

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

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ABSTRACT

Shikonin is a lipophilic red pigments and is found in the outer surface of the roots of *Lithospermum erythrorhizon* 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 erythrorhizon*, Shikonin, Alkannin, Anticancer, Antimicrobial activity, Pharmacological properties.

INTRODUCTION

Shikonin is a natural product isolated from outer surface of Chinese herbal plant *Lithospermum erythrorhizon* (Boraginaceae) roots. Shikonin, derived components are lipophilic red pigments, with naphthazalin 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 *Zicao*². 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 erythrorhizon* in Boraginaceae family⁴. Brockmann and Liebig were the first to define the correct structure of this molecule as 5, 8-dihydroxy-2-[(1*R*)-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 armeniacum*². 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 activity^{7,8}, 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 diversely 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 USA⁵. Shikonin, one of the key active components of *Lithospermum erythrorhizon*, has been shown to exert antitumour effects both *in vitro* and *in vivo*^{12,13}. 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 cells^{14,15}, breast cancer cells¹⁶⁻¹⁸, glioma cells^{19,20}, 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

cell growth and inducing apoptosis through a classic caspases-dependent pathway². 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)- κ B-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.². Furthermore, shikonin and its derivatives have been shown to induce a non-apoptotic cell death known as necroptosis to bypass apoptotic/drug resistance^{23,24}. In addition, shikonin also functions as a proteasome inhibitor²⁵ and topoisomerase I inhibitor²⁶. The anticancer effects of shikonin were also determined in both allografts and xenografts animal models *in vivo*¹³. ROS generation, inactivation of NF- κ B, 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^{27,21,28, 29}. This quinone inhibits Topo I/II activity and results in DNA damage in cancer cells³⁰. Additionally, shikonin also has anti-cancer effects as an anti-estrogen agent by reversing NQO1 expression³¹. It acts as a selective estrogen enzyme modulator by downregulating the expression of steroid sulfatase, important for the biosynthesis of estrogen¹⁶. Furthermore, pyruvate kinase-M₂ and proteasome are also inhibited by shikonin or its analogs³². 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 subtype³³. 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 cancers³³. However, Shikonin induces apoptosis in a classic caspase dependent pathway in cervical, bladder and melanoma cancer cells^{27,34,21} and it besides induces necroptosis regardless of the drug concentration in caspase-3-negative MCF-7 cells³⁵. 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 cells³⁶. 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^{28,37}. Additionally, shikonin also prompt apoptosis via ROS production in osteosarcoma and Bcr/Abl-positive CML cells^{29,38}. 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 xenografts¹³ and inhibits topoisomerase II³⁹, and down-regulates ER2 and activates NFE2-related factor 2 as an anti-estrogen agent in human

breast cancer^{17,31}. Shikonin modulates an estrogen enzyme by down-regulating the expression of steroid sulfatase¹³, which is important for estrogen biosynthesis. Furthermore, shikonin obstructs tumor invasion via the NF-B signaling pathway in human high-metastatic adenoid cystic carcinoma cells²⁸. 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-S2³⁴, COLO205⁴⁰, Tca-8113²⁸, U937⁴¹ and K562³⁸, and cells. The potential mechanism of action is presumably inhibition of the expression of the Bcl-2 family of antiapoptotic proteins⁴⁰. Shikonin may perhaps induce necroptosis in HL60 and K562 cells³⁶ and inhibit cell proliferation by inactivating the NF- κ B pathway in the Tca8113 cell line²⁸. Furthermore, shikonin stimulate the stress-related c-Jun-N-terminal kinase (JNK) pathway in K562 cells³⁸ and inhibit proteasome activity in PC3 cells¹³. In previous studies reported that shikonin reacted with endogenous thiols including glutathione, which in turn induced apoptosis in HL-60 cells²⁷. It induces the p53-mediated cell cycle arrest and cell death in several human malignant cancer cells, and inhibits epidermal growth factor receptor signaling in human epidermoid carcinoma cells. Additionally, shikonin prompts cell death in HL60 human myeloid leukemia cell line^{27,21}. Shikonin inhibited the tumor growth in murine Sarcoma-180⁴². 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^{34,21}. In caspase-3-negative MCF-7 cells, it triggered necroptosis that contributed to overcome Bcl-2- and Bcl-X_L-mediated apoptotic resistance³⁵. Shikonin inhibited the activity of topoisomerase II and NF- κ B, both of which are potential targets for chemotherapy^{28,3}. Shikonin with treatment led to cell cycle arrest over up-regulation of p53 and down-regulation of cyclin-dependent protein kinase 4 in malignant melanoma²⁷. Shikonin induced apoptosis may possibly protected by N-acetylcysteine (NAC) in SKHep-1 hepatoma⁴³, suggested that it targeted an oxidative stress mediated pathway³. Shikonin applies its antitumor activity by prompting apoptosis through ROS accumulation and inactivation of Akt and RIP1/NF- κ B 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^{38,44}.

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

Pharmacological properties	Recent reports	References
Anti-viral properties	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, acetylshikonin and -hydroxyisovalerylshikonin are effective against plant pathogens, such as fungi (<i>Cladosporium herbarum</i>) and virus (TMV-Tobacco Mosaic Virus).	3 55 50
Anti-inflammatory activity	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. -dimethylacrylshikonin, isovaleryl-shikonin and acetylshikonin strongly suppressed carageenan induced paw edema, with acetylshikonin 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.	56 57 9
Leukotrienes Metabolic Pathway Inhibition	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.	58, 59 58, 59
Antioxidant Properties	Two caffeic acid esters of Shikonin possessed the greatest antioxidant capacity. Shikonin has been applied in tobacco smoke detoxification from peroxyradicals. 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.	60 61
Wound Healing effect	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, - dimethylacrylshikonin (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.	28 8 62
Tumor Necrosis Factor Alpha Inhibition	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.	45 63,64,65
		65

open wounds in rats, even though this activity was affected by serum protein⁴⁵. Moreover, another shikonin preparation demonstrated a high antibacterial activity with respect to Gram-positive species, greatly superior to the commercial antimicrobial preparations tested⁴⁶. Recently, shikonin was found to elicit dose-dependent bacteriostatic activity in *Helicobacter pylori* cultures and this study, shikonin inhibited N-acetylation of 2-aminofluorene in the examined *H. pylori* cytosols and intact cells⁴⁷. An extract of the roots of *Onosma argentatum* was effective on *Staphylococcus aureus*, *Bacillus subtilis* and *E. coli* and

also shared very high antioxidant activity⁴⁸. Therapeutic antimicrobial preparations containing *Alkanna tinctoria* have been patented⁴⁹. *In vitro* antifungal activities of several IHN derivatives and *Lithospermum erythrorhizon* root extracts were investigated by several research groups. Hence, Shikonin exhibited a stable fungistatic effect to various cultures of *Candida* genus and one culture of *Trichosporon* genus⁴⁶. Propionylshikonin and b-hydroxyisovalerylshikonin, isolated from the roots of *Lithospermum erythrorhizon*, showed both antifungal (*Cladosporium herbarum*) and antiviral (tobacco mosaic

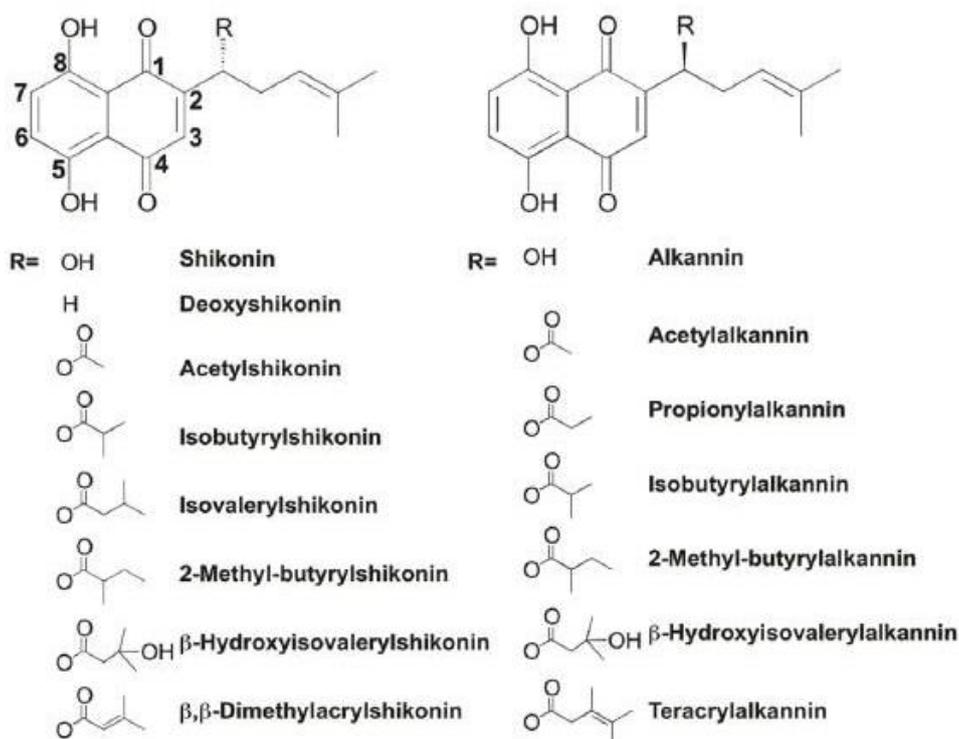


Figure 1: The chemical structures of shikonin and its derivatives (Papageorgiou et al 1999)

virus) activities⁵⁰. Acetylshikonin and b-hydroxyisovalerylshikonin were biologically active against soil-borne bacteria and fungi⁵¹. 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 growth⁵². Succeeding that study, the same group investigated *in vitro* antifungal activities of several naphthoquinones from *L. erythrorhizon* roots against several fungal pathogens⁵³. Shikonin and deoxyshikonin were found to have a much stronger activity than fluconazole against yeast-like fungi (*Candida crusei*, *Saccharomyces cerevisiae*, *C. glabrata*), however acetylshikonin and b-hydroxyisovalerylshikonin lower than the standard. All naphthoquinones tested were found to have a range of activity against the filamentous fungus, *Trichosporon cutaneum*⁵³. Finally, the antifungal activity of shikonin among other quinones was tested and proved moderate against *Colletotrichum fragariae*⁵⁴.

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^{66,67}. 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

REFERENCES

1. Fujita Y, Shikonin: production by plant (*Lithospermum erythrorhizon*) cell cultures. In: Bajaj, Y.P.S. (Ed.). Biotechnology in Agriculture and Forestry. In: Medicinal and Aromatic Plants I, vol. 4. Springer-Verlag, Berlin, 1988, 225–236.
2. Andujar I, Rios JL, Giner RM, Recio MC. Pharmacological properties of shikonin - a review of literature since 2002. *Planta medica*, 2013, 79:1685-1697.
3. Chen X, Yang L, Zhang N, Turpin JA, Buckheit RW, Osterling C, Oppenheim JJ, Howard OM. Shikonin, a component of chinese herbal medicine, inhibits chemokine receptor function and suppresses human

- immunodeficiency virus type 1. *Antimicrob. Agents and Chemother.*, 2003, 47: 2810-2816.
4. Majima R, Kuroda C. The coloring matter of *Lithospermum erythrorhizon*. *Acta Phytochem.*, 1922, 1:43-65.
 5. Papageorgiou VP, Assimopoulou AN, Couladouros EA, Hepworth D, Nicolaou KC. The Chemistry and Biology of Alkannin, Shikonin, and Related Naphthazarin Natural Products. *Angewandte Chemie International Edition*, 1999, 38:270-301.
 6. Yan Y, Furumura M, Gouya T, Iwanaga A, Teye K, Numata S, Karashima T, Li XG, Hashimoto T. Shikonin promotes skin cell proliferation and inhibits nuclear factor- κ B translocation via proteasome inhibition *In Vitro*. *Chin Med J.*, 2015, 128: 2228-2233.
 7. Ozaki Y, Sakaguchi I, Tujimura M, Ikeda N, Nakayama M, Kato Y. Study of the accelerating effect of shikonin and alkannin on the proliferation of granulation tissue in rats. *Biol Pharm Bull.*, 1998, 21:366-370.
 8. Sakaguchi I, Tsujimura M, Ikeda N, Minamino M, Kato Y, Watabe K. Granulomatous tissue formation of shikonin and shikonin by air pouch method. *Biol Pharm Bull.* 2001, 24:650-655.
 9. Zhuravlev JUN, Fedoreev SA, Bulgakov VP, Muzarok TI, Beresneva NV, Golovko EI. Patent RU2141840, 1999.
 10. Toker A, Akcay F, Aksoy H, Suleyman H, Ögen U, Erdem H. The effects of acetyl shikonin isolated from *Onosma armeniacum* on oxidative stress in ethanol-induced ulcer model of rats. *Turk J Med Sci.*, 2013, 43: 315-320.
 11. Chen X, Yang L, Oppenheim JJ, Howard MZ. Cellular pharmacology studies of shikonin derivatives. *Phytotherapy research: PTR.*, 2002., 16:199-209.
 12. Wang WJ, Bai JY, Liu DP, Xue LM, Zhu XY. The antiinflammatory activity of shikonin and its inhibitory effect on leukotriene B4 biosynthesis. *Yao Xue Xue Bao*, 1994, 29:161-165.
 13. Yang H, Zhou P, Huang H, Chen D, Ma N, Cui QC, Shen S, Dong W, Zhang X, Lian W, Wang X, Dou QP, Liu J. Shikonin exerts antitumor activity via proteasome inhibition and cell death induction in vitro and in vivo. *Int J Cancer*, 2009, 124 (10): 2450-2459.
 14. Hashimoto S, Xu M, Masuda Y, Aiuchi T, Nakajo S, Cao J. β -Hydroxyisovalerylshikonin Inhibits the Cell Growth of Various Cancer Cell Lines and Induces Apoptosis in Leukemia HL-60 Cells through a Mechanism Different from Those of Fas and Etoposide. *Journal of biochemistry*, 1999, 125: 17-23.
 15. Duan D, Zhang B, Yao J, Liu Y, Fang J. Shikonin targets cytosolic thioredoxin reductase to induce ROS-mediated apoptosis in human promyelocytic leukemia HL-60 cells. *Free Radical Biology and Medicine*, 2014, 70:182-193.
 16. Zhang Y, Qian RQ, Li PP. Shikonin, an ingredient of *Lithospermum erythrorhizon*, down-regulates the expression of steroid sulfatase genes in breast cancer cells. *Cancer Lett.*, 2009, 284: 47-54.
 17. Yao Y, Zhou Q. A novel antiestrogen agent Shikonin inhibits estrogen-dependent gene transcription in human breast cancer cells. *Breast cancer research and treatment*, 2010, 121:233-240.
 18. Li W, Liu J, Jackson K, Shi R, Zhao Y. Sensitizing the Therapeutic Efficacy of Taxol with Shikonin in Human Breast Cancer Cells. *PLoS ONE*, 2014, 9(4): e94079.
 19. Chen CH, Lin ML, Ong PL, Yang JT. Novel multiple apoptotic mechanism of shikonin in human glioma cells. *Annals of surgical oncology*, 2012, 19:3097-3106.
 20. Zhao Q, Kretschmer N, Bauer R, Efferth T. Shikonin and its derivatives inhibit the epidermal growth factor receptor signalling and synergistically kill glioblastoma cells in combination with erlotinib. *International journal of cancer Journal international du cancer*, 2015, 137:1446-1456.
 21. Yeh CC, Kuo HM, Li TM, Lin JP, Yu FS, Lu HF. Shikonin-induced apoptosis involves caspase-3 activity in a human bladder cancer cell line (T24). *In vivo*, 2007, 21:1011-1019.
 22. Wang H, Wu C, Wan S, Zhang H, Zhou S, Liu G. Shikonin attenuates lung cancer cell adhesion to extracellular matrix and metastasis by inhibiting integrin β 1 expression and the ERK1/2 signaling pathway. *Toxicology*, 2013, 308:104-112.
 23. Xuan Y, Hu X. Naturally-occurring shikonin analogues--a class of necroptotic inducers that circumvent cancer drug resistance. *Cancer Lett.*, 2009, 274:233-242.
 24. Fu Z, Deng B, Liao Y, Shan L, Yin F, Wang Z. The anti-tumor effect of shikonin on osteosarcoma by inducing RIP1 and RIP3 dependent necroptosis. *BMC cancer*, 2013, 13:580.
 25. Wada N, Kawano Y, Fujiwara S, Kikukawa Y, Okuno Y, Tasaki M, et al. Shikonin, dually functions as a proteasome inhibitor and a necroptosis inducer in multiple myeloma cells. *International journal of oncology*, 2015, 46: 963-972.
 26. Zhang FL, Wang P, Liu YH, Liu LB, Liu XB, Li Z. Topoisomerase I inhibitors, shikonin and topotecan, inhibit growth and induce apoptosis of glioma cells and glioma stem cells. *Plos One*, 2013, 8: e81815.
 27. Wu Z, Wu L, Li L, Tashiro S, Onodera S, Ikejima T, p53-mediated cell cycle arrest and apoptosis induced by shikonin via a caspase-9-dependent mechanism in human malignant melanoma A375-S2 cells. *J Pharmacol Sci.*, 2004, 94 (2): 166-176.
 28. Min R, Tong J, Wenjun Y, Wenhui D, Xiaojian Z, Jiakai H, Jian Z, Wantao C, Chenping Z. Growth inhibition and induction of apoptosis in human oral squamous cell carcinoma Tca-8113 cell lines by Shikonin was partly through the inactivation of NF-kappaB pathway. *Phytother Res.* 2008, 22 (3): 407-415.
 29. Chang IC, Huang YJ, Chiang TI, Yeh CW, Hsu LS. Shikonin Induces Apoptosis through Reactive Oxygen Species/Extracellular Signal-Regulated Kinase Pathway in Osteosarcoma Cells. *Biological & Pharmaceutical Bulletin*, 2010, 33(5): 816-824.

30. Bogurcu N, Sevimli-Gur C, Ozmen B, Bedir E, Korkmaz KS. ALCAPs induce mitochondrial apoptosis and activate DNA damage response by generating ROS and inhibiting topoisomerase I enzyme activity in K562 leukemia cell line. *Biochem Biophys Res Commun.*, 2011, 409 (4): 738-744.
31. Yao Y, Zhou Q. A novel antiestrogen agent Shikonin inhibits estrogen-dependent gene transcription in human breast cancer cells. *Breast Cancer Research and Treatment*, 2010, 121 (1): 233-240.
32. Chen J, Xie J, Jiang Z, Wang B, Wang Y, Hu X. Shikonin and its analogs inhibit cancer cell glycolysis by targeting tumor pyruvate kinase-M2. *Oncogene*, 2011, 30 (42): 4297-4306.
33. Duru N, Gernapudi R, Zhou Q. Chemopreventive Activities of Shikonin in Breast Cancer. *Biochem Pharmacol.*, 2014, 3: e163.
34. Wu Z, Wu LJ, Li LH, Tashiro S, Onodera S, Ikejima T. Shikonin regulates HeLa cell death via caspase-3 activation and blockage of DNA synthesis. *J Asian Nat Prod Res.*, 2004, 6:155-166.
35. Han W, Li L, Qiu S, Lu Q, Pan Q, Gu Y, Luo J, Hu X. Shikonin circumvents cancer drug resistance by induction of a necroptotic death. *Mol Cancer Ther.*, 2007, 6: 1641-1649.
36. Han W, Xie J, Li L, Liu Z, Hu X. Necrostatin-1 reverts shikonin-induced necroptosis to apoptosis. *Apoptosis*, 2009, 14:674-86.
37. Nie YK, Zhu LS, Yu HM. Shikonin inhibits the proliferation and induces the apoptosis of human HepG2 cells. *Can J Physiol Pharm.*, 2010, 88:1138-1146.
38. Mao X, Yu CR, Li WH, Li WX. Induction of apoptosis by shikonin through a ROS/JNK-mediated process in Bcr/Abl-positive chronic myelogenous leukemia (CML) cells. *Cell Res.*, 2008, 18:879-888.
39. Yang F, Chen Y, Duan W, Zhang C, Zhu H, Ding J. SH-7, a new synthesized shikonin derivative, exerting its potent antitumor activities as a topoisomerase inhibitor. *Int J Cancer*, 2006, 119:1184-1193.
40. Hsu PC, Huang YT, Tsai M L, Wang YJ, Lin JK, Pan MH. Induction of apoptosis by shikonin through coordinative modulation of the Bcl-2 family, p27, and p53, release of cytochrome c, and sequential activation of caspases in human colorectal carcinoma cells. *J Agric Food Chem.*, 2004, 52:6330-6337.
41. Thangapazham R L, Singh A K, Seth P, Misra N, Mathad VT, Raj K, Maheshwari R K. Shikonin analogue (SA) 93/637 induces apoptosis by activation of caspase-3 in U937 cells. *Front Biosci.*, 2008, 13:561-568.
42. Sankawa U, Ebizuka Y, Miyazaki T, Isomura Y, Otsuka H. Antitumor activity of shikonin and its derivatives. *Chem Pharm Bull (Tokyo)*, 1977, 25:2392-2395.
43. Gao D, Hiromura M, Yasui H, Sakurai H. Direct reaction between shikonin and thiols induces apoptosis in HL60 cells. *Biol Pharm Bull.*, 2002, 25:827-832.
44. Ke G, Wenhua L. Shikonin, a Chinese plant-derived naphthoquinone, induces apoptosis in hepatocellular carcinoma cells through reactive oxygen species: A potential new treatment for hepatocellular carcinoma, *Free Radical Biology & Medicine*, 2011, 2259-2271.
45. Lee YS, Lee DY, Kim YB, Lee SW, Cha SW, Park HW, Kim GS, Kwon DY, Lee MH, Han SH. The Mechanism Underlying the Antibacterial Activity of Shikonin against Methicillin-Resistant *Staphylococcus aureus*. *Evid Based Complement Alternat Med.*, 2015, 520-578.
46. Karyagina TB, Arzumanyan VG, Timchenko TV, Bairamashvili DI. Antimicrobial Activity of Shikonin Preparations. *Pharm Chem J.*, 2001, 35(8): 435.
47. Kuo HM, Hsia TC, Chuang YC, Lu HF, Lin SY, Chung JG. Shikonin inhibits the growth and N-acetylation of 2-aminofluorene in *Helicobacter pylori* from ulcer patients. *Anticancer Res.*, 2004, 24(3a): 1587-1592.
48. Ozgen U, Houghton PJ, Ogundipe Y, Coskun M. Antioxidant and antimicrobial activities of *Onosma argentatum* and *Rubia peregrina*. *Fitoterapia*, 2003, 74(7-8): 682-685.
49. Timothy E, Hanuman J, Jerry N. Patent WO 0141573, 2001.
50. Li C, Fukishi Y, Kawabata J, Tahara S, Mizutani J, Uyeda I. Antiviral and antifungal activities of some naphthoquinones isolated from the roots of *Lithospermum erythrorhizon*. *Nippon Noyaku Gakkaishi*, 1998, 23: 54-57.
51. Brigham LA, Michaels PJ, Flores HE. Cell-specific production and antimicrobial activity of naphthoquinones in roots of *lithospermum erythrorhizon*. *Plant Physiol.*, 1999, 119(2): 417-428.
52. Sasaki K, Yoshizaki F, Abe H. The anti-Candida activity of shikon. *Yakugaku Zasshi*, 2000; 120(6):587-589.
53. Sasaki K, Abe H, Yoshizaki F. In vitro antifungal activity of naphthoquinone derivatives. *Biol Pharm Bull.*, 2002, 25(5): 669 -670.
54. Meazza G, Dayan FE, Wedge DE. Biodistribution anthraquinone conjugates GRH polyamine and polyamine relationship between research. *J Agric Food Chem.*, 2003, 51(13): 3824-3828.
55. Dannenberg AJ, Subbaramaiah K. Treatment of HPV caused diseases. U.S. Patent 2002 0164385, November 7, 2002.
56. Brock TG, McNish RW, Mancuso P, Coffey MJ, Peters-Golden M. Prolonged lipopolysaccharide inhibits leukotriene synthesis in peritoneal macrophages: mediation by nitric oxide and prostaglandins. *Prostaglandins Another Lipid Mediat.*, 2003, 71(3-4):131-145.
57. Kundakovic T, Fokialakis N, Dobric S, Pratsinis H, Kletsas D, Kovacevic N, Chinou I. Evaluation of the anti-inflammatory and cytotoxic activities of naphthazarine derivatives from *Onosma leptantha*. *Phytomedicine*, 2006, 13: 290-294.
58. Chen X, Oppenheim J, Howard OM. Shikonin, a component of antiinflammatory Chinese herbal medicine, selectively blocks chemokine binding to CC chemokine receptor-1. *Int Immunopharmacol.*, 2001, 1(2): 229-236.

59. Chen X, Yang L, Oppenheim JJ, Howard OMZ. Cellular pharmacology studies of shikonin derivatives. *Phytother Res.*, 2002, 16: 199-209.
60. Han J, Weng XC, Bi KS. Antioxidants from a Chinese medicinal herb-Lithospermum erythrorhizon. *Food Chem.*, 2008, 106: 2-10.
61. Nishizawa M, Kohno M, Nishimura M, Kitagawa A, Niwano Y. Presence of peroxyradicals in cigarette smoke and the scavenging effect of shikonin, a naphthoquinone pigment. *Chem. Pharm. Bull.*, (Tokyo) 2005, 53: 796-799.
62. Sidhu GS, Singh AK, Banaudha KK, Gaddipati JP, Patnaik GK, Maheshwari RK. Arnebin-1 accelerates normal and hydrocortisone- induced impaired wound healing. *J Invest Dermatol.*, 1999, 113: 773-781.
63. Chiu SC, Yang NS. Inhibition of tumor necrosis factor- α through selective blockade of Pre-mRNA splicing by shikonin. *Mol. Pharmacol.*, 2007, 71: 1640-1645.
64. Subbaramaiah K, Bulic P, Lin Y, Dannenberg AJ, Pasco DS. Development and use of a gene promoter-based screen to identify novel inhibitors of cyclooxygenase-2 transcription. *J Biomol Screening*, 2001, 6: 101-110.
65. Staniforth V, Wang SY, Shyur LF, Yang NS. Shikonins, phytochemicals from *Lithospermum erythrorhizon*, inhibit the transcriptional activation of human Tumor Necrosis Factor α promoter in vivo. *J Biol Chem.*, 2004, 279: 5877-5885.
66. Ashokkumar K. *Gloriosa superba* (L.): A Brief Review of its Phytochemical Properties and Pharmacology. *International Journal of Pharmacognosy and Phytochemical Research*, 2015, 7(6); 1190-1193.
67. Ashokkumar K, Kumarakurubaran S, Saradha Devi M. *Cynodon dactylon* (L.) Pers.: An updated review of its phytochemistry and pharmacology. *Journal of Medicinal Plants Research*, 7(48): 3477-3483.

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