Review
Pharmacological and analytical aspects of alkannin/shikonin and their derivatives: An update from 2008 to 2022

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Abstract
Alkannin/shikonin (A/S) and their derivatives are naturally occurring naphthoquinones majorly found in Boraginaceae family plants. They are integral constituents of traditional Chinese medicine Zicao (roots of Lithospermum erythrorhizon). In last two decades significant increase in pharmacological investigations on alkannin/shikonin and their derivatives has been reported that resulted in discovery of their novel mechanisms in various diseases and disorders. This review throws light on recently conducted pharmacological investigations on alkannin/shikonin and their derivatives and their outputs. Various analytical aspects are also discussed and brief summary of patent applications on inventions containing alkannin/shikonin and its derivatives is also provided.

Keywords:
alkannin naphthoquinones patents shikonin

1. Introduction
Alkannin and shikonin (A/S) are enantiomeric pair and naphthoquinone pigments (Boulos, Rahama, Hegazy, & Efferth, 2019) which are well known for their therapeutic, cosmetic and coloring applications (Fig. 1). Plants containing these bioactive pigments are traditionally used for curing various ailments since centuries. Alkannin was initially reported as a principle component of the root bark of with records of traditional utilization for 4th century BCE for various ailments, principally for ulcers (Papageorgiou, Assimopoulou, Couladouros, Hepworth, & Nicolaou, 1999; Weigle, 1974). On the other hand, Alkanna tinctoria Tausch. Plant of European Origin belonging to Boraginaceae family shikonin was isolated from the root bark of Chinese medicinal plant Lithospermum erythrorhizon Sieb. et Zucc (Boraginaceae) which is well

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known in China by various traditional names i.e. tzuts’ao, tzu-ken, hung-tzu ken, etc (Huu Tung, Du, Wang, Yuan, & Shoyama, 2013). It is an integral component of traditional Chinese medicine Zicao (roots of L. erythrorhizon) which has successful history in treatment of various inflammatory and infectious diseases (Andújar, Ríos, Giner, & Recio, 2013; Papageorgiou, Assimopoulou, Couladouros, Hepworth, & Nicolau, 1999; Winter, 1984a,b; Khan and Abourashed, 2010).

Apart from these plants, a wide range of plants belonging to Boraginaceae family are known to possess these enantiomers and their derivatives. In fact acetylishikonin was first isolated from L. erythrorhizon by Kuroda and Wada in 1922, later shikonin and its other derivatives were also identified (Kuroda & Wada, 1936). It took almost 14 years to identify accurate structure of shikonin (reported as 5,8-dihydroxy-2-[(1R,1-hydroxy-4-methyl-3-pentenyl]-1,4 naphthoquinone in 1936 by Brockmann); subsequently, it’s another enantiomer alkannin was identified by the same group (Albreht, Vovk, Simonovska, & Srbinoska, 2009; Brockmann, 1936). Approximately, 35 derivatives (Fig. 2) of alkannin and shikonin have been isolated from various plants of Boraginaceae family and extensively investigated for wide range of biological activities including wound healing, antimicrobial (Aburjai, Al-Janabi, Al-Mamoori, & Azzam, 2019), anti-acne (Fang & Shoukang, 1998), antiulcer (Singh & Sharma, 2012), anti-inflammatory (Lee et al., 2016), anticancer (Sun, Zhang, Liu, & Guan, 2019) activities, etc.

In the time frame of 1969 to 2021, a total of 634 full text reports are available in PubMed database and out of these, and 606 reports are published after 2000, showing the increased interest of research groups in A/S and their derivatives. Trend analysis suggests that researchers are more focused on shikonin than alkannin (Fig. 3). An exhaustive review of A/S and their derivatives was first published by Papageorgiou group in 1999 (Papageorgiou, Assimopoulou, Couladouros, Hepworth, & Nicolau, 1999). A decade later, another update was published with prime focus on wound healing and associated bioactivities (Papageorgiou, Assimopoulou, & Ballis, 2008). In 2013, Andújar group published a compilation containing pharmacological investigations on A/S and their derivatives for the period of 2002 to 2013 (Andújar, Ríos, Giner, & Recio, 2013). Subsequently, various review reports were published by different research groups with a focus on either individual bioactivity or on individual derivative. A/S and their derivatives possess enantiomeric properties that make their analysis quite complex. Surface-enhanced Raman Spectroscopy (SERS) and chiral HPLC have been successfully utilized for differentiating A/S and their derivatives (Cañamero, et al., 2022; Azuma et al., 2016). Literature analysis suggests that after 2008 (Papageorgiou, Assimopoulou, & Ballis, 2008), any review update regarding analytical aspects of A/S and their derivatives is not available. Thus, there is a dire need of an updated compilation containing all pharmacological, analytical and miscellaneous investigations on A/S and their derivatives. Forecasting the marketable potential of A/S and their derivatives, wide range of patents have been filed by various research groups around the globe for various applications to safeguard their usage. This review is primarily focused on providing update on various investigations on A/S and their derivatives from year 2008 to 2021 along with thorough insight on the patent applications filed.

2. Pharmacological activities

2.1. Wound healing activity

Dried roots of Arnebia guttata Bung, Arnebia euchroma (Royle) Johnston, and Lithospermum erythrorhizon Sieb. Et Zucc loaded oil based ointment (Zicao) has been widely used for treatment of wounds (Chak, Hsiao, & Chen, 2013; Hsiao, Tsai, & Chak, 2012; Lu et al., 2008; Zeng & Zhu, 2014). The major active components of Zicao include shikonin and its derivatives such as deoxyshikonin, acetylishikonin and β,β-dimethylacrylshikonin. Furthermore, to overcome the demerits of this oil based ointments such as discomfort, irritation and difficulty in cleaning, soluble water based topical preparation such as Zicao-HP-β-CD complex was formulated using 2-hydroxypropyl-β-cyclodextrin to form water-soluble complex which resulted in its enhanced bioavailability and stability. The active ingredients of Zicao enhance collagen synthesis in granuloma tissues and promote inactivation of tumor necrosis factor-α gene expression (Chen, Yu, Hsu, Tsai, & Tsai, 2018). On the other hand, Jawoongo, a Korean traditional medicine has been found highly effective in removing necrotic tissue caused by burn wounds. Jawoongo consists of Lithospermum Radix, Angelicae Gigantis Radix, Ronicerae Flos, Glycyrrhizae Radix, Coptidis Rhizome and Scutellariae Radix. The major active ingredient is Lithospermum Radix which mainly comprises of deoxyshikonin. It significantly increases the phosphorylation of p38 and ERK1/2 in a concentration dependent manner. Additionally, it activates Mitogen-activated protein kinase (MAPK) signaling which promotes cellular migration and angiogenesis. It was observed that deoxyshikonin induced migration and proliferation in HaCaT cells mediated through activation of p38 and ERK respectively. Thus, the study demonstrated that deoxyshikonin possesses strong ability for proliferation, migration and tube formation of HaCaT and HUVEC cells, which in turn promotes angiogenesis (Kim, Lee, & Yook, 2013; Park et al., 2017).

Recently, an increased attention is focused on the herbal medicines attributing to their quality, safety and efficacy. Since ancient times, people have used plant based preparations to promote wound healing process (Fronza, Heinzmann, Hamburger, Laufer, & Merfort, 2009). Various plants especially belonging to Boraginaceae family have been reported to possess excellent therapeutic potential in wound management. The main active metabolites of this family are naphthoquinones which possess anti-inflammatory, anti-microbial, anti-oxidant activities contributing to wound healing (Lee et al., 2016). Meanwhile, additional studies demonstrated that therapeutic benefits of roots of Boraginaceae family plants are wider than its aerial parts. The most active components found in roots are shikonin, alkannin, deoxyshikonin and acetylishikonin. Traditionally, the root extract of Onosma dichroantha Boiss. has been used in Iran for healing burn wounds. Furthermore, the cyclohexane fraction has been found to be most potent inhibitor of lipopolysaccharide induced nitrogen oxide production which accelerates fibroblast proliferation, tissue regeneration and angiogenesis. Active components present in the cyclohexane fraction were found to be shikonin, arnabin-1 and β,β-dimethyl acrylalkannin. Among all of these components, arnabin-1 has proangiogenic and synergistic effects with vascular endothelial growth factor (VEGF) which further augments the wound healing process (Saafari et al., 2019). Similarly, several other phytoconstituents isolated from n-hexane-dichloromethane extract of Onosma argentatum Hub.-Mor. roots i.e. deoxyshikonin, acetyl-
shikonin, 3-hydroxyisovalerylshikonin and 5–8-O-dimethylacetylshikonin were found to be effective in treatment of burns wounds. In another study, the efficacy of mixture of olive oil, beeswax and root extract of Alkanna tinctoria Tausch. was examined on burn wounds which showed rapid epithelization and angiogenesis (Gümüş & Özlü, 2017). Moreover, this extract has been established to increase fibroblasts production which amplifies tissue regeneration and provides better perfusion to wound area resulting in granulation tissue formation (Yazdinezhad, Monsef-Esfahani, & Ghahremani, 2013) (Fig. 4). The healing effects of ointment loaded with Arnebia euchroma extract were also compared with standard silver sulfadiazine on second degree burns and the extract demonstrated higher efficacy. Fibroblast proliferation, cell migration and collagen synthesis were observed to be the major mechanisms in its healing process (Nasiri et al., 2016).
Furthermore, the active constituents of *L. erythrorhizon* such as shikonin, isobutyl-shikonin, β-hydroxy-isovaleryl-shikonin and α-methyl-n-butyl-shikonin were loaded in chitosan/gelatin-based scaffolds and examined for their wound healing potential. The results demonstrated the mechanism of healing via regulation of epithelial-mesenchymal transition (EMT) through TGF-β expression (Table 1) (Hsiao, Tsai, & Chak, 2012; Wang, Kravchuk, & Kimble, 2010; Yao, Chen, Chen, Li, & Huang, 2019).

### 2.2. Antimicrobial activity

Traditional Chinese herb *L. erythrorhizon* has been widely used in treatment of a wide range of infections (Yan, Tan, Miao, Wang, & Cao, 2019). *Candida albicans* is the major opportunistic pathogen and major cause of fungal infections in humans. Shikonin showed significant inhibitory effect on the growth of *C. albicans* through multiple mechanisms. It markedly increases the intracellular ROS (reactive oxygen species) and causes depolarization of mitochondrial membrane potential. It was observed to reduce the ergosterol content also. Further, it could lead to the upregulation of thioredoxin reductase-related gene (TRR1), NADPH oxidoreductase-related gene (EBP1) and mitochondrial respiratory electron transport chain-related gene (MRF1) (Fig. 5) (Miao et al., 2012). Moreover, shikonin was also found effective for periodontal diseases as it has ability to inhibit Porphyromonas gingivalis, Fusobacterium nucleatum, Streptococcus mutans and Lactobacillus acidophilus which are most susceptible bacterial strains involved in dental caries (Li, Xu, Zhu, & Wang, 2012). In the latest studies, shikonin and its derivatives including shikonin glucoside, 4-chlorophenylacetyl shikonin, lithospermidin B and Angelyl shikonin were assessed for protein binding with Main protease (Mpro) of SARS CoV-2 revealed shikonin and some derivatives as potential antiviral agent of Covid (Woo & Das, 2022).

### 2.3. Anticancer activity

Cancer is one of the most fatal diseases and one of the primary causes of deaths globally. The incidence of cancer in India has been expanding in the last two decades as in other developing nations. Not only the incidence but pattern has also changed to a great extent. The active constituents of *L. erythrorhizon* such as shikonin, arnebin 1 and β, β-dimethyl acryl alkannin antagonize the hemolytic activity of PLY thereby reducing the cytotoxicity of PLY. It also inhibits oligomers formation and block pore formation on the cell membrane which leads to decreased production of IFN-γ and IL-6 (Zhao et al., 2017). Moreover, shikonin has been found as therapeutically effective for periodontal diseases as it has ability to inhibit Porphyromonas gingivalis, Fusobacterium nucleatum, Streptococcus mutans and Lactobacillus acidophilus which are most susceptible bacterial strains involved in dental caries (Li, Xu, Zhu, & Wang, 2012). In the latest studies, shikonin and its derivatives including shikonin glucoside, 4-chlorophenylacetyl shikonin, lithospermidin B and Angelyl shikonin were assessed for protein binding with Main protease (Mpro) of SARS CoV-2 revealed shikonin and some derivatives as potential antiviral agent of Covid (Woo & Das, 2022).
wound models. Mechanisms involved and pharmacological outcomes from various investigation of alkannin/shikonin containing plant extracts, alkannin/shikonin and its derivatives on different machinery (Han et al., 2007). In addition, antiapoptotic progression cancer drugs are likely to cause apoptosis. Due to sensitivity to effective cancer treatment. It is known that conventional anti-2012). Anticancer drug resistance is another major obstacle in the cult because of multiple potential targets (Han et al., 2007). transporter-mediated resistance is possible as it works on fewer and upregulation of apoptotic inducers enormously limit the effec-
tion (HPV) long term infection of HPV is the leading cause of cervi-
rative cell death (Degterev et al., 2005). More-
receptor-mediated apoptosis. Necroptosis is a programmed cell death characterized by necrotic cell morphology and activation of autop-
Figure 6 (a/b). Additionally, p73 is responsible for
Table 1
Mechanisms involved and pharmacological outcomes from various investigation of alkannin/shikonin containing plant extracts, alkannin/shikonin and its derivatives on different wound models.

| Test compounds/extract                             | Cell cultures/In vitro/In vivo assays | Mechanism involved/Pharmacological outcomes                                                                 | Wound types                          | References                        |
|-----------------------------------------------------|--------------------------------------|-------------------------------------------------------------------------------------------------------------|--------------------------------------|-----------------------------------|
| Shikonin, isobutylshikonin, 6-hydroxyisovaleryshikonin, 2-methyl-n-butyl-shikonin | Cytotoxicity assay using L929 mouse fibroblasts; In vivo wound healing assay | Proliferation of fibroblasts; Synergistic effect of gelatin and chitosan promote granulation tissue formation Activation of Erk1/2 and p38/β pathway; Induction of hypertropic scar derived fibroblasts apoptosis | Skin wounds                          | Yao, Chen, Chen, Li, & Huang, 2019 Xie et al., 2015 |
| Shikonin, β,β'-dimethylacryl shikonin, β,β'-dimethylnitroshikonin, shikonin | Hypertropic scar derived fibroblasts (HSF) and normal fibroblasts (nHSF) cell lines | Inhibition of LPS-induced NO production thereby promoting tissue regeneration and angiogenesis | Hypertrophic or keloid scars          | Safavi et al., 2019 |
| Shikonin, juglone, 1-naphthoquinone, lapachol, deoxyshikonin, β,β'-dimethylacrylshikonin, acetylshikonin | Murine macrophages (RAW264.7), normal human skin fibroblasts (HS27), human microvascular endothelial cells (HMEC-1), zebrafish line TG (fl1: EGFP) | Human dermal scar-derived fibroblasts (HSF) and Human 'normal' dermal fibroblasts (nHSF) | Inhibition of TGF-β1 induced collagen deposition and cell mediated contraction; Phosphorylation of P-Erk and NF-κβ | Dermal scars | Fan et al., 2019 |
| Alkanna strigosa extract | Excision and incision wound models | Increase in wound contraction rate and promoting granulation tissue formation | Suppurative wounds | Aburjai, Al-Janabi, Al-Mamoorni, & Azazim, 2019 |
| 2-methyl-n-butylshikonin, acetylshikonin, isovaleryshikonin, deoxyshikonin | Anti-oxidant activity using DPPH assay and wound healing activity using Linear incision wound model | Accelerative effect on proliferation and migration thereby promoting re-epithelialization | Incision wounds | Erugyur, Yilmaz, Kutsal, Yücel, & Üstün, 2016 Cardoso et al., 2018 |
| 2-bromo-1,4-naphthoquinone, 2-N-isonicotinoyl-hydrazone-1,4-naphthoquinone, 1-N-isonicotinoyl-hydrazone-[2hydroxy-3-(3-methyl-2-butenyl)]-1,4-naphthoquinone | Mouse fibroblast cell lines 3T3, MTT assay, Scratch assay, Excision wound model | Inhibition of lysophosphatidic acid signaling pathway and MAPK signaling pathway | Diabetic wounds | |
| Shikonin, acetylshikonin, β,β'-dimethylacrylshikonin | Excision wound model | Increase in collagen fibre levels in granuloma tissue via expression of TTN-α | Excision wounds | Chen, Yu, Hsu, Tsai, & Tsai, 2018 Park et al., 2017 |
| Deoxyshikonin | Human umbilical vein vascular endothelial cells (HUVECs), immortalized human keratinocytes (HaCaT) | Stimulation of phosphorylation of p38 and extracellular signal regulated kinase. | Full-thickness dermal wounds | Nasiri et al., 2016 |
| Arnebia euchroma roots | Randomized, single blind clinical trials | Promote angiogenesis via increased expression of matrix mucopolysaccharide deposition, collagen synthesis and fibroblasts proliferation. | Second degree burns wounds | |
| Echium arenarium extract | Murine 218 macrophagic cells (Raw264.7), Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus (MRSA), Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Leishmania major (GLC94) and Leishmania infantum (LV05) | Anti-oxidant, anti-bacterial activity and anti-leishmanial activity | Cutaneous leishmaniamic wounds | Kefi et al., 2018 |
| Allkanna tinctoria extract | Experimental study on patients with second degree burns. | Increased fibroblastic activity and accelerated granulation. | Full-thickness burn wound | Gümüş & Özlu, 2017 |

extent (Ferlay et al., 2010; Jha, 2009; Guddati, 2012; Rocconi et al., 2012). Anticancer drug resistance is another major obstacle in the effective cancer treatment. It is known that conventional anti-
cancer drugs are likely to cause apoptosis. Due to sensitivity to neoplastic cells to apoptosis, they significantly become resistant via antiapoptotic progression and dysregulation of apoptotic machinery (Han et al., 2007). In addition, antiapoptotic progression in neoplastic cells involves overexpression of antiapoptotic proteins (Bcl-2, Bcl-x1, Mcl-1, c-FLIP), proapoptotic proteins mutations (p53, Apaf-1, Bax, FAS) and loss of caspases (Caspace-3 and Caspace-8) which significantly contributes to drug resistance (Bonora et al., 2015). Therefore, defects in the apoptotic signaling and upregulation of apoptotic inducers enormously limit the effect-
iveness of chemotherapy. Presently, overcoming the drug transporter-mediated resistance is possible as it works on fewer targets whereas apoptosis mediated drug resistance is highly diffi-
cult because of multiple potential targets (Han et al., 2007).

Owing to its strong and broad spectrum anti-cancer activity, shikonin and its derivatives are gaining popularity. A study by a research group revealed the necroptotic mechanism of shikonin to promote non-apoptotic cell death (Degterev et al., 2005). More-
over, shikonin could circumvent cancer drug resistance through induction of necroptosis. Necroptosis is a programmed cell death characterized by necrotic cell morphology and activation of autop-
hagy (Han et al., 2007). Also, shikonin promotes topoisomerase mediated DNA cleavage, caspase-dependent apoptosis and cell cycle arrest via activation of tumor suppressor gene p73 and down-
regulation of ICBP90 (Fig. 6). Additionally, p73 is responsible for transcription of various p53 target genes such as p16INK4A, PIUMA (p53-upregulated modulator of cell death) and p21 (Jang, Hong, Jeong, & Kim, 2015). A recent report indicates that ICBP90 is overexpressed in patients with cervical cancer. Cervical cancer is second most malign-
tant tumor in women after breast cancer. Annually, the global rate of cervical cancers is about 60 million cases with 25 million deaths (Kaarthigeyan, 2012). Also, high risk human papillomavirus infec-
tion (HPV) long term infection of HPV is the leading cause of cervi-
cal intraepithelial neoplasia, precancerous lesions and cervical
carcinoma (Cook et al., 2017). Previous studies demonstrated that β-hydroxysovaleryl shikonin (β-HIVS), a shikonin derivative, possesses inhibitory effect on HeLa cells through apoptosis and prevent tumor cell proliferation. β-HIVS retards PI3K activity and downregulates AKT/mTOR signaling along with reduced P70S6K expression levels which ultimately leads to tumor suppression (Lu et al., 2015).

On the other side, breast cancer is most prevalent malignancy in women. Recently, triple-negative breast cancer (TNBC) accounts for about 20% of all new cases of breast cancer accompanied with higher grade and distinct metastatic potential. Therefore, suppression of metastasis might be a promising therapy for TNBC patients (Lambert, Pattabiraman, & Weinberg, 2017; Temian, Pop, Irime, & Berindean-Neagoe, 2018). Essentially, epithelial-to-mesenchymal transition (EMT) plays a pivotal role in regulating metastasis process. EMT involves loss of epithelial phenotypes and the gain of mesenchymal features. It is characterized by downregulation of epithelial cell-surface markers such as occludin, E-cadherin and zona occludens-1 whereas upregulation of mesenchymal markers such as N-cadherin and vimentin. Of particular interest, shikonin has been established as an effective strategy with good therapeutic potential for TNBC patients. It significantly reduces the expression of miR-17-5p which leads to activation of tumor suppressor gene (PTEN). However, overexpression of PTEN downregulates the Akt expression thereby inhibiting metastasis (Bao et al., 2020).

In recent years, the incidence of colon cancer is considerably increasing in western countries attributing to unhealthy lifestyles. The safety and efficacy of shikonin was determined against colon cancer. Studies demonstrated that shikonin promotes cell death via mitochondrial dysfunction which is induced by downregulation of Bcl-2 and upregulation of Bax, Caspase-3 and Caspase-9. In addition, activation of MAPK pathway and increased endoplasmic reticulum stress triggers apoptosis (Han et al., 2019; Liang et al., 2017). Specifically, anti-cancer activity of shikonin against gefitinib-resistant non-small cell lung cancer (NSCLC) was investigated. Shikonin showed strong cytotoxicity against NSCLC cell lines. Also, it effectively generates ROS and stimulates EGFR degragation resulting in inhibition of TrxR thereby inducing apoptosis (Li et al., 2017). Another study on paclitaxel-resistant non-small cell lung cancer, shikonin induces dysregulation of NEAT1 expression which leads to deactivation of PI3K/Akt pathway hence, inhibiting cell proliferation. Simultaneously, shikonin considerably increases expression of PARP and caspase-3 and caspase 9 cleavages (Zang, Rao, Zhu, Wu, & Jiang, 2020). Researchers reported that activation of STAT3 and PKM2 regulates cell proliferation (Cao et al., 2020; Hoshino, Hirst & Fujii, 2007). Therefore, STAT3 and PKM2 can be considered as key targets for tumor suppression. Recent studies indicated that shikonin markedly reduced the expression of STAT3dimer and PKM2 gene thereby inhibiting inhibits melanoma cell growth (Cao et al., 2020; Liu et al., 2020). Furthermore, the deactivation of NfkB also contributes in inhibiting cancer-inducing inflammation by decreasing release of inflammatory cytokines such as COX-2, iNOS and IL-6 (Table 3). In the recent studies shikonin was tested against Acute Myeloid Leukemia. Shikonin impairs the mitochondrial activity and electron transport chain complex-II to selectively target leukemia cells (Roma et al., 2022). Moreover, inhibitory potential of shikonin was reported on Sunitinib-Resistant renal carcinoma cells. It acts by necrodoxin complex formation and downregulation of AKT/mTOR signaling pathway (Markowitsch et al., 2022). Lately, shikonin was tested and found effectively active against Mutant-non small lung cancer cells. It induces necrosis and apoptosis of cancer cells via thioresdoxin reductase 1 inhibition following SecTRAPs generation and oxygen-coupled redox cycling pathway (Zhang et al., 2022). One of the study demonstrated antitumor growth of leukemia cells. It triggers the apoptosis of cancer cells by checking the cancer cell growth in S phase of cell cycle (Chen et al., 2021). Shikonin is found to be a potential inhibitor in pancreatic cancer as it mediates PD-L1 degradation which in turn suppresses immune evasion in pancreatic cancer cells via NF-kB/STAT3 and NF-kB/CSN5 signaling pathway (Ruan et al., 2021). The antitumor potential of shikonin co delivered with siTGF-β against triple negative breast cancer cells was investigated by Li et al and this codelivery approach was found to be magnificently efficacious for the same (Li et al., 2022). In a nutshell, shikonin/alkannin and their

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**Fig. 5.** Mechanistic action of shikonin and its derivatives on various microbial strains viz. *Staphylococcus aureus*, *Candida albicans* and *Streptococcus pneumoniae* where ROS is reactive oxygen species and MMP is mitochondrial membrane potential.
Table 2
Mechanisms involved and pharmacological outcomes from various antimicrobial investigations on alkannin/shikonin and its derivatives.

| Shikonin and its derivatives | Cells/Targeted strains | Mechanism involved | References |
|-----------------------------|------------------------|--------------------|------------|
| Shikonin                    | Human lung epithelial cells (A549) | Antagonistic effect on haemolytic activity of pneumolysin (PLY); Reduce the cytotoxicity of PLY by inhibiting oligomers formation and blocking pore formation on the cell membrane; | Zhao et al., 2017 |
|                             | Murine model of endonasal pulmonary infection; Streptococcus pneumoniae strain D39 serotype 2 (NCTC 7466) | | |
|                             | Aspergillus terreus (NCCP860035) | Decreased production of IFN-γ and IL-6. Upregulation of Mpkc, spm1, protein kinase (Pkc-c), protein kinase (disk1) serine/threonine-protein kinase and small GTPase ras-1 proteins; | Shishodia & Shankar, 2020 |
| Iranian Arnebia euchroma extract | S. aureus ATCC 35591 (MRSA) and S. aureus ATCC 25923 (MSSA) | Moderate increase in CAMP. | Liao et al., 2016 |
| Lithospermum erythrorhizon seeds | Bacillus subtilis 613R, Clavibacter michigenensis subsp. nebraskensis CN74-1, Agrobacterium radiobacter K84, Agrobacterium tumefaciens C58, Escherichia coli ESS, Erwinia carotovora ATCC 15713, Pseudomonas aureofaciens, Pseudomonas fluorescens, Pseudomonas syringae B, Raistonia solanacearum, and Serratia marcescens | | |
| Acetylsyshikoninshikonin, β-sitosterol, β,β-dimethylacyl shikonin and deoxyshikonin from L. erythrorhizon | Porphyromonas gingivalis (ATCC 33277), Streptococcus mutans (UA 159), Pseudobacterium nucleatum (ATCC 25586) and Lactobacillus acidophilus (ATCC 4356) | | Li, Xu, Zhu, & Wang, 2012 |
| | Escherichia coli (ATCC-59008), Klebsiella pneumoniae (ATCC-59008) and Enterobacter cloacae (ATCC25924), Bacillus subtilis (ATCC-10031), Staphylococcus aureus (ATCC-25923), Streptococcus pneumoniae (ATCC-10032), Aspergillus niger, Rhizoctonia phaseoli, Aspergillus flavus, Penicillium chrysogenum and Candida albicans | | Singh & Sharma, 2012 |
| Shikonin                    | Candida albicans (SC5314) | Increased intracellular ROS and depolarization of mitochondrial membrane potential | Miao et al., 2012 |
| Deoxyalkaninn, alkannin, acetylalkaninn, isobutyryl alkannin, β-hydroxyiso valeryshikonin and shikonin isovalerate from Arnebia hispidissima (Lehm.) DC. | Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Staphylococcus epidermidis (ATCC 12228), Klebsiella pneumoniae (ATCC 13883), Enterobacter cloaceae (ATCC 13047), Pseudomonas aeruginosa (ATCC 227853) Candida albicans (ATCC 10231), Candida tropicalis (ATCC 13801) and Candida glabrata (ATCC 28838), S. aureus, E. faecalis and MRSA. | | Damianakos et al., 2012 |
| Alkannin, shikonin, acetyl alkannin, acetyl shikonin, β,β-dimethyl acryloyl alkannin isovaleryl alkannin, and β-methylbutyryl alkannin, Cinnamoyl alkannin, 3,4-(methylenedioxy)cinamoyl alkannin, isobutyryl alkannin from Arnebia euchroma | | | Shen et al., 2002 |

derivatives are promising candidates for anticancer activity which act by various signaling pathways.

2.4. Miscellaneous activities

Apart from pharmacological activities discussed above alkaninn/shikonin and their derivatives also possess therapeutic potential against phytophagototoxicity, bronchial asthma, peptic ulcer, spasmogenicity, atherosclerosis, inflammatory diseases, ischemic heart diseases, cataract, hepatotoxicity and impotency (Fig. 7) (Yildirim, 2020). Onosma, the biggest genus of Boraginaceae family, is being used as traditional medicine since centuries (Davis, 1970). Shikonin and its derivatives have also been reported to inhibit oxidized low-density lipoprotein (LDL) induced monocyte adhesion by deactivation of NFκB and hence used in treatment of atherosclerosis. It is well known that oxidized LDL plays a key role in thrombosis, endothelium apoptosis and vascular smooth muscle proliferation. In addition, it also stimulates release of inflammatory mediators such as cytokines and reactive oxygen species. Moreover, activation of NFκB further upregulates the expression ofMoreover,
intracellular adhesion molecule (ICAM-1), E-selectin, vascular cell adhesion molecule and monocyte chemotactic protein-1. Hence, the accumulation of oxidized low-density lipoprotein (oxLDL) and inflammatory cells lead to atherosclerosis. Shikonin has also been found effective in retarding oxLDL mediated ROS production through induction of expression of PI3K/Akt/Nrf 2-dependent antioxidant genes such as SOD-1, HO-1, Catalase, GPx-1, GCLM, and GSR (Huang et al., 2015).

Furthermore, the oxidative stress is the major cause of various other medical conditions such as ageing, diabetes, stroke, neurodegenerative disorders, cancer etc. Oxidative stress is often accompanied with higher blood sugar levels. The skeletal muscle cells are rich in insulin-sensitive glucose transporters named as glucose transporter 4 (GLUT4). Their main function is translocation of glucose from cytoplasm to cell membrane aiding in glucose uptake. Therefore, it plays imperative role in regulation of homeostasis of glucose. However, the contraction-induced release of reactive oxygen species (ROS) and activation of AMP activated protein kinase (AMPK) may also lead to increased glucose uptake in skeletal muscle cells (Mao, Yu, Li, & Li, 2008; Su, Huang, & Zhu, 2016). Subsequently, acetylshikonin-induced glucose uptake was significantly inhibited by reduction of PLC-β3 in L6 myotubes, which makes it evident that acetylshikonin-induced glucose uptake may be triggered by activation of inositol lipid signaling and increased DAG release (Huang et al., 2019). On the other hand, ageing is also considered as biggest cause of Alzheimer’s disease. Various studies have shown that oxidative stress, neuronal apoptosis and neuroinflammation plays critical role in pathogenesis of Alzheimer’s disease (Heneka, 2015). SIRT1 is essentially involved in cognitive functions and shows protective effect against ageing-related neuronal degeneration. Thus, SIRT1 can be the most promising therapeutic target for Alzheimer’s disease. Multiple studies reported that chronic inflammation associated with raised levels of pro-inflammatory mediators such as IL-6, IL-10, TNF-α and IL-1β. Notably, acetylshikonin reduced the levels of these mediators via inhibiting the activation of NFκB and thereby reducing inflammation. Simultaneously, it also inhibits the activation of p21/p53 signaling pathway (Chang et al., 2015). Furthermore, overexpression of thymic stromal lymphopoietin (TSLP) is a major factor contributing to allergic diseases such as asthma, allergic rhinitis etc. Epithelial cell-derived TSLPs control the allergic condition via regulating the activation of T-cells, mast cells, and dendritic cells. The findings of the study elucidated that shikonin as well as L. erythrorhizon aqueous extract was able to downregulate TSLP production as well as markedly attenuated the levels of IKKα, NLRP3 and Caspase-1 (Yen et al., 2017). Besides having multiple pharmacological effects, naphthoquinones are also considered as potent allelochemicals as they hold good potential to defend against predators. Previous studies demonstrated that juglone, 1,4-naphthoquinone, plumbagin and 2-methoxy-1,4-naphthoquinone showed anti-feedant activity against the cabbage looper Trichoplusia ni (Akhtar, Isman, Niehaus, Lee, & Lee, 2012). Naphthoquinones were also found effective against the dry bean pests Epilachna varivestis and Acanthoscelides obtectus (Cespedes et al., 2016). Moreover, the extreme toxicity of juglone against Myzocallis walshii and plumbagin against Tetranychus cinnabarinus, Myzus persicae and Illinois liriodendri were also investigated. These studies substantiate that juglone and plumbagin are effective insecticidal and acaricidal agents. The inhibitory and toxicity potential of Onosma visianii roots against Spodoptera littoralis was also investigated. The main active constituents of O. visianii roots include isovalerylshikonin and isobutyrylshikonin. Being highly lipophilic in nature, these active moieties easily enter the insect exoskeleton and hinder the physiological processes. Moreover, the ester groups of these moieties increases cuticle penetration via linkage with hydroxyl groups and significantly inhibits acetylcholinesterase (AChE).
| Compounds                  | Cell lines/In vitro/vivo assay                                                                 | Mechanism involved                                                                                                                                                                                                 | Types of cancer                                      | References |
|---------------------------|-----------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|------------|
| Shikonin                  | Human normal lung fibroblast cell line CCD19 and human NSCLC cell lines (HCCB27, H1650 and H1975) | Induces EGFR degradation causes deactivation of Tyr1173 and Tyr1068 of EGFR; Inhibits TrxR1 to activate ROS-mediated apoptosis                                                                                       | Gefitinib-resistant non-small cell lung cancer       | Li et al., 2017 |
|                           | Human epithelial colorectal adenocarcinoma Caco-2 cells. AOM/DSS model.                       | Inhibition of COX-2, iNOS and IL-6 via deactivation of NFκB; Inhibits Bcl-2 and activates Caspase-3                                                                                                                  | Colon cancer                                         | Andrújar, Ríos, Giner, & Recio, 2013 |
|                           | Normal human colon epithelial cell line (NCM460), well-differentiated colon carcinoma cell lines (HT29 and HCT116), poorly differentiated colon carcinoma cell line (SW480). | Overexpression of SIRT2; Inhibits the viability of SW480 cells and arrests the cell cycle at the G2/M stage; Inhibition of ERK1/2 phosphorylation                                                                 | Colorectal cancer                                    | Zhang et al., 2017 |
| MRI mouse tumor xenograft model MCF-7 and SK-BR-3 cells |                                                                                         | Downregulation of ERα, GPER, EGFR and p-ERK expressions; Inhibits the proliferation in MCF-7 and SK-BR-3 cells; Arrest cell cycle at G0/G1 phase in MCF-7 and induce apoptosis in SK-BR-3 cells | Breast cancer                                        | Yang et al., 2019 |
|                           | Human lung cancer cells (A549)                                                               | Significant increase in RIP1 levels leading to necroptosis                                                                                                                                                    | Non-small cell lung cancer                           | Kim et al., 2017 |
|                           | Nude mouse tumor xenograft model                                                             | Decreased Bcl-2 and Bcl-xl expression; Increased caspase 3 and 9 activities. Depolarization of mitochondrial membrane potential                                                                                 | Colon cancer                                         | Liang et al., 2017 |
|                           | Human colon cancer cell lines HCT116, SW480 and human normal colon mucosal epithelial cell line NCM460. | Irreversible inhibition of human recombinant CDC25 phosphatases; Inhibit dephosphorylation of CDK1 thereby inducing cell cycle arrest at G2/M phase Suppression of NEAT1 and Akt signaling | Cancer                                               | Zhang et al., 2019 |
|                           | Xenograph tumour model                                                                       |                                                                                                    |                                                     | Zhang, Ras, Zhu, Wu, & Jiang, 2020 |
|                           | Human lung cancer cells (A549)                                                               | (continued on next page)                                                                                                                                                                                                                           |                                                     |            |
Table 3 (continued)

| Compounds                                | Cell lines/In vitro/In vivo assay                                                                 | Mechanism involved                                                                 | Types of cancer                                                                 | References          |
|------------------------------------------|--------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------|
| b,b-Dimethyl acrylshikonin               | (CRL-6475) and mouse nonmetastatic melanoma cell line B16-F0 (CRL6322), Reticulum (ER) stress.   | Activation of p-ERK, p-p38, Caspase-3 and Caspase-9                                 |                                                                                  | & Fan, 2020         |

Fig. 7. Mechanistic activity of shikonin and acetyl shikonin for treatment of asthma, arthrosclerosis, diabetes and inflammation. LDL, low density lipoprotein; DAG, diacylglycerol; ROS, reactive oxygen species; TSLP, thymic stromal lymphopoietin; IL, interleukins; GCM, global compact on migration.

Table 4

Mechanism involved and pharmacological outcomes from miscellaneous investigations on alkannin/shikonin containing plant extracts, alkannin/shikonin and its derivatives.

| Test compounds                                | Cell lines/In vitro/In vivo assay                                                                 | Mechanism involved                                                                 | Disease targeted                                                                 | References          |
|-----------------------------------------------|--------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------|
| Lithospermum erythrorhizon and Angelica sinensis extract | Human Bronchial Epithelial cell line (BEAS-2B)                                                  | Anti-inflammatory effect in Der-p2-stimulated BEAS-2 β cells;                      | Allergic diseases such as asthma, atopic dermatitis and allergic rhinitis      | Yen et al., 2017    |
| Onosma tauricum extract                      | Anti-oxidant assays (DPPH, CUPRAC, ferrous ion chelating, FRAP, plumbagin, ABTS) and Enzyme inhibitory assays (AChE, α-amylase, BChE, tyrosinase, α-glucosidase) | Anti-oxidant and enzyme inhibitory activity                                       |                                                                                  | Kirkan et al., 2018 |
| Onosma sieheana and Onosma stenoloba extracts | Total phenolic assay, total flavonoid assay, anti-oxidant assay, tyrosinase assay, α-amylase assay. | Anti-tyrosinase activity. Increased expression of p-Erk1/2 and reduced expression of tyrosinase related protein 1 and 2 | Diabetes                                                          | Sahinler, Ceylan, & Tepe, 2020 |
| Lithospermum radix aqueous extract           | Sub-acute oral toxicity                                                                          | Suppression of spinal inflammation                                               | Chemotherapy induced neuropathy, Fibrinolysis                                   | Han et al., 2016    |
| Shikonin                                     | PA-1 activity assay, Clot lysis assay, mouse arterial thrombosis model, Mouse liver fibrosis model. | Inhibition of plasminogen activator inhibitor-1 activity; Anti-thrombotic and anti-fibrotic effect | Atherosclerosis                                                                 | Huang et al., 2015  |
|                                              | Human endothelial cell line derived from human lung carcinoma cells and human umbilical vein endothelial cells | Induction of expression of PI3K/ Akt/NF2-dependent antioxidant genes such as SOD-1, HO-1, Catalase, GPx-1, GCLM and GSR; Inhibition of oxLDL-induced intracellular ROS accumulation via NF-κB adhesion | Rheumatoid arthritis                                                          | Liu et al., 2020    |
|                                              | Human umbilical vein endothelial cells (HUVEC), human fibroblast-like synoviocyte (HFLS),Collagen-induced arthritis |                                                                                   |                                                                                  |                     |
Table 4 (continued)

| Test compounds | Cell lines/In vitro/In vivo assay | Mechanism involved | Disease targeted | References |
|----------------|----------------------------------|--------------------|-----------------|------------|
| Isovaleryl shikonin and isobutyryl shikonin | Acute toxicity, chronic toxicity, growth inhibition, antifeedant activity, AChE inhibitory activity and antioxidant assay. | Inhibition of AChE enzymes; Inhibition of mitochondrial respiration thereby inhibiting larval growth | Inhibition of larval growth of Tobacco cutworm Spodoptera littoralis | Li, Zeng, Su, He, & Zhu, 2018 |
| Acetylshikonin | Behavioral testing (Morris Water Maze test) | Inhibition of activation of p53/p21 signaling pathway; Upregulation of SIRT1 in hippocampus; Anti-apoptotic activity in neuronal cells and attenuated H2O2 induced oxidative stress | Alzheimer’s disease | Huang et al., 2019 |
| Acetylshikonin and isobutyryl shikonin | Anti-genotoxic properties (Umu-test) and cytotoxicity assay (lung fibroblast cell line (V79)) | Activation of p2C-β3/PKCö cascades via activation of inositol lipid signaling and increase in DAG release | Diabetes. | Skrzypczak et al., 2015 |

Table 5

| Plant | Extraction process | Solvent systems | Methods | Constituents | References |
|-------|-------------------|----------------|---------|--------------|------------|
| Lithospermum erythrorhizon | Sonication | Gradient elution: Petroleum ether-ethyl acetate, petroleum ether – dichloromethane, petroleum ether – acetone, and petroleum ether – ethyl acetate and acetone | Silica gel column chromatography | Acetylshikonin, shikonin, deoxyshikonin, β-sitosterol and β,β-dimethylacrylshikonin | Li, Xu, Zhu, & Wang, 2012 |
| | Solid liquid extraction | – | Open column of silica gel chromatography | Shikonin, acetylshikonin, 5,8-dihydroxy-1,4-naphthoquinone (DH), 1,4naphthoquinone (NAP) and β,β'-dimethylacrylshikonin. Shikonin (an improved method) | Cheng et al., 2008 |
| | Solid-liquid extraction | n-hexane/2- propanol (90:10, volume percentage) | Chiral HPLC | Isobutyrylshikonin | Azuma et al., 2016 |
| | Maceration | 50% hexane in CH2Cl2, CH2Cl2, 5% and 33% acetone in CH2Cl2, and 5% and 33% methanol in CH2Cl2 | Silica gel column chromatography and Sephadex column with methanol. | Shikonin, β-hydroxyisovalerylshikonin, acetylshikonin, β,β-dimethylacrylshikonin, deoxyshikonin, isobutyrylshikonin, and methyl-n-butyrilshikonin. | Yen et al., 2017 |
| | Maceration | 0.085% H3PO4 buffer and acetonitrile: 10%–25% for 20 min; 25%–70% for 30 min; 70%–90% for 40 min; 80%–90% for 60 min; and 100% for 65 min. | Reverse phase column chromatography | Shikonin, bhydroxyisovalerylshikonin, acetylshikonin, β-acetoxyisovalerylshikonin, deoxyshikonin, isobutyrylshikonin, β,β-dimethylacrylshikonin, and methyl-n-butyrilshikonin. | Deoxyshikonin |
| | Ultrasonic extraction. | Methanol and water with 0.5% acetic acid. | Reverse phase column chromatography | Deoxyshikonin | Park et al. (2017) |
| Onosma visianii | Soxhlet extraction | methanol and water (0.1% formic acid) (90:10) | Semipreparative HPLC | Isovalerylshikonin, isobutyrylshikonin, acetylshikonin, hydroxyisovalerylshikonin, shikonin-β,β-dimethylacrylate, proponyshikonin, 5,8-dimethoxy acetylshikonin, 1-(5,8-dimethoxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-4-methylpent-3-en-1-yl 2-methylbutanoate, 5,8-dimethoxy isobutyrylshikonin, 5,8,0-dimethyldioxoexshikonin, 2-(4-hydroxy-4-methylpent-2-ene-1-yl)-5,8-dimethoxynaphthalene-1,4-dione. | Sut et al., 2017 |
| Alkanna strigosa | Soxhlet extraction | (CHCl3; MeOH: H2O) (5:4:1) | Preparative tlc | Alkannin and shikonin | Aburjai, Al-Janabi, Al-Mamoori, & Azzam, 2019 |
| Echium italicum | Maceration | Hexane-Etilacetae | silica gel column chromatography | 2-Methyl-n-butyrilshikonin, isovalerylshikonin, acetylshikonin and deoxyshikonin | Eryugur, Yilmaz, Kutsal, Yıcıel, & Üstün, 2016 |
| Lomandra hastilis | Sonication | n-hexane-acetone (3:1) | Preparative TLC | 5,8-Dihydroxy-2-ethyl-3,6,7-trimethoxy-1,4-naphthoquinone, lomazarin, 2-(1'- | Utkina & Pokhilo, 2017 |

(continued on next page)
Table 5 (continued)

| Plant          | Extraction process | Solvent systems                  | Methods                                      | Constituents                                                                 | References                      |
|----------------|--------------------|-----------------------------------|----------------------------------------------|-------------------------------------------------------------------------------|---------------------------------|
| *Alkanna tinctoria* | Extraction with 95% EtOH | Hexane-EtOAc (20:1-0:1, volume percentage) | Silica gel column chromatography Sephadex column | Alkannin, shikonin, 5,8-dihydroxy-3,6,7-trimethoxy-1,4-naphthoquinone          | Akgun, Erku, Pilavci, & Yesil, 2011 |
|                |                    | Methanol                           | Reverse phase column chromatography           |                                                                               | Albreht, Vovk, Simonovska, & Srbinska, 2009 |
|                |                    | Hexane-EtOAc (5:1-4:1, volume percentage) | HPLC-PDA analysis                            |                                                                               | Mohapatra et al., 2016          |
|                |                    | supercritical CO2 extraction       |                                              |                                                                               | Yusufoğlu et al., 2018          |
| *Echium italicum*       | Solid-liquid extraction | MeOH:HC0OH (20:1, volume percentage) and THF:MeCN: H2O:HC0OH (30:20:50:0.5, volume percentage) | Chiral thin-layer chromatography and semi-preparative HPLC | Alkannin, 2-methyl-n-butyrylshikonin, and isovaleryl shikonin                  |                                 |
| *Arnebia nobilis Reichb.f.* | Perculation      | –                                 |                                              | Acetyl alkanin, acetoxyisorvaleryl alkanin and β,β'-dimethylacyl alkanin       |                                 |
| *Alkanna hispidissima*  | Perculation       | Hexane - acetone - acetic acid     |                                              | Arnebin-1, arnebin-2, arnebin-3, arnebin-4, arnebin-5, arnebin-6 arnebin-7, tiglicacid, arnebinone, alkanin, arnebinol, and cycloarnebin-7 |                                 |

3. Isolation and analytical aspects of A/S and their derivatives

A/S and their derivatives have been reported to be isolated from various Boraginaceae family plants (Table 5) amongst which L. erythrorhizon (Lee et al., 2016; Rajasekar et al., 2012; Han et al., 2007; Azuma et al., 2016) and Alkanna tinctoria Tausch. (Mohammed, 2016; Rashan et al., 2018; Jaradat et al., 2018) yield high content of shikonin and alkannin derivatives, respectively. Adding on, the petroleum ether and chloroform fraction of dried roots of L. erythrorhizon elute β,β'-dimethylacrylshikonin, isobutyl shikonin, shikonin, 5,8-dihydroxy-3,6,7-trimethoxy-1,4-naphthoquinone, and 5,8-dihydroxy-3,6,7-trimethoxy-2-(1'-methoxymethyl)-1'-naphthoquinone. In another report, solid liquid extraction and HPLC-VIS technique was also used to obtain acetyl alkanin and β,β'-dimethylacyl alkanin from the dry roots of Echium etalicum (Albreht, Vovk, Simonovska, & Srbinska, 2009). In addition to the conventional methods of extraction and isolation, a novel method called supercritical CO2 method was used to

Table 6

| Titles                                              | Targeted diseases                                      | Mechanism of action               | References    |
|-----------------------------------------------------|--------------------------------------------------------|-----------------------------------|---------------|
| Acylatedalkannin or shikonin deriva. - useful as dermatological, bactericial and fungidal medicaments | Treatment of skin lesions: ulcers, burns, wounds, scurf, skin cancers, Measles, rashes, ulcer sores, eczema, burns | Antibacterial and anti-inflammatory effect | Papageorgiou, 1980 |
| Process for preparing arnebia root medicine with broad-spectrum medical functions | Proliferation of fibroblasts                             |                                   | Song, 2004    |
### Table 6 (continued)

| Titles | Targeted diseases | Mechanism of action | References |
|--------|-------------------|---------------------|------------|
| Alkannin derivatives as immune inhibitors and metal complexes thereof | Arthritis, scleroderma, lupus erythematosus, HIV infection and malignant tumor | Immunological suppression of chemokines and HIV-type 1 | Li & Hu, 2004 |
| Use of alkannin in preparing medicine for treating tumor disease | Treatment of tumor, effective on the tumor, effective on cancer cells | Killing tumor cells with p-glycoprotein | Hu & Fang, 2005 |
| Application of shikonin in preparing medicine for inducing apoptosis | Treatment of tumor | Shikonin induces ROS production and cytoreduction release in cancer cells | Hu & Han, 2007 |
| Application of Xinjiang radix macrotomiae for treating flat wart, common wart and fig wart | Treatment of verrucous disease, flat wart, common wart, fig wart | Diminishing the inflammation of tumor on an afflicted part, healing of wounds without leaving scar | Li & Chen, 2009 |
| Method of treatment of virus infections using shikonin compounds | Virus infections, mycoplasma infections, malignant tumor | Promoting idiosyncratic cell mediated immunity and improves immune response of T-lymphocytes | Wang, 2008 |
| Antineoplastic sulphur-containing alkannin and naphthoquinone derivatives | Antineoplastic | Inhibition of tumor cell growth | Li, Zhao, Xie, He, & Guo, 2008 |
| Antineoplastic alkannatioria ketoximes derivatives | Antineoplastic | Retard tumor cell growth | Li & Zhao, 2010 |
| Application of alkannin in preparation of pyruvate kinase inhibitor | Psoriasis, herpes simplex keratitis | Inhibition of PMK2 activity | Hu, 2011 |
| Medical application of radix arnebiaeAusulithospermi naphthoquinone compounds | Crohn’s disease | Inhibition of NF-kB and STAT-3 | Liu & Fan, 2014a |
| Medical application of gromwell naphthoquinone compounds | Ulcerative colitis | Inhibition of COX-2 and cytokines (INF-γ and IL-6) | Liu & Fan, 2014b |
| Medical application of lithospermumnaphthoquinone compounds | Chronic obstructive pulmonary disease (COPD) | Resisting inflammation, killing viruses and realizing quick apoptosis of skin vegetation cells. | Yuan & Wang, 2016 |
| Pharmaceutical composition for treating flat wart and verruca vulgaris and preparation method for pharmaceutical composition | Flat wart, verruca vulgaris | Inhibition of influenza virus, gram positive and gram negative bacteria | Liu, Zhao & Wang, 2017 |
| Compound traditional Chinese medicine for preventing and treating stigmatosis of freshwater fish | Stigmatosis | Activation of AMPK (AMP activated protein kinase) | Yoon, Lee, Jung, & Jeong, 2017 |
| Compositions for metabolic disorders comprising alkannin as an active ingredient | Obesity, hyperlipidemia, fatty liver | Activation and differentiation of TH cells and cytokine secretion | Liu & Yu, 2017 |
| Application of alkannin in preparation of medicine for treating upper and lower respiratory tract allergic disease | Allergic rhinitis and allergic asthma | Inhibition of EGFR kinase activity and induction of apoptosis in cancer cells | Liu, Leung, Li, & Fang, 2018 |
| Hydroxynaphthoquinone compounds for treatment of non-small cell lung cancer | Non– small cell lung cancer | Synergistic effect of shikokin and herqueiazole | Zhuang & Zhang, 2017 |
| Herqueiazole-containing medicine for controlling inflammation | Inflammation | Eliminating vaginal bacteria and maintaining vaginal flora and acid base balance | Wang & Chen, 2017 |
| External biological preparation for feminine vagina prophylaxis and health care as well as treatment of gynaecological genital tract inflammation, and preparation method | Cervical erosion, vaginitis, pelvic inflammation | Inhibition of H1, K+ ATPase enzyme activity | Li, 2018 |
| Omeprazole enteric-coated capsules capable of inhibiting gastric acid secretion | Gastric and duodenal ulcer | Anti-inflammatory effect | Liu, Wei, Zhong, Sun, Yi, & Yang, 2017 |
| Composition for treating burns and scalds | Treating burns and scalds. | Inhibition of TNF-α | Ling, Wang, Wang, & Zhang, 2017 |
| Shikonin and derivant thereof are as the application of gene therapy sensitizer | Cancer | Inhibition of STAT 3 (Signal transducers and activators of transcription) pathway | Chu, 2018; Chu, 2018b |
| Externally-applied anti-inflammatory agent containing radix lithospermii extract | Inflammation | | |
acetylshikonin, shikonin, deoxyshikonin, L. erythrorhizon extract was subjected to sonication to obtain tilis (Park et al., 2017; Utkina & Pokhilo, 2017). On the similar lines, thoqimone derivatives from the residual extract of Lomandra has-also exploited to primarily obtain deoxyshikonin and other naph-

Subsequently, the ultrasonication technique was isolate A/S from the powdered roots of A. tinctoria. Supercritical CO₂ functions as non-polar, lipophilic solvent with alkannin/shikonin. It was reported that highest yield was obtained at higher tem-

Furthermore, Micro-

Table 7 (continued)

| Patent No./ Filling date | Plants | Titles | Conditions | Methods of extraction | Compounds | References |
|--------------------------|--------|--------|------------|-----------------------|-----------|------------|
| 1999) CN1384149A (17-05-2002) | Comfrey roots | Gromwell haematocramehaematochrome extracting process | Liquid CO₂ | Super critical CO₂ extraction | alkannin, β hydroxyl isovalerylshikonin, 2,3 dimethyl pentene shikonin. | Wang, 2002 |
| | Arnebia euchroma | Method for promoting Xinjiang alkannatinctoria callus growth using rare earth element | N₂, solid medium | Callus growth culture | Shikonin | Wang, Fang, & Wang, 2005 |
| CN1546450A (08-01-2004) | Dried Arnebia roots | Preparation method of high purity alkannaphthoquinone | Supercritical CO₂ | Super critical CO₂ extraction | Alkannin, deoxyshikonin | Li & Hu, 2004 |
| CN101434530A (12-12-2008) | Comfrey dried purple roots | Method for extracting alkannin from alkanet | Ethanol | Solid liquid extraction | Shikonin | Zu et al., 2009 |
| CN10194212A (15-07-2010) | Comfrey powder | Method of extracting alkannin naphthoquinone pigment | 1,1,1,2-Tetrafluoroethane | Molecular distillation | Dimethyl acrylamide shikonin, isovalerylshikonin | Liu & Liu, 2011 |
| CN101906028B (26-08-2010) | Comfrey roots powder | Method for extracting benzoxquinone compound in lithospermum | Petroleum ether | Multiple reflux extraction | Alkannin | Yan, Xu, Yu, & Lei, 2013 |
| CN10228499A (20-06-2011) | Arnebia roots | Method for separating naphthoquinone active ingredients from sinkangarnea root | Petroleum ether, ethylacetate | Ultrasonic extraction | Deoxyshikonin, acetyl shikonin, shikonin, β,β-metho acetylloxyshikonin, isovalerylshikonin, β hydroxyl isovalerylshikonin | Yuan & Yuan, 2011 |
| CN10337913A (15-04-2012) | Comfrey purple grass powder | Extraction method of alkannin | Cyclohexane | Maceration | Shikonin | Pan, Wang, Tang, Li, Wang, & Zhou, 2013 |
| CN103664566A (02-12-2013) | Comfrey purple grass | Alkannin extraction device | Petroleum ether | Ultrasonic crusher extraction | Shikonin | Tang, Wang, & Zhou, 2014 |
| CN105949045A (28-07-2014) | Arnebia roots | Method for extracting alkannin from arnebia roots | Super critical CO₂ | Super critical fluid extraction | Shikonin | Guo, Zhang, & Xu, 2016 |
| CN105348065A (04-12-2015) | Lithospermum ericythroniz | Preparation method for high-purity alkannin from lithospermumerythroniz | Petroleum ether | Percolation | Shikonin | Yang & Yang, 2016 |
| CN104774151A (30-01-2015) | Lithospermum mongolia | Preparation technology of mount taishan Radix Lithospermum naphthoquinone active monomers | Petroleum ether, hexanoic acid, Capro lactone Hexylalcohol-water, n-Hexane, ethylacetate, acetonitrile, water | High performance counter current chromatography | Iospenyl shikonin, hexylshikonin, isobutyryl shikonin | Lei, Haiwei, Jiang, Zhai, Yi, & Jiang, 2015 |
| CN10751203A (03-03-2016) | Arnebia euchroma | Method for separating and preparing natural naphthoquinone compounds | Petroleum ether, ethylacetate, acetonitrile, water | High speed counter current chromatography | Deoxyshikonin, propionyl shikonin, β,β dimethylacryl shikonin, isovalerylshikonin. | He, Qing, & Zhang, 2017 |
| CN108409570A (06-03-2018) | Arnebia euchroma | Fast and efficient purification method comfrey acetyl shikonin | Ethylacetate/petroleum ether | Reverse phase silica gel chromatography | Acetylshikonin | Jiang, Lin, & Zhao, 2018 |

isolated from the powdered roots of A. tinctoria. Supercritical CO₂ functions as non-polar, lipophilic solvent with alkannin/shikonin. It was reported that highest yield was obtained at higher tem-

Forecasting the market potential, numerous patent applications on inventions containing alkannin/shikonin and its derivatives have been filed by various research groups across the world. Brief details of these applications are divided into two categories viz. therapeutic and analytical and are summarized in Tables 6 and 7.

5. Conclusion

Alkannin/shikonin and its derivatives possess a wide variety of pharmacological activities. These constituents are majorly investi-
gated for their wound healing, antimicrobial and anticancer potential. In the last decade, various mechanisms of alkannin/shikonin and their derivatives are explored implicated in wide variety of diseases. The present study suggests the higher applicability of alkannin/shikonin and its derivatives are in the development of potent and safer wound healing and anticancer agents. Various analytical investigations are also discussed that will help the analysts for more efficient analysis of alkannin/shikonin and its derivatives from different sources. Brief patent summary is provided to highlight the future marketable potential of alkannin/shikonin and its derivatives. The appropriate knowledge of the pharmacological aspects of A/S and their derivatives will not only benefit the novel product researchers but also the pharmaceutical/formulation scientists in their future course of action. Further, the advanced and novel drug delivery systems could be used to mask the limitations of these derivatives including their low solubility and photo-degradation. Despite having magnificent pharmacological potential, there is a dire need to collect remarkable data related to their toxicological and safety profile which can establish the clinical utility of these components.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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