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The Role of *Andrographis paniculata* in Modulating the Immune Response in Cancer-Associated Chronic Inflammation, Angiogenesis, and Metastasis

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**Running Title:** *Andrographis paniculata* inhibits cancer by modulating the immune response

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**Abstract**

The immune system plays an essential role in cancer pathogenesis through providing an inflammatory immune response. Chronic inflammation causes tumor growth, angiogenesis, and metastasis facilitated by interactions between tumor, immune, and stromal cells in the tumor microenvironment (TME). Various inflammatory mediators and growth factors secreted by cells
in the TME exert a synergistic effect on cancer promotion. Thus, the development of cancer therapies that lead to inhibition of the activity of immune cells, cytokines, chemokines, and cancer-inducing growth factors is a promising therapeutic strategy. *Andrographis paniculata* (*A. paniculata*) is an ethnomedicinal plant with immunomodulatory and anticancer activity. *A. paniculata* can also inhibit the resistance of chemotherapy agents associated with the TME as adjuvant chemotherapy. This review focuses on the mechanism of *A. paniculata* in suppressing cancer-associated chronic inflammation, angiogenesis, and metastasis through modulation of the immune response. The results show that *A. paniculata* exerts anticancer effects directly targeting cancer cells, inhibiting cancer growth by modulating immune responses. *A. paniculata* exerts anticancer effects by inhibiting the production of cytokines, growth factors, and chemokines via the nuclear factor-kappa B (NF-κB), mitogen-activated protein kinase (MAPK), Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathways. In addition, this review provides a new hypothesis regarding the potential of *A. paniculata* to serve as an anticancer agent that can inhibit cancer cell proliferation at the angiogenesis and metastatic stages through regulating inflammation due to interactions between cancer cells, immune cells, and stromal cells in the TME.

**Keywords:** *Andrographis paniculata*, immunomodulatory, anticancer, angiogenesis, metastasis, tumor microenvironment

**Introduction**

The immune response in inflammation can either suppress or trigger cancer growth. Acute inflammation has an anticancer effect derived from the activity of immune cells, including natural killer (NK) cells, dendritic cells (DC), M1 polarized macrophages, N1 polarized neutrophils, and effector T cells (CD8+ cytotoxic T cells and effector CD4+ T cells). Immune cell activity in the
acute inflammatory response to cancer growth includes direct killing, phagocytosis, and secretion of proinflammatory cytokines. Chronic inflammation affects the TME, which exerts an immunosuppressive response that causes cancer cells to grow and promotes metastasis and angiogenesis. The TME comprises the surrounding immune cells, fibroblasts, lymphocytes, blood vessels, extracellular matrix, inflammatory cells of the bone marrow, and various signaling molecules. The interaction between cancer cells and cells in the TME influences cancer development.

Immune cells that play a role in chronic inflammation are tumor-associated macrophage (TAM)-associated M2 polarized macrophages, tumor-associated neutrophil-associated N2 polarized neutrophils (TAN), Treg regulatory T cells, and myeloid-derived suppressor cells (MDSC). The activity of immune cells in chronic inflammation in the TME includes the secretion of proinflammatory cytokines, chemokines, and growth factors that promote cancer cell growth. The proinflammatory cytokines are produced mainly by activated macrophages and are involved in the upregulation of inflammatory cytokines, including interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α). Chemokines are a large family of cytokines with chemotactic activity. Chemokines and their receptors are expressed by cancer cells and cells around the TME that play a role in leukocyte activation, angiogenesis, metastasis, and cell proliferation. Chemokines and their receptors have become targets for cancer immunotherapy. Some examples of chemokines and their receptors that play a role in cancer development are (1) CXC-chemokine ligand 16 (CXCL16) and its receptor CXC-chemokine receptor 6 (CXCR6) serve as a potent angiogenic mediators, and (2) CC-chemokine receptor 7 (CCR7) mediates tumor cell migration to lymph nodes through binding to CC-chemokine ligands 19 and 21 (CCL19 and CCL21). In some tumors, the expression of CXCR4 gives cancer cells the ability to migrate and metastasize to
CXCL12-secreting organs.\textsuperscript{6,7} Growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and transforming growth factor-beta (TGF-\(\beta\)) stimulate the proliferation, invasion, and angiogenesis of cancer cells.\textsuperscript{8}

The production and expression of inflammatory mediators such as cytokines, chemokines, and growth factors are regulated by the transcription factors nuclear factor-kappa B (NF-\(\kappa\)B), mitogen-activated protein kinase (MAPK), and Janus kinase (JAK)/signal transducer and activator of transcription (STAT). The NF-\(\kappa\)B signaling pathway controls the expression of genes such as TNF-\(\alpha\), IL-6, VEGF, B-cell lymphoma pro-apoptotic protein (Bcl-2), Bcl-XL, and Bcl-Xs. JAK/STAT signaling pathways,\textsuperscript{9} especially those including transcription factor STAT3, facilitate cell cycle progression by activating cyclin-dependent kinases (CDKs) and inducing VEGF expression, and the transcription factor STAT5 inhibits apoptosis by increasing the production of the anti-apoptotic protein Bcl-xL.\textsuperscript{10} The MAPK signaling pathway p38-MAPK also plays a central role in producing the inflammatory cytokines IL-1\(\beta\), TNF-\(\alpha\), and IL-6.\textsuperscript{11} These signaling pathways promote cellular proliferation through apoptosis resistance, angiogenesis, invasion, and metastasis.

Chronic inflammation associated with cancer can also be caused by chemotherapy treatment. Several chemotherapeutic agents induce inflammation, leading to TME-associated resistance and metastasis.\textsuperscript{12} These insights encourage therapeutic approaches that modulate the immune response to cancer-associated chronic inflammation in the development of chemotherapeutic agents or adjuvant chemotherapy.

\textit{A. paniculata}, known as the "king of bitters," is an ethnomedicinal plant widely used traditionally in Indonesia, India, Thailand, China, Pakistan, Bangladesh, Philippines, Hong Kong, and Malaysia. \textit{A. paniculata} contains various phytochemical compounds, including diterpenoids, flavonoids, and polyphenols.\textsuperscript{13} The main phytochemicals of \textit{A. paniculata} are andrographolide,
14-deoxy-11,12-didehydroandrographolide, and neoandrographolide (Figure 1). Several flavonoid compounds from *A. paniculata* have been isolated, and their activities are known to be those of polymethoxy flavones and flavanones.\textsuperscript{14–16} *A. paniculata* has been used for generations as a traditional medicine to treat various diseases, including cancer, and its use has been supported by scientific evidence. Currently, synthetic anticancer drugs have side effects such as immunosuppression; toxicity to the heart, kidneys, and liver; alopecia; nausea; and vomiting.\textsuperscript{17} Therefore, herbal plants without such side effects, such as *A. paniculata*, have become an alternative cancer treatment or cancer adjuvant therapy. The advantage of *A. paniculata* compared with other herbal plants is that *A. paniculata* is not only proven to have anticancer activity on various cancer cells, but its bioactive metabolite andrographolide when combined with chemotherapeutic agents (doxorubicin, gemcitabine, cisplatin, and topotecan) can increase their effectiveness. Moreover, andrographolide could reduce the side effects of the chemotherapeutic agent bleomycin.\textsuperscript{18–22} Another advantage is that both *A. paniculata* extract and its bioactive compound andrographolide can inhibit various inflammatory mediators and signaling pathways that play a role in cancer angiogenesis and metastasis. In addition, these activities can be an alternative in suppressing the potential for chemotherapy resistance, which will be discussed in this review.

![Chemical structures](image)

**Figure 1. Chemical structures of (a) Andrographolide; (b) 14-deoxy-11,12-didehydroandrographolide; (c) Neoandrographolide**

Natural products are currently widely used for cancer therapy, namely for the induction or inhibition of inflammatory activity through specific signaling pathways.\textsuperscript{23} Most of the immunomodulatory agents with anticancer activity are divided according to two mechanisms of
action: the first through blocking the initiation of cancer cells and the second through inhibiting the proliferation of cancer cells in the early stages of cancer angiogenesis and metastasis.\textsuperscript{24,25} This review describes the immunomodulatory and anticancer mechanisms of \textit{A. paniculate} and focuses on discussing the mechanism of \textit{A. paniculata} in modulating the immune response to suppress cancer growth. In addition, it provides a new hypothesis regarding the activity of \textit{A. paniculata} as a suppressor of cancer cell proliferation at the angiogenesis and metastatic stages and its potential to inhibit chemotherapeutic agent resistance through the regulation of inflammation in the TME.

**Pharmacological Activities**

The pharmacological activities of \textit{A. paniculata} extract and its bioactive metabolites as immunomodulatory, anticancer, anti-hyperglycemic, antipyretic, antibacterial, anti-malarial, anti-diarrheal, filaricidal, hepatoprotective, anti-inflammatory, anti-allergic, anti-parasitic, anti-ulcerogenic, antiviral, anti-hyperlipidemic, antioxidant, analgesic, renoprotective, anti-fertility, and anti-platelet agents have been demonstrated in vitro and in vivo.\textsuperscript{15,26,27}

**The role of the immune system in cancer**

The immune system is divided into innate and adaptive immune systems, which function to maintain tissue homeostasis from exposure to harmful agents such as infectious organisms (viruses, bacteria, etc.), or toxic compounds.\textsuperscript{28} The form of defense of the innate and adaptive immune system is an inflammatory response produced by the activity of immune cells. The innate immune system functions as a short-term first defense include phagocytic cells such as macrophages, neutrophils, natural killer cells, and various receptors such as the Tall Like Receptor (TLR).\textsuperscript{29} The adaptive immune system is an antigen-specific system mediated by T cells and B cells with highly specialized receptors.\textsuperscript{30,31} This immune system explicitly targets the types of pathogens that cause an infection and has been previously identified. In conclusion, the adaptive
immune system works more slowly than the innate immune system. The advantage of adaptive immune system is being able to "remember" the type of pathogen. When the same pathogen has exposed the body, the adaptive immune system can respond quickly.\textsuperscript{32}

The immune response to cancer has the dual potential of suppressing or promoting cancer development. This activity depends on the inflammatory response produced by immune cells. The mediators of the inflammatory response in the initiation of cancer is divided into extrinsic and intrinsic factors.\textsuperscript{2} Extrinsic factors are pathogenic infections, autoimmune attacks, UV exposure, excessive alcohol consumption, smoking, and obesity. Conversely, cancer-inducing mutations cause intrinsic factors to activate various inflammatory cells associated with cancer development. These extrinsic and intrinsic factors have been reported to form a immunosuppressive TME that is favorable for cancer development. Several immune system inflammatory mediators and growth factors play a role in the promotion and suppression of cancer. Their functions and roles are described below.

**IL-6**

IL-6 is a proinflammatory cytokine secreted by monocytes in response to infection and tissue injury. IL-6 increases proliferation and inhibits apoptosis through binding its receptor (IL-6R\(\alpha\)) and coreceptor glycoprotein 130 (gp130), thereby activating the transcription factors STAT1 and STAT3 in the JAK/STAT signaling pathway.\textsuperscript{33} STAT3 is involved in the proliferation and suppression of apoptosis in breast tumor cells through upregulating the target genes cyclin D1, c-Myc, Mcl-1, Bcl-2, and Bcl-xL. The protumorigenic effect of IL-6 has been shown in various types of cancer, including gastric cancer, lung cancer, and colorectal cancer.\textsuperscript{34–36} When NOD scid gamma female mice received intraductal mammary gland injections of IL-6-overexpressing T47D (ER+) cells (xenograft model), there was a significant increase in p-STAT33 in primary tumors.
accompanied by increased tumor growth. Activation of the IL-6/STAT3 pathway significantly increased lung metastases by approximately 5-fold. These results indicate that activating the IL-6/STAT3 pathway in ER+ tumors promotes the spread of metastases.37

**TNF-α**

TNF-α is an inflammatory cytokine with two types of functions in tumor development: antitumor and protumor. This cytokine has two receptors: TNF-α receptor-1 (TNF-αR1) and TNF-αR2. TNF-α, an antitumor cytokine that triggers apoptosis through TNF-αR1, forms a signaling complex I with TNF receptor-associated domain protein (TRADD), receptor interactions protein kinase (RIPK)-1, and TNF receptor-associated factor (TRAF) 2 (sometimes TRAF2 can be activated by TRAF 5). Signaling complex I recruits the adapter protein Fas-associated death domain protein (FADD), forming a death-inducing signaling complex (DISC) (also known as complex 2). DISCs can recruit and promote automatic catalytic activation of pro-caspases 8 and 10 (also known as FADD [Fas-associated death domain]-like IL-1β–converting enzyme [FLICE] and FLICE 2); caspases 8 and 10 are activated and then activate effector caspases, such as caspases 3 and 6, until apoptosis occurs. TNF-α acting as a protumor cytokine plays a role in cell proliferation, invasion, and angiogenesis of cancer cells. The binding between TNF-α and TNF-αR1 induces cell proliferation, cell activation, and differentiation by activating the transcription factors NF-κB, AP-1, and Elk-1. TNF-αR2 can regulate cell proliferation, cell-to-cell interactions, and migration through Etk phosphorylation, activation of PI3K and Akt pathways, and activation of VEGFR2 receptors, thereby increasing angiogenesis.38

**IL-1β**
IL-1β is an IL-1 family cytokine produced and secreted by immune cells, fibroblasts, and cancer cells. To produce a response, IL-1β binds to its receptor IL-1R1. IL-1β mediates the process of angiogenesis and metastasis of cancer cells through interactions with VEGF. Several studies have shown that angiogenesis and VEGF depend on IL-1. IL-1β interacts with IL-1R1 on endothelial cells and activates p38 MAPK and MAPK activated protein kinase 2, inducing cell migration. IL-1β also induces the production of IL-6, TGF-β, TNF-α, and epidermal growth factor (EGF) in breast cancer cells. IL-1β activates extracellular signal-regulated protein kinase (ERK) 1/2, activator protein (AP)-1, and matrix metalloproteinases (e.g., MMP-9) in invasive breast ductal carcinoma. IL-1β can act as an anticancer agent through induction of Th1 and Th9 responses to produce IL-9, IL-2, and IFN-γ, which play a role in inhibiting cancer growth. IL-1β also activates CD4 and CD8 to exert anticancer effects.

**VEGF**

VEGF is a growth factor in cancer cell angiogenesis in a family consisting of placental growth factor (PGF), VEGFA/VEGF, VEGFB, VEGFC, and VEGFD. VEGFs binds to three endothelial receptor tyrosine kinases with different affinities, such as VEGFR1, VEGFR2, VEGFR3, as well as co-neuropilin receptors and heparan sulfate proteoglycans. VEGF is a crucial mediator of angiogenesis in cancer, and is regulated by oncogenes; various growth factors such as fibroblast growth factor (FGF), epidermal growth factor (EGF), and TNF; IL-1 cytokines; and hypoxia. In addition to acting as a mitogenic signal for vascular endothelial cells, VEGF also induces anti-apoptotic factors that protect against vascular apoptosis and mediate the secretion and activation of enzymes that degrade the extracellular matrix. The blood vessels that form around the tumor are irregular, have blind ends, are tortuous and disorganized, and have high interstitial
pressure, which results in hypoxia and further VEGF production.\textsuperscript{45} VEGF is also immunosuppressive through inhibiting T cell function, increasing recruitment of Treg cells and MDSCs, and inhibiting DC cell differentiation and activation. The binding of the VEGF ligand to the VEGFR2 receptor inhibits T cell activity and increases the activity of immunosuppressive Treg cells. VEGF and VEGFR2 activate MDSCs via the JAK2/STAT3 signaling pathway, whereas the VEGF ligand and VEGFR1 receptor interaction inhibit DC cell maturation.\textsuperscript{44} Various studies have shown that the binding of VEGF to VEGFR1 blocks the activation of the transcription factor NF-κB and results in inhibition of DC maturation in murine models.\textsuperscript{46–48} VEGF promotes the development of TAMs, which can inhibit T cell activation and proliferation by releasing IL-10, TGF-β, and prostaglandins.\textsuperscript{49,50}

**TGF-β**

TGF-β exerts a tumor suppressor effect in the early stages of cancer through inhibiting cell cycle progression and inducing apoptosis. In the late stages of cancer, TGF-β exerts a promotion, invasion, and metastasis effect. Receptors play an essential role in apoptosis by signaling via the suppressor of mothers against decapentaplegic (SMAD) pathway. The TGF-β ligand binds to the TGF-β RII receptor and activates TGF-β RI to form a hetero-tetrameric receptor complex. Ligand binding and subsequent oligomerization trigger TGF-β RII to phosphorylate TGF-β RI on serine or threonine residues. In the canonical pathway, activated receptor complexes phosphorylate R-SMAD (SMAD2 or SMAD3), which forms a heteromeric complex with SMAD4, which then translocates to the nucleus to regulate the expression of target genes.\textsuperscript{51} Non-canonical pathways that play a critical role in cancer cell invasion and metastasis occur through activation of the
RAS/MAPK, PI3K/Akt, RHO/Rho-associated protein kinase (ROCK), Jagged/Notch, WNT/β-catenin, and mammalian target of rapamycin (mTOR) signaling pathways.\textsuperscript{8,52}

**Chemokines**

Chemokines are the largest cytokine subfamily and can be further subdivided into four main classes, depending on the location of two cysteine residues (C). They are, namely, CC chemokines, CXC chemokines, chemokines, and CX3C chemokines. In cancer development, chemokines have dual protumor and antitumor roles.\textsuperscript{53} Several protumor chemokines have been identified, including CCL2, CCL3, and CCL5, which can increase tumor invasion and metastasis. CCL2 activates the JAK/STAT and p38 MAPK signaling pathways and influences vascularization and metastasis by inducing MMP-9. CCL2, and CCL5 and recruiting MDSCs and macrophages into the TME. CCL18 also plays a role in invasion and metastasis, but this depends on the type of cancer. CXCL12 and VEGF promote angiogenesis in vascular endothelial cells.\textsuperscript{54} CXCL12 and its receptor CXCR4 induce cancer cell invasion and metastasis. Gefitinib-resistant A549 cells expressed high levels of CXCR4+ and showed increased phosphorylation of Akt, mTOR, and STAT3 (Y705). The antitumor effect of chemokines is through the activity of several chemokines such as CXCL8, CXCL9, and CXCL10. CXCL8 enhances the immunogenicity of cancer cells by translocating calreticulin to the cell surface. CXCL9 and CXCL10 inhibit angiogenesis in endogenous tumors.\textsuperscript{54,55} CXCL10 inhibits FGF-induced angiogenesis formation by CXCL8 in vivo and in vitro.\textsuperscript{56,57}

The activity of the immune system on the growth of cancer cells in the form of an inflammatory response is divided into acute inflammation and chronic inflammation. Acute inflammation can prevent cancer facilitated by NK cells, DC, M1 polarized macrophages, N1
polarized neutrophils, CD8+ cytotoxic T cells, and CD4+ T cells. These cells trigger the secretion of cytokines (IL-12, IL-15, IFN-γ, and IL-1) that induce an inflammatory response to kill cancer cells. A schematic illustration of the role of the immune system in the development of cancer is shown in Figure 2. The mechanism of each immune cell in acute inflammation in inhibiting cancer growth is as follows: NK cells produce granzymes and perforins, DC activated by tumor antigens induce the production of cytokines (IL-12, IL-15, and IFN-γ), mature DCs activate B cells, CD8+ T cells, and CD4+ T cells, and activate M1 macrophages and N1 neutrophils to phagocytose tumor cells. During infection M1-TAM and N1-TAN produce the cytokines TNF-α, IL-6, IL-1β. Continuous production of these proinflammatory cytokines will cause chronic inflammation and induce DNA damage through the formation of reactive oxygen and nitrogen species (RONS). This condition will recruit tumor-inducing immune cells consisting of TAM, TAN, Treg regulatory T cells, and MDSC. These cells stimulate the secretion of cytokines (IL-10, IL-6, and PGE-2), CXC motif chemokine ligand (CXCL1, CXCL6, and CXCL8) and CC motif chemokine ligand (CCL5 and CCL22), and growth factors (TGF-β, VEGF, EGF, and platelet-derived growth factor [PDGF]) that contribute to the formation of a proangiogenic and protumor microenvironment. In this TME, cancer cells also prevent tumor antigen presentation on DC. Continuous chronic inflammation will cause angiogenesis and metastasis in cancer cells associated with the TME.

Many studies have reported that inflammation is related to angiogenesis or neovascularization. This activity is mediated by tumor-inducing immune cells in the TME. Tumor-inducing immune cells, especially TAM and MDSC, secrete proangiogenic factors such as VEGF, EGF, PDGF, TGF-B, and cytokines (IL-8 and IL-10) that trigger angiogenesis. This new blood vessel formation recruits inflammatory cells to the site of inflammation, allowing it to continue.
Cancer cells can produce proangiogenic factors and inflammatory mediators through themselves or from the extracellular matrix, so cancer cells are constantly surrounded by inflammatory cells.\(^{59}\) In the TME, cancer cells, immune cells, and other stromal cells work together to form blood vessels and promote endothelial cell proliferation. This condition is the initiation of metastases.

The main requirement for cancer cells to metastasize is that they migrate to other sites in the body. Cancer cells must leave their primary site, invade surrounding tissues, and intravasate into blood and lymph vessels. Immune cells that play a role at this stage are TAM and TAN, which affect the organization of the extracellular matrix (ECM), cancer cell motility, and the formation of blood and lymph vessels.\(^{60}\) Tumor cells, immune cells, and stromal cells in the TME can affect the ECM by secreting ECM-remodeling enzymes (MMPs, cathepsins, and other proteases). ECM remodeling can support the invasion of tumor cells that change the shape of the surrounding tissue and increase the release of growth factors.\(^{61,62}\) The main route of migration of cancer cells to other places is through the blood and lymph vessels, called intravasation. After intravasation, circulating cancer cells must defend against immune cells such as NK cells to colonize distant sites. Treg cells can increase the survival of cancer cells in circulating blood vessels and lymph through the production of receptor activator of NF-κB ligand (RANKL).\(^{63}\) The next stage is establishing a pre-metastatic "niche," which is facilitated by bone marrow-derived cells (BMDCs) recruited to vital organs such as lung, liver, brain, and bone to provide a response to VEGFA and cancer cell-derived growth factors. Before the arrival of tumor cells in specific organs and after the establishment of a pre-metastatic "niche," cancer cells must extravasate, that is, migrate through modulation and attachment to the endothelium. Immune cells that play a role in the extravasation stage are neutrophils that release extracellular neutrophil traps (NETs). The final step is colonization and persistent growth of new cancer cells.\(^{60}\)
Immunomodulatory effects of A. paniculata and Its Bioactive Metabolites

Several in vitro and in vivo studies reported the immunomodulatory activity of A. paniculata extract and its bioactive metabolites to modulate the immune system, as summarized in Table 1. Regarding the immunomodulatory effects of A. paniculata, one study indicated that A. paniculata ethanolic extract (1–16 µg/ml) and andrographolide (0.5–8 µg/ml) inhibited in a dose-dependent manner the nitric oxide (NO) and PGE2 production resulting from NF-κB and MAPK signaling pathways induced by lipopolysaccharide (LPS) in RAW 264.7 cells. A. paniculata also exerts an effect on oxidative stress and inflammation in dermal fibroblasts (human dermal fibroblasts, adult [HDFa]). The production of reactive oxygen species (ROS) that play a role in oxidative stress was reduced in hydrogen peroxide-treated HDFa after incubation with methanolic extract of A. paniculata (5 µg/mL) and 14-deoxyandrographolide (1 µg/mL). The methanolic extract of A. paniculata (5 µg/mL) and andrographolide (5 µg/mL) exerted an anti-inflammatory effect by decreasing the expression and production of the inflammatory mediators TNF-α and IL-6 in HDFa cells stimulated by LPS and TNF-α, respectively. The production of TNF-α decreased after administration of A. paniculata extracts (16.4 µM and 18.7 µM) in a human monocytic cell line (THP-1) stimulated with LPS.

The results of previous in vitro studies reported that 19 diterpenoid lactone compounds isolated from A. paniculata decreased the production of TNF-α and IL-6 in LPS-treated RAW 264.7 cells. Andrographolide significantly inhibited NO production in LPS-treated RAW 264.7 cells, with an IC₅₀ value of 13.4 µM. Andrographolide concentrations of 6.25, 12.5, and 25 µg/ml significantly reduced IL-1β, IL-6, and TNF-α production and expression via NF-κB/MAPK signaling pathway in LPS-induced RAW 264.7 cells. Andrographolide activates the AMP protein kinase (AMPK) pathway.
inhibit the NF-κB/MAPK signaling pathway and the production of IL-1β, IL-6, inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein-1 (MCP-1), and cyclooxygenase (COX)-2 in LPS-treated macrophages.\textsuperscript{71,72} AMPK activation also provides anti-inflammatory effects on numerous immune cells, including mast cells, T cells, neutrophils, and macrophages.\textsuperscript{73,74}

Andrographolide suppressed expression of COX-2 and inhibited NF-κB in RAW 264.7 cells stimulated by Toll-like receptor 3 (TLR3) agonists (e.g., Poly [I: C]) and TLR4 agonists (e.g., LPS). Andrographolide also suppresses the toll-interleukin-1 receptor domain-containing adapter inducing interferon-β (TRIF) signaling pathway by targeting TANK-binding kinase 1 (TBK1). The TLR adapter molecules TRIF and TBK1 are kinases involved in the TRIF-dependent TLR signaling pathway. TRIF will facilitate TLR4 and TLR3 to activate transcription factors interferon regulatory factor (IRF3) and NF-κB. Phosphorylated IRF3 will be translocated into the nucleus to bind to an IFN-stimulated response element in the promoter regions of the genes interferon-γ-inducible protein 10 (IP-10) and IFN-β. This IRF3-regulated gene plays a crucial role in antibacterial and antiviral immune responses.\textsuperscript{75}

In the atherosclerosis rat model, \textit{A. paniculata} extract doses of 0.6 and 2.4 mg/kg body weight (BW) significantly reduced the number of Th17 cells. Moreover, \textit{A. paniculata} extract at 1.2 mg/kg BW significantly increased the number of Treg cells. The ratio of Treg cells to Th17 cells significantly increased after administration of \textit{A. paniculata} extract at 0.6 and 2.4 mg/kg BW.\textsuperscript{76} Ethyl acetate extract of \textit{A. paniculata} (0.78, 1.56, 3.12, and 6.25 mg/kg BW) reduced TNF-α, IL-12, macrophage inflammatory protein (MIP)-2, and NO in serum and peritoneal macrophages isolated from BALB/c mice treated with LPS.\textsuperscript{77} Andrographolide at 200 mg/kg BW and \textit{A. paniculata} extract at 100, 200, and 400 mg/kg BW given orally once a day for 28 days significantly inhibited the NF-κB signaling pathway through the upregulation of Iκβα
phosphorylation, p65 protein expression, and nuclear translocation in male Wistar rat treated with fine particulate matter (PM2.5) at 8 mg/kg BW.\textsuperscript{78}

As shown in previous studies, \textit{A. paniculata} and its bioactive metabolites also enhance the immune response. Water extract of \textit{A. paniculata} (200 and 400 µg/ml) can stimulate the proliferation of human peripheral blood mononuclear cells (PBMCs) and the production of IFN-γ and TNF-α.\textsuperscript{79} \textit{A. paniculata} ethanol extract (1 µg/mL) can stimulate the proliferation of lymphocyte cells by approximately 38% compared with control lymphocyte cells without treatment.\textsuperscript{80} Extract of \textit{A. paniculata} (100 mg/kg BW) increased the number of lymphocytes and macrophages significantly.\textsuperscript{81}

\textbf{Anticancer effects of \textit{A. paniculata} and Its Bioactive Metabolites}

\textit{A. paniculata} ethanolic extract has anticancer activity in neuroblastoma cancer cells (IMR-32) and colon cancer cells (HT-29) with an IC\textsubscript{50} value of 200 µg/mL. An aqueous ethanol and acetone extract of \textit{A. paniculata} 200 µg/mL also exerted anticancer activity on HT-29 cancer cells.\textsuperscript{91} A methanolic extract of \textit{A. paniculata} (1.0 µg/mL) inhibited the growth of leukemia cancer cells (P388).\textsuperscript{92} MCF-7 breast cancer cells treated with \textit{A. paniculata} extract (111 and 222 ppm) significantly inhibited cell proliferation by 47.98% and 30.50%, respectively, compared with the control group.\textsuperscript{93} \textit{A. paniculata} hydroalcoholic extract at a dose of 100 µg/mL can inhibit the growth of an ovarian cancer cell line (ovcar-5) by as much as 51.12%.\textsuperscript{94} A water extract of \textit{A. paniculata} (850.3 g/mL) significantly reduced EGF receptor (EGFR) and Akt mRNA expression in human EC-109 cells. The suspected compounds involved were panicolin and moslosooflavone, which were confirmed with liquid chromatography–mass spectrometry (LC-MS).\textsuperscript{95}
The major phytochemicals of A. paniculata are andrographolide, 14-deoxy-11,12-didehydroandrographolide, and neoandrographolide, which each have anticancer activity on various cancer cells.

It was shown that the 10 µM concentration of andrographolide could significantly induce apoptosis. Moreover, it can significantly decrease IL-6 protein and reduce phosphorylation of STAT3 and ERK in PC-3 and DU145 cells. Andrographolide (5, 10, and 25 µM) significantly inhibited proliferation and invasion of nasopharyngeal carcinoma (NPC) cells in a dose- and time-dependent manner. Andrographolide inhibited the transcriptional activity of NF-κB in TNF-α induced NPCs. Inhibition of NF-κB by andrographolide decreased the expression of surviving cyclin-D1 and EGFR, which play a role in cell proliferation and survival. Furthermore, andrographolide also decreased MMP-9, intercellular adhesion molecule 1 (ICAM-1), and VEGF expression, leading to metastasis and invasion of cancer. Andrographolide also induced the G2/M cell cycle arrest.

Andrographolide (10 µM) suppressed clonogenicity, induced apoptosis, and promoted G1/S cell cycle arrest. Andrographolide inhibits the TLR4/NF-κB signaling pathway through decreased expression of the proteins TLR4, myeloid differentiation primary response 88 (MyD88), p-Iκβα, and p-p65 in B16 cells. This study also demonstrated the anti-melanoma effect of andrographolide with siRNA for TLR4 knockdown in B16 cells. The results showed no significant difference between TLR4 knockdown B16 cells administered 10 M andrographolide. In the NF-κB pathway, andrographolide inhibits the expression of Bcl-6, an antitumor gene, and CXCR4 as a promoter of the NF-κB target gene that activates transcription factors. Andrographolide at 100 µM induces DNA damage through increasing the expression of phosphor-H2AX in HepG2 and HeLa cells. Andrographolide at 100 µM induced the G2/M phase in HepG2 cells.
Andrographolide at 10 µg/mL significantly induced apoptosis in Jurkat cells through decreasing the activation of the P13K/Akt signaling pathway.\textsuperscript{100}

14-deoxy-11,12-didehydroandrographolide had antiproliferative activity on THP-1 and Jurkat leukemia cells with IC\textsubscript{50} values of 35,209 ± 2.209 µg/ml and 30,062 ± 1,241 µg/ml, respectively. 14-deoxy-11,12-didehydroandrographolide also decreased glutathione (GSH) levels by 19.76% and increased pro-caspase-3 expression in THP-1 cells, indicating redox-mediated cell death.\textsuperscript{101} 14-deoxy-11,12-didehydroandrographolide showed cytotoxic activity on PANC-1 and PSN-1 pancreatic cancer cells with IC\textsubscript{50} values of 10.0 and 9.27 µM, respectively.\textsuperscript{102} 14-deoxy-11,12-didehydroandrographolide has cytotoxic activity on leukemia cancer cells (U937) with an IC\textsubscript{50} value of 13 µM. These compounds can also induce apoptosis in a concentration-dependent manner and increase the activation of caspase-3 and caspase-9. Cell cycle analysis revealed that 14-deoxy-11,12-didehydroandrographolide induced G\textsubscript{0}/G\textsubscript{1} cell cycle arrest.\textsuperscript{103} 14-Deoxy-11,12-didehydroandrographolide is cytotoxic to T47D breast cancer cells, induces stress on the endoplasmic reticulum (ER), and causes the autophagosomes of the T47D. 14-Deoxy-11,12-didehydroandrographolide also upregulates LC3-II. The signaling pathway involved is GADD45A/p38 MAPK/DDIT3.\textsuperscript{104}

Neoandrographolide is one of the major compounds from \textit{A. paniculate}. It also has a cytotoxic effect on several human cancer cells, including PC-3 prostate cancer cells (IC\textsubscript{50} 12.7 ± 0.34 µM), lung cancer cells A549 (IC\textsubscript{50} 5.53 ± 0.34 µM), and colon cancer cells SW-620 (IC\textsubscript{50} 9.5 ± 0.89 µM). In this study, the same cancer cell was also treated with a neoandrographolide derivative compound with a modified carbohydrate structure, namely 4′,6′-benzylidene neoandrographolide. This compound had more potent cytotoxic activity than neoandrographolide.
on SW-620, PC-3, and A549 cancer cells, with IC₅₀ values of 6.2 ± 0.21, 2.65 ± 0.78, and 1.75 ± 0.33 µM, respectively. Administration of 4′,6′-benzylidene neoandrographolide also increased cellular ROS production and induced apoptosis through increased expression of caspase-3 Bax and decreased expression of Bcl-2.¹⁰⁵

*Annuus paniculata* hot water extract (1600 mg/kg) decreased the number of regulatory T cells (CD4+CD25+Foxp3+) in lymph nodes and spleen. IL-10 and IL-12 cytokines decreased significantly in leukocytes isolated from the spleen of C57BL/6 mice induced by 4-nitroquinoline 1-oxide (NQO) at 60 µg/mL. This extract also worked synergistically with cisplatin + 5-fluorouracil in inhibiting tumor growth. The side effects of chemotherapy agents can also be reduced by administering an aqueous extract of *A. paniculata*.⁷⁹ Andrographolide at 25 and 100 mg/kg BW inhibited the expression of p-p65, c-Jun, p-Akt, osteopontin, and downregulated cyclin D1 and VEGF in the orthotopic NOD/SCID mouse model induced with MDA-MB-231 cells. This study showed that andrographolide downregulates the expression of p-p65, c-Jun, p-Akt, osteopontin, VEGF, and Flk 1 in anticancer and antiangiogenic activity.¹⁰⁶

**Table 2. The mechanisms of anticancer *A. paniculata* and its bioactive metabolites.**

**The potential of *A. paniculata* in inhibition of metastasis and angiogenesis**

Andrographolide inhibits A549 non-small human lung cancer invasion and migration by decreasing MMP-7 expression via the PI3K/Akt/AP-1 signaling pathway.¹⁰⁹ Andrographolide downregulates MMP-9 expression by inhibiting NF-κB in H3255 lung cancer cells. These effects will inhibit the migration and invasion of cancer cells.¹¹⁰ Andrographolide inhibits 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced migration and invasion of MCF-7 breast cancer
cells by reducing MMP-9 activation through inhibiting the ERK1/2, P3K/Akt, AP-1, and NF-κB signaling pathways. Anandrographolide inhibits the expression of VEGF and hypoxia-inducible factor-1 (HIF-1α), which play an essential role in angiogenesis and metastasis in non-small cell lung cancer cells. An aqueous extract of *A. paniculata* inhibited the migration of esophageal cancer cells and endothelial cells by suppressing MMP-2 activity. Andrographolide inhibits angiogenesis by blocking the VEGFA-induced activation of VEGFR2 and the MAPK signaling pathway in Hep3B cells. Andrographolide significantly decreased the expression of hVEGF-A165 and VEGF, EGFR, cyclin A, and cyclin B, which exerted an antiangiogenic effect.

**The potential of *A. paniculata* in inhibition of chemotherapy resistance**

Chemotherapy induces signaling pathways that inhibit and kill cancer cells and stimulate signals that lead to metastasis. One mechanism for cancer resistance to chemotherapy is mediated by the interaction of tumor cells with the TME, which induces stromal cells to produce cytokines, chemokines, and growth factors. Inflammation will form the TME, which plays a vital role in chemotherapy resistance to metastasize cancer cells. Several chemotherapy drugs, cisplatin, paclitaxel, 5-fluorouracil, and doxorubicin, are known to induce inflammation leading to metastasis of cancer cells.

Cisplatin can induce MAPK, ERK, and epithelial-mesenchymal transition (EMT) signaling pathways in cancer that play a role in cancer cell invasion and metastasis. Paclitaxel regulates the inflammatory mediators IL-1β, IL-8, IL-6, and VEGFA via the AP-1 and NF-κB signaling pathways. Previous studies have shown that paclitaxel increases the production of cytokines via TLR4 in breast cancer cells. Paclitaxel induces the Ras/Raf/mitogen-activated protein kinase/ERK kinase (MEK)/ERK pathway activation in human breast cancer and lymphoma. Paclitaxel activates the TLR4-MyD88-ERK signaling pathway closely associated with
tumor growth, development, invasion, and chemoresistance.\textsuperscript{118} 5-fluorouracil activated the p38 MAPK signaling pathway in murine macrophage cells by increasing the production of IL-1β, IL-6, and TNF-α.\textsuperscript{119} Other studies have shown that 5-fluorouracil upregulates NF-κB, MAPK, and thymidylate synthase in breast cancer cells. Administration of doxorubicin to various cancer cells increased the production of prostaglandin E2 and IL-1β. Doxorubicin also activates NF-κB through p53 induction in neuroblastoma cells. Doxorubicin increased the expression of IL-1β and IL-6 mRNA in murine macrophages, which was mediated by p38 MAPK.\textsuperscript{120}

All inflammatory mediators, growth factors, and signaling pathways involved in chemotherapy-induced inflammation play an essential role in regulating the TME leading to resistance, invasion, metastasis, and angiogenesis of cancer cells. As indicated in previous reports, \textit{A. paniculata} suppresses inflammation through inhibiting the production of inflammatory mediators and related signaling pathways. Thus, \textit{A. paniculata} can be used as an adjunct to chemotherapy to inhibit the development of resistance.

Table 1 and Table 2 show that \textit{A. paniculata} has immunomodulatory and anticancer activity. The activity of \textit{A. paniculata} in inhibiting inflammatory cytokines from various signaling pathways in immune cells can be developed as an anticancer agent. As in previous reports, immunomodulatory-anticancer agents can be obtained through two mechanisms; the first is blocking the initiation of cancer cells and the second is inhibiting the proliferation of cancer cells at the stage of angiogenesis and cancer metastasis. The first mechanism of inhibiting the initiation of cancer is a form of acute inflammation, whereas the second mechanism of inhibiting angiogenesis and metastasis can be in the form of suppressing the activity of pro-tumor immune cells, cytokines, chemokines, and growth factors. The molecular mechanism of \textit{A. paniculata} in
modulating the immune response in cancer-associated chronic inflammation, angiogenesis, and metastasis is shown in Figure 3.

The mechanism of *A. paniculata* in inhibiting inflammation can be the basis for developing immunomodulatory-anticancer agents through the second mechanism. It is also supported by previous studies showing that *A. paniculata* inhibits angiogenesis and metastasis through suppressing the production of MMP-7, MMP-9, MMP-2, VEGF, EGFR, cyclin A, cyclin B, and HIF-1α through the PI3K/Akt/ATP-1, NF-kB, ERK1/2, and MAPK signaling pathways. Andrographolide is the primary bioactive metabolite that plays a role in inhibiting the inflammatory response. Interestingly, Table 1 provides information that *A. paniculata* can also enhance the immune response. These activities can be used as the basis for developing immunomodulatory-anticancer agent therapy. The first mechanism is to block the initiation of cancer cells in the form of an acute inflammatory response. The previous report also stated that the activity of the immune response in the form of acute inflammation could inhibit the development of cancer cells.

Several chemotherapy drugs such as cisplatin, paclitaxel, 5-fluorouracil, and doxorubicin can increase inflammation through various pathways such as NF-kB, MAPK, ERK, and TLR that play a role in the resistance, metastasis, invasion, and angiogenesis of cancer cells. *A. paniculata* is known to have a mechanism that inhibits these signaling pathways, so combination chemotherapy with *A. paniculata* can be an alternative in suppressing potential chemotherapy resistance. *A. paniculata* also can be a chemotherapy adjuvant through overcoming chemotherapy resistance associated with TME regulation.

Much research on *A. paniculata* and its bioactive metabolites as immunomodulatory and anticancer agents has been performed in the last ten years. However, there are still many problems
to be solved. First, this study shows that *A. paniculata* extract suppresses the immune response and stimulates an immune response in the form of inflammation. The activity of *A. paniculata* in promoting the inflammatory response can be developed as an anticancer agent at the stage of cancer initiation. Meanwhile, the effect of *A. paniculata* in suppressing the inflammatory response can use for cancer therapy in cancer-related chronic inflammation, angiogenesis, and metastasis. Its action in stimulating and suppressing the immune response in inflammation may be dose-dependent, so to be developed as an anticancer agent, further research is needed to determine the dose of *A. paniculata*. Second, the bioactive metabolite that plays a role in inhibiting inflammation is andrographolide, so further research is needed on compounds that play a role in immunostimulant activity using a bioassay-guided isolation method. If the active compounds are known, it will make it easier to develop products by maximizing the extraction method to obtain these compounds. Third, the use of *A. paniculata* as an anticancer agent and inhibition of chemotherapy resistance associated with the TME needs to be explored. Research on the effect of *A. paniculata* on other components of the TME, such as TAM, MDSCs, and TAN, is necessary.

**Conclusion**

*A. paniculata* and its bioactive metabolites have immunomodulatory and anticancer activities. *A. paniculata* suppresses cancer-associated chronic inflammation, angiogenesis, and metastasis through the modulation of immune responses. *A. paniculata* and its bioactive metabolites can be developed as anticancer agents that inhibiting the proliferation of cancer cells at the angiogenesis and metastatic stages. *A. paniculata* can also be developed as an adjuvant chemotherapy that inhibits chemotherapy-induced TME-associated inflammation.

**Author Contribution**
Rengganis Ulvia participated in the study concept and design, data collection, analysis, and interpretation of results, wrote the paper, and drafted the manuscript. Retno Murwanti and Andayana Puspitasari Gani participated in the development of study concept and design, editing the manuscript, critical interpretation, and scientific guidance throughout the development of the paper. All authors approved the final version of the manuscript.

Conflict of Interest

The authors have no conflict of interests to declare.

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Table 1. The mechanisms of immunomodulatory effects of *A. paniculata* and its bioactive metabolites.
<table>
<thead>
<tr>
<th>Extract/Compound</th>
<th>Study Type</th>
<th>Stimulated Cells</th>
<th>Effect on NO, PGE-2</th>
<th>Effects on Inflammation Mediators</th>
<th>Additional Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract of <em>A. paniculata</em></td>
<td>In vitro</td>
<td>LPS-stimulated RAW 264.7 cells</td>
<td>↓</td>
<td>↓NO, PGE-2</td>
<td>Inhibits phosphorylation of p38, JNK, ERK (MAPK pathway), and IkBα.</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>In vitro</td>
<td>LPS-stimulated RAW 264.7 cells</td>
<td>↓</td>
<td>↓NO</td>
<td></td>
</tr>
<tr>
<td>Diterpenoid Lactone</td>
<td>In vitro</td>
<td>LPS-stimulated RAW 264.7 cells</td>
<td>↓</td>
<td>↓TNF-α and IL-6</td>
<td></td>
</tr>
<tr>
<td>Andrographolide</td>
<td>In vitro</td>
<td>LPS-stimulated RAW 264.7 cells</td>
<td>↓</td>
<td>↓TNF-α, IL-6, and IL-1β</td>
<td>Inhibits phosphorylation of IkBα, p38, JNK, ERK 1/2.</td>
</tr>
<tr>
<td>Neoandrographolide, Dehydroandrographolide, 5, 7, 2’, 3’-sitosterol</td>
<td>In vitro</td>
<td>RAW 264.7 cells</td>
<td>↓</td>
<td>↓NO</td>
<td></td>
</tr>
<tr>
<td>Andrographolide</td>
<td>In vitro</td>
<td>Cigarette smoke extract-stimulated RAW 264.7 cells</td>
<td>↓</td>
<td>↓TNF-α dan IL-1β</td>
<td></td>
</tr>
<tr>
<td>Andrographolide</td>
<td>In vitro</td>
<td>LPS-stimulated RAW 264.7 cells</td>
<td>↓</td>
<td>Inhibits of NF-κB/MAPK pathway, activation of AMPK pathway</td>
<td></td>
</tr>
<tr>
<td>Andrographolide</td>
<td>In vitro</td>
<td>Poly [I: C] and LPS-stimulated RAW 264.7 cells</td>
<td>↓</td>
<td>Inhibits TLR from TRIF and TBK1 pathway, ↓IRF3 and ↓IFN-β, IP-10 mRNA expression</td>
<td></td>
</tr>
<tr>
<td>Methanol extract of <em>A. paniculata</em> and 14-deoxyandrographolide</td>
<td>In vitro</td>
<td>LPS-stimulated HDFa</td>
<td>↓</td>
<td>↓IL-6, TNF-α</td>
<td></td>
</tr>
<tr>
<td><em>A. paniculata</em> extract and andrographolide</td>
<td>In vitro</td>
<td>LPS-stimulated human monocytic cell line THP-1</td>
<td>↓</td>
<td>↓TNF-α</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate extract of <em>A. paniculata</em></td>
<td>In vivo</td>
<td>LPS-stimulated BALB/c mice</td>
<td>↓</td>
<td>↓TNF-α, ↓IL-12, ↓MIP-2, ↓NO</td>
<td></td>
</tr>
</tbody>
</table>
A.paniculata extract | In vivo | Atherosclerosis rat model | ↓ | Th17 cell ↑ Treg cell | 90

A.paniculata extract and andrographolide | In vivo | PM2.5-stimulated rat | ↓ | NF-κB p65 and IκBα phosphorylation | 78

Water extract of A. paniculata | In vitro | Peripheral blood mononuclear cells (PBMCs) | ↑ | PBMC proliferation, ↑ TNF-α, IFN-γ | 79

Ethanol Extract of A. paniculata | In vitro | Lymphocytes | ↑ | Lymphocyte cell proliferation | 80

Extract of A. paniculata | In vivo | Type 2 diabetic rats | ↑ | Macrophage, lymphocytes | 81

Table 2. The mechanisms of anticancer A. paniculata and its bioactive metabolites.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Study design</th>
<th>Subject</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. paniculata ethanolic extract</td>
<td>In vitro</td>
<td>IMR-32 and HT-29 cells</td>
<td>IC_{50} value: 200 µg/mL</td>
</tr>
<tr>
<td>A. paniculata aqueous, ethanol and acetone extract</td>
<td>In vitro</td>
<td>HT-29 cells</td>
<td>IC_{50} value: 200 µg/mL</td>
</tr>
<tr>
<td>A. paniculata methanolic extract</td>
<td>In vitro</td>
<td>P388 cells</td>
<td>1.0 µg/mL mL inhibit growth of P388 cells</td>
</tr>
<tr>
<td>A. paniculata hydroalcoholic extract</td>
<td>In vitro</td>
<td>ovcar-5 cells</td>
<td>100 µg/ml can inhibit 51.12% ovcar-5 cells</td>
</tr>
<tr>
<td>Water extract of A. paniculata</td>
<td>In vitro</td>
<td>EC-109 cells</td>
<td>↓ EGFR and Akt mRNA expression</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>In vitro</td>
<td>DU145 and PC-3 cells</td>
<td>↓ apoptosis and ↓ IL-6 ↓ phosphorylation of STAT3 and ERK</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>In vitro</td>
<td>IMDA-MB-231 cells</td>
<td>20 µM ↓ viability, proliferation, migration, ↑ apoptosis, cell cycle arrest G2/M phase</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>In vitro</td>
<td>NP cells</td>
<td>5, 10, 25 µM ↓ proliferation an invasion in time and dose-dependent, ↓ NF-κB, survivin, cyclin D1, EGFR, MMP-9, VEGF, and ICAM-1, ↑ cell cycle arrest G2/M phase</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>In vitro</td>
<td>A375 and C8161 cell</td>
<td>↓ proliferation ↑ cell cycle arrest G2/M phase, apoptosis, ↑ JNK and p-p38</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>In vitro</td>
<td>B16 cells</td>
<td>IC_{50} : 10 µM ↑ cell cycle arrest G1/S phase, apoptosis, ↓ TLR4, MyD88, pIκBα, pp65, Bcl-6 and CXCR4</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>In vitro</td>
<td>HepG2 cells</td>
<td>↑ cell cycle arrest G2/M phase,</td>
</tr>
<tr>
<td>Compound</td>
<td>Cell Type</td>
<td>In Vitro Model</td>
<td>Concentration</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>----------------------------</td>
<td>----------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>Jurkat cells</td>
<td>In vitro</td>
<td>10 µg/mL</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>MDA-MB-231 cells</td>
<td>In vitro</td>
<td>IC₅₀ 35.209 ± 2.209 g/ml and 30.062 ± 1.241 µg/ml</td>
</tr>
<tr>
<td>14-deoxy-11 12-didehydroandrographolide</td>
<td>THP-1 and Jurkat cells</td>
<td>In vitro</td>
<td>IC₅₀ 10.0 µM and 9.27 µM</td>
</tr>
<tr>
<td>14-deoxy-11 12-didehydroandrographolide</td>
<td>PANC-1 and PSN-1 cancer cells</td>
<td>In vitro</td>
<td>IC₅₀ 13 µM</td>
</tr>
<tr>
<td>Neoandrographolide</td>
<td>PC-3 cells, A5-49 cells, SW-620 cells</td>
<td>In vitro</td>
<td>IC₅₀ 6.2 ± 0.21 µM, IC₅₀ 2.65 ± 0.78 µM, IC₅₀ 1.75 ± 0.33 µM</td>
</tr>
<tr>
<td>4′,6′-benzylidene neoandrographolide</td>
<td>PC-3 cells, A5-49 cells, SW-620 cells</td>
<td>In vitro</td>
<td>IC₅₀ 1600 mg/kg</td>
</tr>
<tr>
<td>Hot water extract of A. paniculata</td>
<td>C57BL/6 mice carcinogen-induced esophageal tumorigenesis</td>
<td>In vivo</td>
<td>5 µg/g reduced tumor volume, ↓cell proliferation and angiogenesis</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>C57BL/6J mice induced B16 cells</td>
<td>In vivo</td>
<td>25 mg/KgBW and 100 mg/KgBW andrographolide</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>NOD/SCID mouse model induced MDA-MB-231 cells</td>
<td>In vivo</td>
<td>100 mg/KgBW andrographolide</td>
</tr>
</tbody>
</table>
Figure 1. Chemical structure of (a) Andrographolide; (b) 14-deoxy-11 12-didehydroandrographolide; (c) Neoandrographolide
Figure 2. Schematic illustration of the role of the immune system in cancer development.

a. acute inflammation; b. Chronic inflammation; c. Angiogenesis and metastasis.
Figure 3. The molecular mechanism of *A. paniculata* in modulating the immune response in (a) chronic inflammation; (b) angiogenesis and metastasis; *A. paniculata* (AP).