Anticancer, Antimicrobial and other Pharmacological Properties of Shikonin and its Derivatives

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ABSTRACT
Shikonin is a lipophilic red pigment and is found in the outer surface of the roots of Lithospermum erythrorhizori of the Boraginaceae family. It is a potent pharmaceutical substance with a well-known and extensive range of anticancer, antimicrobial, anti-inflammatory, antioxidant and wound healing activity. Significant research has been conducted on shikonin effectiveness on several tumours and on their mechanism of anticancer action. Shikonin and its derivatives have some antiproliferation and antimetastasis activities in various cancer types both in vitro and in vivo. Few decades onward, a wealth of new information arising from research efforts, on the anticancer and antimicrobial properties of shikonin has been accumulated. Therefore, the aim of this review is to provide an updated data published on antimicrobial, anticancer activities, and other health properties of shikonin and their derivatives. Furthermore, the novel mechanisms for shikonin and its derivatives reported in the contemporary study make these compounds attractive candidates for the treatment of deadly diseases.

Keywords: Lithospermum erythrorhizori, Shikonin, Alkannin, Anticancer, Antimicrobial activity, Pharmacological properties.

INTRODUCTION
Shikonin is a natural product isolated from outer surface of Chinese herbal plant Lithospermum erythrorhizori (Boraginaceae) roots. Shikonin, derived components are lipophilic red pigments, with naphthazarin skeleton are present as low molecular weight fatty acid ester in the roots1. European countries, Shikonin and its stereoisomer, alkannin, have been used as a food pigment1. The naphthoquinones shikonin and its derivatives are the main active molecules present in traditional Chinese herbal medicine Zicao2. It is widely planted in Korea, Japan, and China, and it is used as a dye for food colorants and fabrics staining. This plant has been classified as edible by the KFDA (Korea Food & Drug Administration)3. Shikonin was first isolated as acetylshikonin from the roots of Lithospermum erythrorhizior in Boraginaceae family4. Brockmann and Liebig were the first to define the correct structure of this molecule as 5, 8-dihydroxy-2-[(1R)-1-hydroxy-4-methyl-3-pentenyl]-1,4-naphthoquinone and identify shikonin’s enantiomer alkannin in 19365. Furthermore, shikonin, alkannin and other derivatives also found in several other species of Boraginaceae family, such as Arnebia euchroma, Echium lycoris, Eritrichium sericeum and Onosma armeniacum6. Recent strategies such as cell tissue cultures and total synthesis have been successfully applied for production of shikonin and its derivatives5. Over the past 40 years several biological investigations have demonstrated therapeutic effects of shikonin and its derivatives, including antioxidant, antimicrobial, anti-inflammatory, anticancer2, and, wound healing effects6, granulation tissue-forming activity7,8, antibacterial9 and antiulcer activity10. Shikonin derivatives are widely used for external injuries such as hemorrhoids, burns and cuts. Furthermore, it is believed to be able to remove heat from the blood and possess properties of detoxification, and also it has been used effective treatment for a variety of inflammatory and infectious diseases, macular eruptions, measles, sore-throat, carbuncles and oozing dermatitis11. Their diversified beneficial properties stand for a sound scientific basis for the use of Zicao in folk medicine to treat a variety of inflammatory and infectious diseases. Antitumour activity of Shikonin
Shikonin and its derivatives were first found exhibiting in vitro cytotoxicity against cancer cells in 1974 during a mass screening programs of natural products conducted by the National Cancer Institute of the USA12. Shikonin, one of the key active components of Lithospermum erythrorhizori, has been shown to exert antitumour effects both in vitro and in vivo12,13. Several in vitro and in vivo studies have been demonstrated the antitumour activities of shikonin and its derivatives towards various types of cancer cells, such as leukemia cells14,15, breast cancer cells16-18, glioma cells19,20, bladder cancer cells21 and lung cancer cells22. The molecular mechanisms underlying shikonin and derived components antitumor activities are differ depending on the cell type and treatment method2. Primarily they exert their antitumor effects by inhibiting

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cell growth and inducing apoptosis through a classic caspases-dependent pathway. A wide range of anticancer mechanisms are involved and potential role in shikonin-induced apoptosis, such as generation of reactive oxygen species (ROS), suppression of nuclear factor (NF)-κB-regulated gene products, activation of caspases-9, -8 and -3, release of the mitochondrial proteins cytochrome c, cleavage of poly (ADP-ribose) polymerase (PARP), upregulation of p53, cell cycle arrest with concomitant downregulation of cyclin dependent proteins, decreased Bcl-2 expression and increased Bax expression, etc. Furthermore, shikonin and its derivatives have been shown to induce a non-apoptotic cell death known as necroptosis by bypass apoptotic/drug resistance. In addition, shikonin also functions as a proteasome inhibitor and topoisomerase I inhibitor. The anticancer effects of shikonin were also determined in both allografts and xenografts animal models in vivo. ROS generation, inactivation of NF-κB, regulation of epidermal growth factor receptor and insulin-like growth factor 1, and activation of caspases may be involved in the anti-cancer mechanisms of shikonin. This quinone inhibits Topo II activity and results in DNA damage in cancer cells. Additionally, shikonin also has anti-cancer effects as an anti-estrogen agent by reversing NQO1 expression. It acts as a selective estrogen enzyme modulator by downregulating the expression of steroid sulfatase, important for the biosynthesis of estrogen. Furthermore, pyruvate kinase-M2 and proteasome are also inhibited by shikonin or its analogs. Shikonin performs as a strong candidate for being an innovative therapeutic for breast cancer and its mechanism of action should be further studied for the identification of novel targets specific to breast cancer subtype. The previous studies reports noticed that shikonin has been used to induce antitumor activity in breast cancer through targeting multiple signaling pathways. In addition, shikonin is able to sensitize breast cancer cells to some chemotherapeutic drugs, which potent to help solving the problem of drug resistance. Moreover, it has significant anti-migration and anti-invasion characteristics in several cancers. However, Shikonin induces apoptosis in a classic caspase dependent pathway in cervical, bladder and melanoma cancer cells and it besides induces necroptosis regardless of the drug concentration in caspase-3-negative MCF-7 cells. Furthermore, various concentrations of shikonin prompt either apoptosis or necroptosis, and necroptosis converts to apoptosis in the presence of Nec-1 in HL-60 and K562 cells. Shikonin induces growth inhibition and apoptosis in some cancer cells may be attributed to the inactivation of NF-B activity or increasing Annexin V signal and CD95 (Fas/APO) expression. Additionally, shikonin also prompt apoptosis via ROS production in osteosarcoma and Bcr/Abl-positive CML cells. Several different mechanisms contribute to the anticancer activities of shikonin. For example, shikonin suppresses proteasomal activities thereby inhibiting tumor growth in both H22 allografts and PC-3 xenografts and inhibits topoisomerase IIα and down-regulates ER2 and activates NFE2-related factor 2 as an anti-estrogen agent in human breast cancer. Shikonin modulates an estrogen enzyme by down-regulating the expression of steroid sulfatase, which is important for estrogen biosynthesis. Furthermore, shikonin obstructs tumor invasion via the NF-B signaling pathway in human high-metastatic adenoid cystic carcinoma cells. Consequently, shikonin may directly or indirectly inhibit or modulate cancer related cellular targets. Shikonin has been shown that ability to induce apoptosis by sequential activation of caspases in A375-S2 and COLO205 cells. Shikonin suppresses osteosarcoma signaling pathways and induces apoptosis in osteosarcoma cells. For example, shikonin suppresses osteosarcoma signaling pathways and induces apoptosis in osteosarcoma cells. Additionally, shikonin prompts cell death in HL60 human myeloid leukemia cell line. Shikonin inhibited the tumor growth in murine Sarcoma-180. Shikonin inhibited the tumor growth in murine Sarcoma-180. Consequent studies discovered that shikonin prompted apoptotic cell death in several cancer types, which involved multiple cellular targets. It induced apoptosis by activation of caspase-3 in leukemia, bladder and cervical cancer cells. In caspase-3-negative MCF-7 cells, it triggered necroptosis that contributed to overcome Bcl-2 and Bcl-XL mediated apoptotic resistance. Shikonin inhibited the activity of topoisomerase II and NF-κB, both of which are potential targets for chemotherapy. Shikonin with treatment led to cell cycle arrest over up-regulation of p53 and down-regulation of cyclin-dependent protein kinase 4 in malignant melanoma. Shikonin induced apoptosis may possibly protected by N-acetylcysteine (NAC) in SKHep-1 hepatoma, suggested that it targeted an oxidative stress mediated pathway. Shikonin applies its antitumour activity by prompting apoptosis through ROS accumulation and inactivation of Akt and RIP1/NF-κB pathways in liver cancer cells. It inhibits cancer cell growth by blocking cell cycle progression in G1 phase, inhibiting antitelomerase, DNA topoisomerase activity, and antiangiogenesis. However, shikonin inducing cancer cell apoptosis in human malignant melanoma, colorectal carcinoma, and leukemia cells through successive activation of caspases. It induced apoptosis through a ROS/JNK-mediated process in Bcr/Abl-positive chronic myelogenous leukemia cells. Antimicrobial effects of Shikonin

Shikonin exhibited similar antibacterial activities against both Methicillin-Sensitive S. aureus and Methicillin-Resistant S. aureus in vitro, almost the same as silver sulfadiazine. Subsequently, a shikonin ointment was prepared in PEG 400 and PEG 4000 base (0.1% shikonin) and exhibited an antibacterial effect against S. aureus in...
Table 1: Other medicinal properties of Shikonin and its derivatives

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<thead>
<tr>
<th>Pharmacological properties</th>
<th>Recent reports</th>
<th>References</th>
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<tr>
<td>Anti-viral properties</td>
<td>Shikonin has been anticipated for the development of novel anti-HIV drugs due to its chemokine receptor inhibitor properties and it has ability to inhibit the replication of several HIV strains. It has been patented for downregulating human papilloma virus protein expression in human papilloma infected patients. Additionally, acetylschikonin and -hydroxyisovalerylshikonin are effective against plant pathogens, such as fungi (Cladosporium herbarum) and virus (TMV-Tobacco Mosaic Virus).</td>
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<td>Anti-inflammatory activity</td>
<td>Shikonin has been shown to inhibit the biosynthesis of leukotriene B4 and 5-hydroxyeicosatetraenoic acid, which play a key role in the mechanism of its anti-inflammatory effects. -dimethylacyrshikonin, isovaleryl-shikonin and acetylschikonin strongly suppressed carageenan induced paw edema, with acetylschikonin revealing the greatest anti-inflammatory activity. Shikonin esters also been patented for their anti-inflammatory, antibacterial activity and treatment of several inflammatory diseases. Shikonin is recommended as a useful anti-inflammatory agent for selectively blocking the binding of CCR1 ligands.</td>
<td>50, 56, 57, 9</td>
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<td>Leukotrienes Metabolic Pathway Inhibition</td>
<td>Shikonin inhibits the biosynthesis of leukotriene B4 and 5-hydroxyeicosatetraenoic acid and it indicates an inhibitory effect upstream of 5-hydroxyeicosatetraenoic acid. Leukotrienes are eicosanoid lipids they act as major Chemokine (C-C motif) ligand 5 (CCL5) and macrophage inflammatory protein-1 (MIP-1) are selectively blocked by Shikonin.</td>
<td>58, 59</td>
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<td>Antioxidant Properties</td>
<td>Two caffeic acid esters of Shikonin possessed the greatest antioxidant capacity. Shikonin has been applied in tobacco smoke detoxification from peroxysteradicals. Evidence implicating the ROS scavenging property of Shikonin as causative to NF-kappa beta downregulation comes from studies that link in vitro induction of apoptosis of squamous cell carcinoma cell lines to downregulation of NF-kappa beta activity by Shikonin.</td>
<td>60, 61</td>
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<td>Wound Healing effect</td>
<td>Shikonin treated wounds, as determined by histological examination, experienced an increase in re-epithelialization, neovascularization, collagen synthesis, fibronectin as compared to non-treated. Increased levels of Transforming growth factor beta-1 (TGFβ1) in wound healing were observed immunohistologically after application of -dimethylacyrshikonin (Arnebin-1). Shikonin ointments were prepared and existing an enhancing effect on wound healing in experimental burn and open wound healing models in rats, with the 0.1% shikonin concentration being the most effective.</td>
<td>28, 8</td>
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<td>Tumor Necrosis Factor Alpha Inhibition</td>
<td>Shikonin and isobutylshikonin are able to reduce the levels of functional TNF-both at the transcriptional and post transcriptional level. It inhibited TFIID protein complex (a multi-component transcription factor containing TATA box-binding protein that controls polymerase II driven promoters) to the TATA box of the TNF-promoter.</td>
<td>63, 64, 65</td>
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Acetylshikonin and b-hydroxyisovalerylshikonin were biologically active against soil-borne bacteria and fungi. In another study, extracts of Zicao plant (Lithospermum erythrorhizon root) containing shikonin derivatives and Arnebia euchroma containing alkannin derivatives, shared anti-Candida albicans properties. Likewise, acetylshikonin inhibited the fungal growth. Succeeding that study, the same group investigated in vitro antifungal activities of several naphthoquinones from L. erythrorhizon roots against several fungal pathogens. Shikonin and deoxyskikonin were found to have a much stronger activity than fluconazole against yeast-like fungi (Candida crusei, Saccharomyces cerevisiae, C. glabrata), however acetylshikonin and b-hydroxyisovaleryl-shikonin lower than the standard. All naphthoquinones tested were found to have a range of activity against the filamentous fungus, Trichosporon cutaneum. Finally, the antifungal activity of shikonin among other quinones was tested and proved moderate against Colletotrichum fragariae.

Other health benefits
Shikonin and its derivatives has several health benefits like antiviral, anti-inflammatory, antioxidant activities and wound healing effects were summarized in Table 1.

CONCLUSION
Medicinal plants are natural sources of bioactive compounds to treat life threatening diseases. Shikonin and their derivatives are now well-established a pharmacological benefits and wide spectrum of, antimicrobial, anti-inflammatory, antioxidant, anticancer, free radical scavenging and wound healing activity. The earliest medicinal properties claimed for Shikonin and their derivatives have been confirmed by scientific experimentation within the last 30 years. The clinical application of preparation that contain ester derivatives of Alkannin and Shikonin for the treatment of burns, wounds and ulcers is, perhaps, the most dramatic development. The last years, research on Shikonin and their derivatives has revealed their effectiveness on various aspects of cancer treatment and has approached their mechanisms of action. These rare qualities establish this naturally occurring family of drug and their derivatives as superior to current pharmacological treatments. Obviously, Shikonins and their derivatives serve as starting points for new exciting science and additional discovery of modern drugs, which is eagerly wait in years to come.

CONFLICT OF INTEREST
There are no conflicts of interests.


