase-2 (COX-2) enzyme activity and reduce the levels of several cytokines, such as IL-1β, IL-6, TNF-α, and prostaglandins.[24] Saponins and tannins are also involved in increasing the expression of transforming growth factor beta (TGF-β).[25] The function of TGF-β is to stimulate the migration of monocytes to the injury site, followed by cytokines (IL-4, IL-10, and IL-13) that induce the differentiation of monocytes into macrophages, thereby increasing macrophage infiltration.[26]

On day 7 of inflammation, the polyphenol content in galam leaves demonstrated anti-inflammatory effects by inhibiting TNF-α and NF-κB signaling pathways. Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine produced through the activation of NF-κB. A study by Apriasari *et al*. (2020)[27] confirmed that tannins are capable of reducing macrophage numbers by inhibiting TNF-α and NF-κB pathways. Inhibition of NF-κB and TNF-α is a key mechanism of anti-inflammatory action that supports wound healing. Flavonoids also regulate cellular functions by stimulating the production of TGF-β1, which induces the proliferation of odontoblast-like cells and triggers reparative dentin formation. Flavonoids have the ability to promote dentin bridge formation during direct pulp capping procedures by enhancing fibroblast proliferation and collagen production. A study by Kusuma *et al*. (2022),[8] compared the number of macrophages following application of Biodentine® and miswak extract, and found that flavonoids could reduce macrophage counts on day 7 due to their antioxidant effects, which mitigated reactive oxygen species (ROS) by neutralizing free radicals.[8]

Effect of Galam leaf extract on VEGF expression

Vascular endothelial growth factor (VEGF) expressions in the various groups are presented in Figure 3. Vascular endothelial growth factor (VEGF) expressions were interpreted in terms of the intensity of the immunohistochemical staining. The stronger the intensity of the observed stain, the higher the level of VEGF expression by the cells. Strong intensity is indicated by a prominent and dense brown staining in the cytoplasm of the cells (Figure 3A). High VEGF expression suggests an active healing or regeneration process in the rat dental pulp, typically as a response to injury or inflammation. On the other hand, moderate intensity is characterized by a clear dense brown stain (Figure 3B), though not as dense as that observed in the strong intensity. Moderate VEGF expression indicates that angiogenesis is still occurring but at a less intense level. This is usually observed during the intermediate phase of healing or in pulp tissue experiencing mild to moderate stimulation.

A weak intensity is represented by very faint brown stain, sometimes barely visible in the cytoplasm of the cells (Figure 3C). This reflects low VEGF expression, indicating minimal or nearly absent angiogenic activity. Vascular endothelial growth factor (VEGF) is primarily distributed in the endothelial cells of blood vessels, fibroblasts, and odontoblasts.

Figure 3: Photomicrograph of rat dental pulp showing VEGF expression **(A)** strong intensity, **(B)** moderate intensity, and **(C)** weak intensity

The results of this study indicate that the combination of tricalcium silicate cement and galam (*Melaleuca cajuputi* var. *cumingiana*) leaf extract enhances VEGF expression in the dental pulp tissue of Wistar rats (*Rattus norvegicus*) subjected to mechanical perforation, particularly on days 7 and 14 post-perforation. This increase in VEGF expression supports the role of endothelial progenitor cells in accelerating vascularization and dentin tissue regeneration through the stimulation of pulp cells, including odontoblast progenitors and neural cells. These findings align with a previous study that reported that VEGF plays a key role in angiogenesis and tissue regeneration and can be induced by various growth factors and biomaterials.[28] Although the p-value for intergroup comparison indicated no statistically significant difference in VEGF expressions among the groups, the higher mean VEGF expression in the combination group suggests a synergistic potential between tricalcium silicate cement and galam leaf extract in stimulating angiogenesis.

These findings also demonstrated that the application of this combination as a direct pulp capping material can increase VEGF expression more effectively than other treatment groups. This is supported by the work of Sequeira *et al*. (2023), who reported a significant increase in VEGF expression in tissues induced by lipoteichoic acid (LTA).[29] Vascular endothelial growth factor (VEGF) as a growth factor involved in the formation of reparative dentin. Rahayu *et al*. (2020) also confirmed that VEGF expression increased with the administration of calcium hydroxide and propolis. Vascular endothelial growth factor (VEGF) plays a crucial role in both physiological and pathological dentinogenesis and angiogenesis in healthy dental pulp.[30] The presence of VEGF in dentin and the pulp cell promotes the presence of endothelial progenitor cells in the pulp, along with odontoblast progenitors and neural cells. The role of these endothelial progenitor cells in vascularization is essential for dentin regeneration.[3]

Galam leaf extract is known to enhance VEGF release, an effect attributed to the presence of several bioactive components. For example, flavonoids present in galam leaves have been shown to promote angiogenesis and odontoblast differentiation during pulp healing.[23] This occurs because flavonoids stimulate VEGF release by neutralizing free radicals, thus reducing reactive oxygen species (ROS) and stimulating macrophages to secrete VEGF.[29] The phenolic compounds in galam leaves have also been shown to increase VEGF expression by binding to superoxide radicals (O^{2-}) and releasing nitric oxide (NO), which enhances VEGF via the PI3K-Akt signaling pathway.[32]

Molecular docking results

From the *in silico* molecular docking analysis, dammarane-3,12,25-triol, one of the bioactive compounds of galam leaves demonstrated the strongest binding affinity (-8.8 kcal/mol) to VEGF (Table 2). Dammarane-3,12,25-triol is a type of dammarane triterpenoid, known for its potential anti-inflammatory properties.[31] This compound, derived from plants, have also been studied for other biological activities like antioxidant and anticancer effects.[34]

Other bioactive phytochemicals such as saponins have been shown to enhance FGF and VEGF secretion by promoting monocyte proliferation, thereby increasing macrophage numbers, which are the cells responsible for secreting FGF and VEGF. Alkaloids, another active phytochemical, contribute to VEGF upregulation by activating the Wnt/β-catenin pathway.[32] These assertions are supported by the

study of Tao *et al*. (2023)[35], which found that saponins from *Aralia taibaiensis* extract promoted angiogenesis by enhancing VEGF and VEGFR2 signaling.[35] Additionally, hesperidin, a flavonoid derivative was reported to increase VEGF expression by binding to superoxide radicals (O^{2-}) and releases nitric oxide (NO), which activates the PI3K-Akt pathway to transmit intracellular signals.[36]

Table 2: Binding affinities of bioactive compounds (Ligands) of galam (*Melaleuca cajuputi*) leaf with VEGF receptors

Ligand	Binding affinity (kcal/mol)
3-Cyclohexen-1-ol	-3.8
Caryophyllene 1,4,8-Cycloundecatriene	-5.6
Caryophyllene oxide	-5.4
Alpha-Tetralone	-5.3
Alpha-Tocopherol	-5.4
Betulin	-8.1
Methyl-lathodoratin	-6.2
1,4-Naphthalenedione	-5.9
2H,6H-Pyrano(3,2-b)xanthen-6-one, 7,12-dihydroxy-2,2-dimethyl-	-7.9
gamma-Sitosterol	-7.4
Dammarane-3,12,25-triol	**-8.8**
Phytol 2-Hexadecen-1-	-4.9
Naphthalene	-5.9
Caryophyllene oxide 5-Oxatricyclo[8.2.0.0(4,6)]dodecane	-5.6
Ethanone	-5.8
3,7,11,15-Tetramethyl-2-hexadecen-1-OL	-4.9
6,7-Dimethoxy-4H-1-benzopyran-4-one	-6.4

Angiogenesis is a complex process of neovascular formation regulated by major angiogenic growth factors such as FGF-2 and VEGF. During angiogenesis, physiological hypoxia occurs, leading to systemic hemodynamic and vascular changes that induce hypoxia-inducible factor-1α (HIF-1α) to trigger VEGF expression. This process involves heat shock protein 90α (HSP90α), which regulates angiogenesis and enhances the proliferation, differentiation, and migration of endothelial cells.[37]

Key growth factors involved in pulp angiogenesis include FGF, TGF-*β*1, and VEGF. VEGF is a major regulator of angiogenesis that increases vascular permeability and induces chemotaxis, proliferation, and differentiation of human dental pulp cells.[38] VEGF is primarily expressed by M2 macrophages, and its presence in dentin pulp cell promotes the recruitment of endothelial progenitor cells, odontoblast progenitors, and neural cells into the pulp tissue, facilitating dentin regeneration.[3] Shen *et al*. (2021) study supported this by showing that VEGF significantly increases human dental pulp cell (hDPC) expression compared to the control group on days 7 and 14.[23] This indicates that VEGF influences both osteoblasts and odontoblasts, which are crucial for dentin regeneration.

Additionally, it has been reported that VEGF levels peak on day 7 during inflammation when granulation tissue forms, before declining by day 14.[30] This may be attributed to the role of *platelet-derived growth factor* (PDGF) as a promoter of proteoglycan and collagen synthesis. When fibroblasts receive PDGF signals, they produce other growth factors such as KGF (keratinocyte growth factor), VEGF, and bFGF (basic fibroblast growth factor), which promote blood vessel formation. VEGF also plays a significant role in the differentiation and mineralization of hDPCs,

Conclusion

The findings from this study demonstrated that the use of the combination of galam (*Melaleuca cajuputi* var. *cumingiana*) leaf extract and tricalcium silicate cement as a dental pulp capping material significantly affected pulpal macrophage counts and VEGF expression in Wistar rats. On day 3 of pulp inflammation, this combination resulted in the highest increase in macrophages, followed by a decrease on day 7, indicating a role in inflammation resolution. These results suggest a synergistic effect of the combination in promoting angiogenesis during pulp wound healing. These findings were supported by results from *in silico* study, which showed that dammarane-3,12,25-triol, a bioactive compound in galam leaves has a strong binding affinity with VEGF. The promising outcomes of this study open new avenues for the development of biologically active dental pulp capping materials that combine natural plant extracts with bioactive cements. The synergistic interaction between galam (*Melaleuca cajuputi* var. *cumingiana*) leaf extract and tricalcium silicate cement suggests potential for enhanced inflammatory modulation, angiogenesis stimulation, and tissue regeneration in dental pulp therapy.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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