

PHYTOCHEMISTRY, STRUCTURE–ACTIVITY RELATIONSHIP, AND CLINICAL PHARMACOLOGICAL POTENTIAL OF *ALKANNA* *TINCTORIA*: A COMPREHENSIVE REVIEW

Navan Garg^{*1}, Neha Khatun²

¹Faculty of Pharmaceutical sciences, PCTE Group of Institutes, Punjab, Ludhiana, India, 142021.

²Chandigarh College of Pharmacy, Landran, Mohali, India, 140307.

Article Received: 18 August 2026 | Article Revised: 08 September 2026 | Article Accepted: 28 September 2026

***Corresponding Author: Navan Garg**

Faculty of Pharmaceutical sciences, PCTE Group of Institutes, Punjab, Ludhiana, India, 142021.

DOI: <https://doi.org/10.5281/zenodo.23233900>

How to cite this Article: Navan Garg, Neha Khatun (2026) PHYTOCHEMISTRY, STRUCTURE–ACTIVITY RELATIONSHIP, AND CLINICAL PHARMACOLOGICAL POTENTIAL OF *ALKANNA TINCTORIA*: A COMPREHENSIVE REVIEW. World Journal of Pharmaceutical Science and Research, 5(10), 464-479.



Copyright © 2026 Navan Garg | World Journal of Pharmaceutical Science and Research.

This work is licensed under creative Commons Attribution-NonCommercial 4.0 International license (CC BY-NC 4.0).

ABSTRACT

Alkanna tinctoria, commonly known as Ratanjot, is a medicinal herb widely recognized for its therapeutic efficacy in neurodegenerative diseases such as alzheimer's disease, parkinson's disease, and vascular dementia. The present review summarizes the phytochemical composition, structure–activity relationship, pharmacological potential, and emerging clinical relevance of bioactive constituents isolated from this plant. Major phytochemicals including alkannin, shikonin, flavonoids, phenolic acids, and naphthoquinone derivatives exhibit significant antioxidant, anti-inflammatory, antimicrobial, wound-healing, neuroprotective, and anticancer activities. Mechanistic investigations indicate that these compounds regulate important molecular pathways such as NF-κB, MAPK, and Nrf2/ARE signalling, thereby reducing oxidative stress and inflammatory responses associated with chronic diseases. Experimental and limited clinical studies suggest promising applications of *Alkanna tinctoria* in neurodegenerative disorders, skin regeneration, wound management, and pharmaceutical formulations. Despite encouraging findings, the available evidence is predominantly preclinical, and challenges related to toxicity, standardization, pharmacokinetics, and clinical validation remain unresolved. Present review highlights existing research gaps and future perspectives to support the development of safe, effective, and evidence-based therapeutic products derived from *Alkanna tinctoria*.

KEYWORDS: *Alkanna tinctoria*, neuroprotection, structural activity relationships, phytochemicals, clinical pharmacological potentials.

1. INTRODUCTION

Ratanjot i.e. *Alkanna tinctoria* L., belonging to the Boraginaceae family, commonly known as dyer's alkanet and dyer's bugloss is a natural herb native to Asia, Arabia, Southeast zone of Europe and Mediterranean regions. (Alshamar, H. A. and Dapson, R. W et al., 2023; Rana, S. and Chauhan, P et al., 2022). In Indian system of medicine, roots of Ratanjot are used to treat different disorders related to skin, gastro intestinal tract, renal calculi, menstruation, and respiration. (Figure 1). Ratanjot is used as an antimicrobial, antioxidant and skin disorders. (Arampatzis AS et al., 2022;

Kourounakis AP et al., 2002). Major role as an antioxidant to treat neurodegenerative disease especially Alzheimer's Disease, Dementia and Parkinson Disease by decreasing the oxidative stress and increase the flow of ions in the arteries was investigated scientifically researchers in the past. (Saraf S et al., 2006). The plant is most renowned for the vibrant red dye extracted from its dark red roots. Presence of phenolic acids, flavonoids, glucosides, resins, waxes, tannin, alkaloids, pyrrolizidine and volatile oil has been also reported in various extracts of the roots. Pharmacologically active Naphthazarin and phenolic compounds are responsible for color and specific flavour of *Alkanna tinctoria* respectively. (Das A et al., 2024; Khan U A et al., 2015). Natural red pigments and bioactive phytochemicals of ratanjot finds broader application in medicine, food, and cosmetic industries, owing to their antiwrinkle antipyretic, antinociceptive, sedative, anticancer, antioxidant, antiulcer, antiaging, anti-inflammatory, antimicrobial, and wound-healing properties Shikonins and alkannin exist as natural enantiomer and the pharmacological activities are attributed due to their specific stereochemistry. (Dwivedi C et al., 2014; Pluhár Zs et al., 2001; Gadhave D G et al., 2024). Macroscopically alkanet roots appear as chopped reddish black roots covered with a crust layer with inner yellowish white part. Pigments are biosynthesized mostly in the surface of the crust layer of alkanet roots. European medical descriptions and various pharmacopoeias contain information related to phytochemicals extracted from *Alkanna tinctoria*. (Lamprey R N L et al., 2022). Present review will be helpful in highlighting structural insights and pharmacological activities of bioactives isolated from *Alkanna tinctoria*. (Wilson D M et al., 2023). Phytoconstituents biosynthesized in the roots are Alkannin, Shikonins, β , β -dimethyl-acryl-alkannin, isovalerylalkannin, acetyl alkannin and α -methyl-n-butyl-alkannin etc. whereas the non-ariel parts consist of different phytoconstituents like inositol, volatile oils, β -eudesmol, α -eudesmol, γ -eudesmol, kaempferol-3-glucoside, kaempferol-3-rutinoside, quercetin-3-glucoside, quercetin-3-rutinoside, kaempferol-3,6-dimethylether, kaempferol-3,7-dimethylether, 6-methoxy kaempferol 3,7-dimethylether, and kaempferol-3-methylether etc. Shikonins, Alkannin and other derivatives of 5,8-dihydroxy-1, 4-(naphthazarin) are the major elements of the combination of lipid-soluble red pigments found in the root bark and dried roots of *A. tinctoria*. The roots and dried roots also contain isovaleric acid, β -dimethyl acrylic acid, pyrrolizidine alkaloid, β -acetoxoy isovaleric acid, and angelic acid. (Ganos C et al., 2024; Shahzad Khan M et al., 2023; Das R et al., 2023; Zannou O et al., 2020). Figure 2 and 3 represents the phytoconstituents obtained from roots and leaves of *Alkanna tinctoria*. Pharmacokinetic parameters and pharmacological activities of phytoconstituents obtained from *Alkanna tinctoria* mentioned in Table 1.

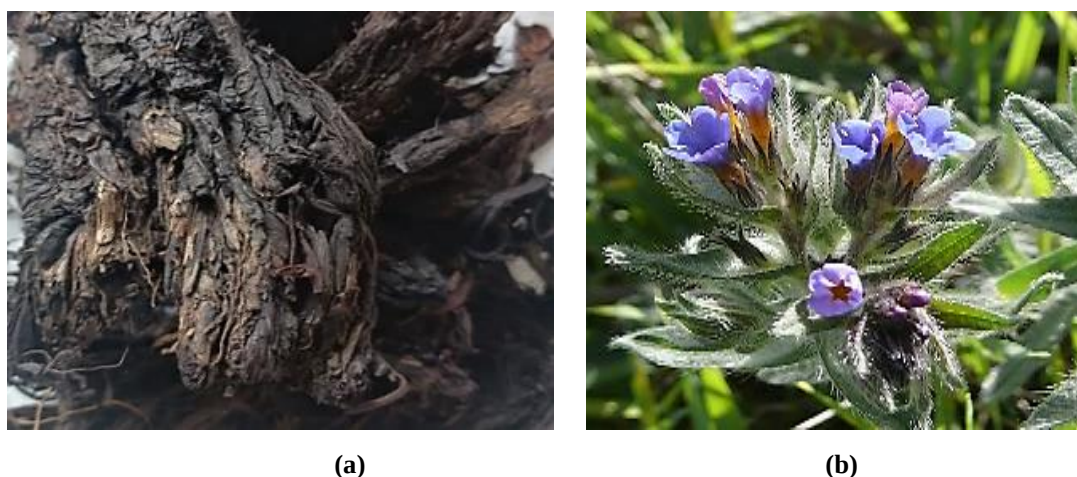


Figure 1: *Alkanna tinctoria* (a) roots and (b) leaves with flowers.

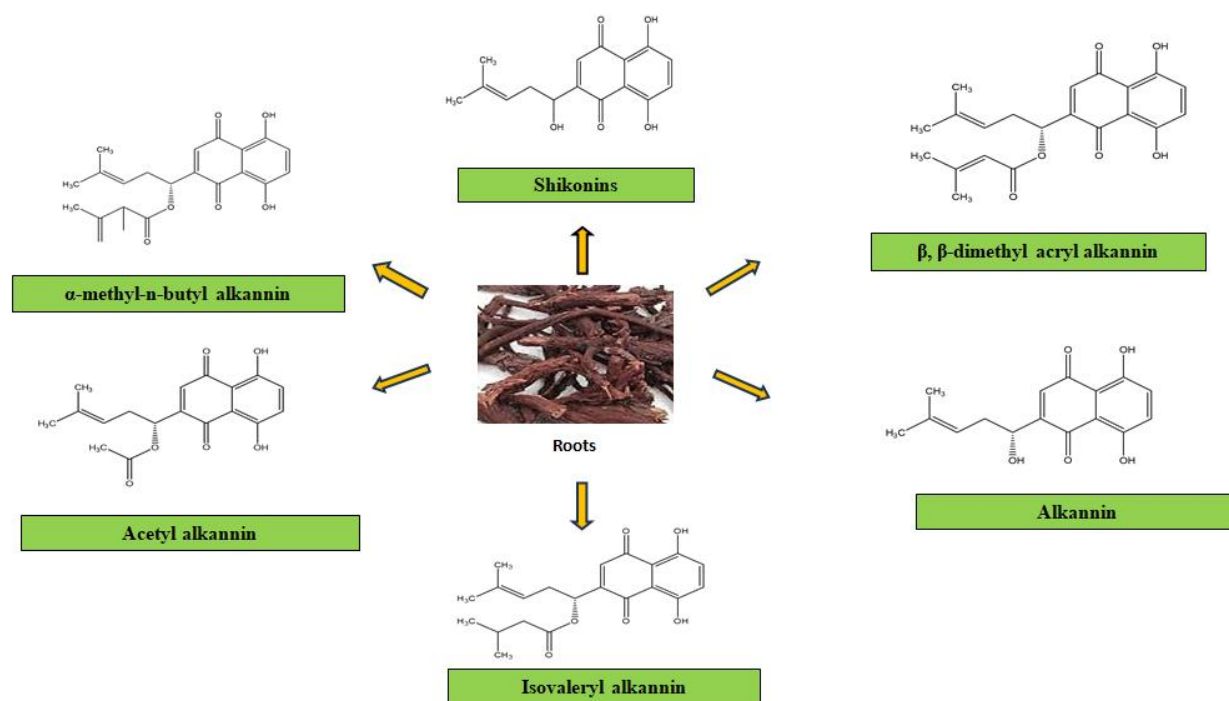


Figure 2: Major phytochemical constituents isolated from roots of *Alkanna tinctoria* including alkannin, shikonin, flavonoids, phenolic acids, and naphthoquinone derivatives.

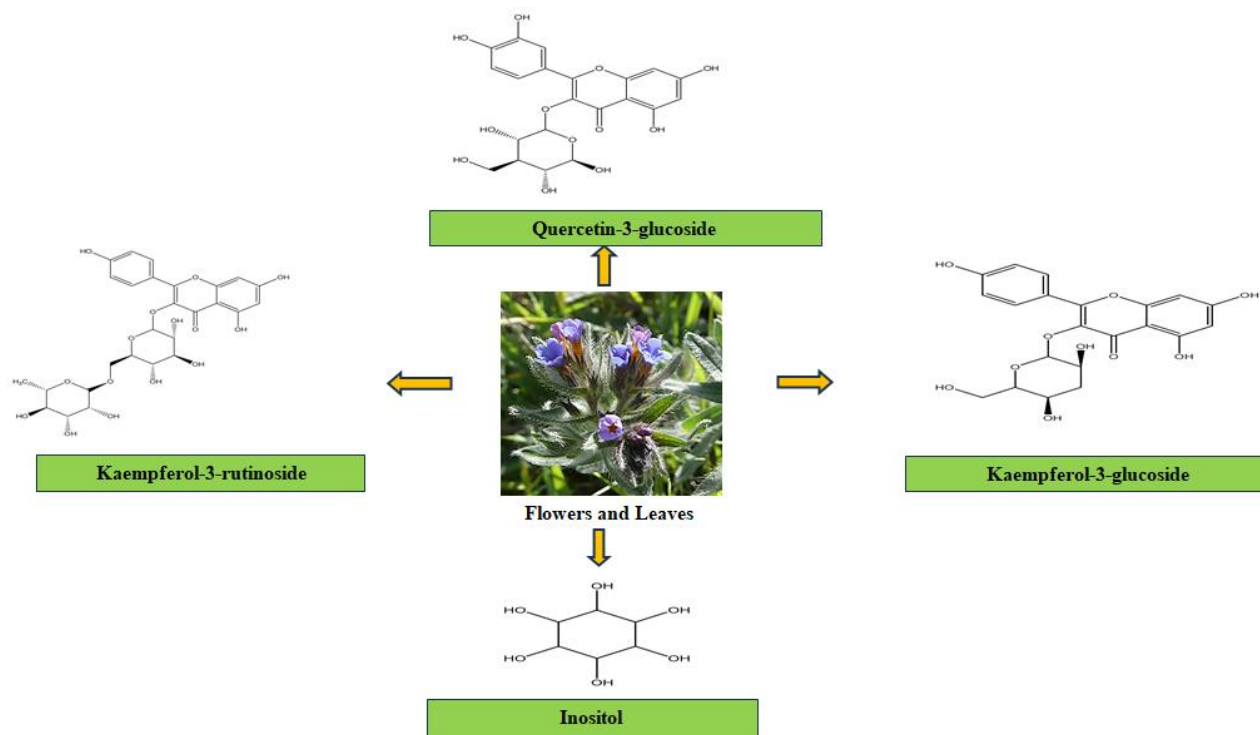


Figure 3: Major phytochemical constituents isolated from flowers and leaves of *Alkanna tinctoria*.

Table 1: Pharmacokinetic parameters and pharmacological activities of phytoconstituents obtained from. *Alkanna tinctoria*

S. No.	Metabolites	Phytochemicals	Lipophilic / Hydrophilic	Log P	Molecular Weight (g/mol)	Molecular Formula	Pharmacological Activities	Reference
Naphthoquinones								
1.	Alkannin	Naphthoquinones	Lipophilic	3.2	288.30	C ₁₆ H ₁₆ O ₅	Antibacterial, Wound Healing, Antioxidant, Anticancer	Kumari P et al., 2024; Kaur K et al., 2022
2.	Shikonins	Naphthoquinones	Lipophilic	3.1	288.30	C ₁₆ H ₁₆ O ₅	Antimicrobial, Anti-inflammatory, Anticancer	Depeursinge A et al., 2010; Kaur K et al., 2022
3.	Acetyl alkannin	Naphthoquinones	Lipophilic	3.6	330.33	C ₁₈ H ₁₈ O ₆	Antioxidant, Cytotoxic, Antimicrobial	Jaradat N A et al., 2018
Triterpenes								
4.	β-Sitosterol	Triterpenes	Highly Lipophilic	8.5	414.71	C ₂₉ H ₅₀ O	Anti-inflammatory, Cholesterol-Lowering, Immunomodulatory	Ganos C et al., 2024; Das A et al., 2024
Fatty Acids								
5.	Linoleic acid	Fatty Acids	Highly Lipophilic	6.1	280.45	C ₁₈ H ₃₂ O ₂	Skin Health, Anti-inflammatory, Cardioprotective	Das A et al., 2024; Kaur K et al., 2022
6.	Palmitic acid	Fatty Acids	Highly Lipophilic	7.2	256.42	C ₁₆ H ₃₂ O ₂	Antimicrobial, Skin Barrier Protection	Das A et al., 2024; Kumari P et al., 2024
7.	Myristic acid	Fatty Acids	Highly Lipophilic	6.3	228.38	C ₁₄ H ₂₈ O ₂	Skin Moisturizing, Antimicrobial	Das A et al., 2024; Kumari P et al., 2024
Alkaloids								
8.	Retronecine	Alkaloids	Both	0.7	153.22	C ₉ H ₁₅ NO	Hepatotoxic (Toxic), Antimicrobial	Kaur K et al., 2022
Sugars								
9.	Ononitol	Sugar Alcohol	Hydrophilic	-1.1	194.18	C ₇ H ₁₄ O ₆	Antidiabetic, Antioxidant	Kaur K et al., 2022; Oberg A I et al., 2011; Saeed M et al., 2024
10.	Glucose	Sugar	Highly Hydrophilic	-3.2	180.16	C ₆ H ₁₂ O ₆	Energy Source	Oberg A I et al., 2011; Assimopoulou A N et al., 2004
11.	Sucrose	Disaccharides	Highly Hydrophilic	-3.7	342.30	C ₁₂ H ₂₂ O ₁₁	Energy Source	Assimopoulou A N et al., 2004
Flavonoids								
12.	Quercetin-3-rutinoside (rutin)	Flavanol	Hydrophilic	-1.9	609.14	C ₂₇ H ₃₀ O ₁₆	Anti-inflammatory, Antioxidant, Cardioprotective	Kaur K et al., 2022; Cheng Y W et al., 2008
13.	Luteolin-7-glucopyranoside	Flavone	Hydrophilic	—	447.08	C ₂₁ H ₂₀ O ₁₁	Antioxidant, Neuroprotective, Antiviral	Zannou O et al., 2020; Alshamar H A et al., 2023
14.	Quercetin-3-glucopyranoside	Flavanol	Hydrophilic	1.0	463.08	C ₂₁ H ₂₀ O ₁₂	Anti-inflammatory, Antioxidant	Yadav S et al., 2022
15.	Quercetin-3-galactopyranoside	Flavanol	Hydrophilic	0.4	463.08	C ₂₁ H ₂₀ O ₁₂	Antioxidant, Cardioprotective	Assimopoulou A N et al., 2004

16.	Kaempferol-3-rutinoside	Flavanol	Hydrophilic	0.07	593.14	C ₂₇ H ₃₀ O ₁₅	Antioxidant, Anticancer	Kaur K et al., 2022; Nikolova M et al., 2022
17.	Kaempferol-3-glucopyranoside (astragalin)	Flavanol	Hydrophilic	2.4	447.08	C ₂₁ H ₂₀ O ₁₁	Anti-inflammatory, Antioxidant	Yadav S et al., 2022; Nakanishi K et al., 2024
18.	Flavones	Flavonoids	Lipophilic	2.6	224.26	C ₁₅ H ₁₀ O ₂	Antioxidant, Anti-inflammatory, Neuroprotective	Kaur K et al., 2022;
19.	Luteolin-7-rutinoside	Flavone	Hydrophilic	-1.6	593.14	C ₁₅ H ₁₀ O ₆	Antioxidant, Anti-inflammatory	Yadav S et al., 2022; Assimopoulou A N et al., 2004
20.	5,7,4'-trihydroxy-3,6,8-trimethoxyflavone (sarothrin)	Flavone	Lipophilic	–	359.07	C ₁₈ H ₁₆ O ₈	Antioxidant, Anticancer	Kaur K et al., 2022
21.	Kaempferol-3,4'-dimethylether (ermanin)	Flavanol	Lipophilic	–	313.05	C ₁₇ H ₁₄ O ₆	Antioxidant, Anticancer	Nikolova M et al., 2022
22.	Kaempferol-3-methylether (isokaempferide)	Flavanol	Lipophilic	3.1	299.05	C ₁₆ H ₁₂ O ₆	Antioxidant, Anti-inflammatory	Assimopoulou A N et al., 2004, Nikolova M et al., 2022
23.	6-methoxykaempferol-3,7-dimethylether (penduletin)	Flavanol	Lipophilic	–	343.08	C ₁₈ H ₁₆ O ₇	Antioxidant, Anti-inflammatory	Kaur K et al., 2022; Cheng Y W et al., 2008
24.	Quercetin	Flavonoids	Moderately Lipophilic	1.8	302.24	C ₁₅ H ₁₀ O ₇	Antioxidant, Antiviral, Anti-inflammatory, Cardioprotective	Kaur K et al., 2022; Cheng Y W et al., 2008; Nikolova M et al., 2022
Phenolic Compounds								
25.	Rabdosiin	Phenolic Compound	Hydrophilic	–	717.14	C ₃₆ H ₃₀ O ₁₆	Antioxidant, Antiviral	Assimopoulou A N et al., 2004; Cheng Y W et al., 2008
26.	Caffeic acid	Phenolic Compounds	Moderately lipophilic	1.4	180.16	C ₉ H ₈ O ₄	Antioxidant, Antimicrobial, Anti-inflammatory	Kaur K et al., 2022
Phenolic Acids								
27.	Rosmarinic acid	Phenolic Acids	Lipophilic	2.4	360.32	C ₁₈ H ₁₆ O ₈	Neuroprotective, Antiviral, Antioxidant, Anti-inflammatory	Nikolova M et al., 2022; Gonelimali F Det al., 2018
28.	Lithospermic acid	Phenolic Acid	Hydrophilic	3.5-3.75	537.10	C ₂₇ H ₂₂ O ₁₂	Antioxidant, Hepatoprotective, Antidiabetic	Saeed M et al., 2024; Nakanishi K et al., 2024
29.	Salvianolic acid C	Phenolic Acid	Hydrophilic	3.9	491.09	C ₂₆ H ₂₀ O ₁₀	Antioxidant, Cardioprotective, Anti-inflammatory	Yadav S et al., 2022;

Structure-Activity relationship of Alkannin and Shikonin

(Figure 4) represents the structural insights of alkannin and shikonins come under naphthazarin moiety reveals that by assisting hydrogen atom donation on the side chain –OH group at C-1 exhibit positive effect to the radical scavenging properties of alkannin and shikonins. (Ordoudi, S.A et al., 2011; Yadav S et al., 2022; Assimopoulou, A.N et al., 2008). (Figure 5 and Figure 6 represents the SAR of Shikonins and Alkannin). The chiral carbons of alkannin and shikonins make them potent phytochemicals with a wide spectrum of biological and pharmacological activities viz. antimicrobial, wound healing, anti-inflammatory, antioxidant and anticancer activity. Further enantiomeric forms of shikonin and alkannin influence their biological activities and potency. (Papageorgiou, V.P et al., 1999; Assimopoulou, A.N et al., 2004; Assimopoulou, A.N et al., 2005). The presence of free hydroxy group at C-1 has positive influenced on biological activities of alkannin and shikonins. On esterification of –OH group at C-1, pharmacological activity of acetylshikonin, deoxyshikonin, alkannin and shikonins was observed. Further activity of these esterified compounds depends upon to a great extent on acyl group size and molecule stereochemistry. (Wang, F et al., 2019; Haruka Oshikiri et al., 2024; Arampatzis, A.S et al., 2022). Structurally activities relationship studies reveal that presence of oxygen is essential for exhibiting biological activities of alkannin and shikonins. Moreover, deoxyshikonin is comparatively less potent than alkannin and shikonin due to absence of hydroxyl group at C-1' in the side chain in deoxyshikonin. (Kontogiannopoulos, K.N et al., 2014).

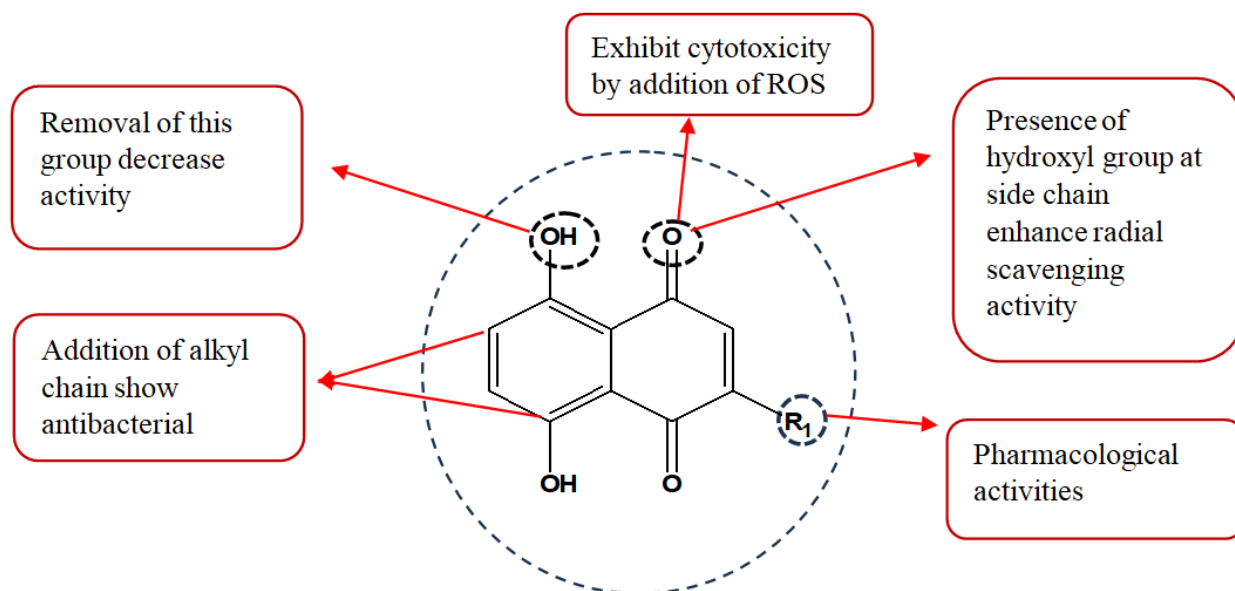


Figure 4: Structure–activity relationship (SAR) analysis of naphthazarin derivatives demonstrating the influence of functional groups on antioxidant, anti-inflammatory, and neuroprotective activities.

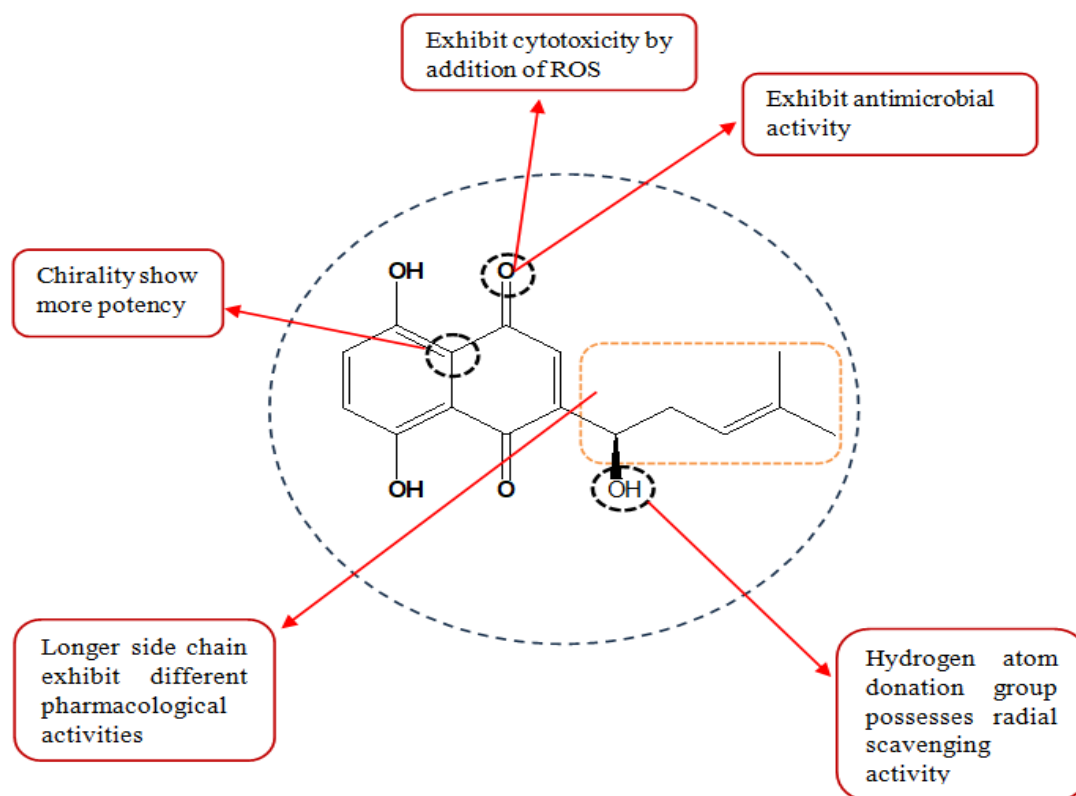


Figure 5: Correlation between structural modifications of alkannin/shikonin analogues and their biological activities based on structure–activity relationship studies.

3. Collection of *In Vitro*/*In Vivo* Study on species of *Alkanna* Genus on Various Activity

The Greek physician Hippocrates stated that the root of *A. tinctoria* may be used to treat a variety of skin conditions, and the botanist Theophrastus noted that it might be used as a dye and medication. (Khan U A et al., 2015). Additionally, Dioscorides, a Greek physician and pharmacologist, recommended *A. tinctoria* for the treatment of a few dermatological conditions. Because of its astringent qualities and status as a natural source of red pigments, *A. tinctoria* roots have been utilized for centuries in cosmetics, including hair colouring and anti-aging plants. (Stella A et al., 2011). *A. tinctoria* root bark has been shown in recent research to have chemo preventive, antiproliferative, free radical scavenging, burn wound healing, antibacterial, antipyretic, and sedative properties. The root bark and dried Roots of *A. tinctoria* is employed as a natural colorant in the food and nutraceutical supplement sectors as well as in cosmetic formulations like red hair dye. (Assimopoulou AN et al., 2009; Papageorgiou VP et al., 1999).

3.1 Anti-Inflammatory Activity

In vitro investigation of anti-inflammatory activity of alkannin derivatives extracted from the *Alkanna tinctoria* roots with egg albumin denaturation and HRBC membrane stabilization method.

The experimental studies demonstrate about the antioxidant action through the modulation of inflammatory pathways, which are key contributors to oxidative stress. Both pro-inflammatory signalling pathways *i.e.* nuclear factor-kappa B (NF- κ B) and mitogen-activated protein kinases (MAPKs) were suppressed by shikonins and its derivatives isolated from *Alkanna tinctoria*. (Kheiri A et al., 2017). By downregulating NF- κ B, shikonins suppresses the expression of pro-inflammatory cytokines (such as TNF- α , IL-1 β , and IL-6), which lowers ROS generation at its source. (Fu D et al., 2016).

Furthermore, the methanolic root extract showed a concentration-dependent inhibition of protein denaturation with an IC_{50} value of 3.15 $\mu\text{g/ml}$ (similar to the standard drug indomethacin) and also exhibited significant membrane stabilization with an IC_{50} value of 3.30 $\mu\text{g/ml}$. (Kumar N et al.,)

3.2 Antioxidant Activity

Clinical and pre-clinical studies have been conducted to evaluate the antioxidant effect of alkannin and shikonins isolated from dried roots of *Alkanna tinctoria*, indicating their preventive role in neurodegenerative disease, cognitive function and vascular dementia by regulating various pathway that enhance the flow of blood increases within the arteries of affected organ. (Singleton V L et al., 1999; Tufa T et al., 2019).

The extract demonstrated strong DPPH Radical Scavenging Activity with an IC_{50} value of 2.73 $\mu\text{g/ml}$, indicating its potential as an antioxidant. Comparatively, butanol fractions of methanolic extract obtained from different species of *Alkanna* dried roots shown highest antioxidant activity with EC_{50} value of 0.17-0.20 mg/mL and free radical scavenging activity. (Salimikia I et al., 2015). Methanolic root extract exhibited significant scavenging effect against ABTS radicals with an IC_{50} value of 2.74 $\mu\text{g/ml}$, in comparison with standard ascorbic acid. (Kumar N et al., and Khan R A et al., 2022).

Multiple mechanism that increases the flow of blood by reducing the oxidative stress through Nrf 2 /ARE pathway and DPPH pathway were proposed. Further studies highlighting the improvement in the natural defences by human cell against oxidative stress with increase in the activity of endogenous antioxidant enzymes. The extracts obtained with sodium acetate: lactic acid and sodium acetate: formic acid from the roots of *Alkanna tinctoria* reveals total phenolic compounds (170.96-175.97 mg GAE/g), total flavonoid content (3.82-4.78 mg ECE/g) and DPPH radial scavenging (112.31- 130.91 m mol TE/g) exhibit strong antioxidant activity. (Zannou O et al., 2020).

3.3 Neurodegenerative Activity

Potential cholinesterase inhibitory activity of phytochemicals extracted from the *Alkanna tinctoria* roots in Alzheimer's disease. *In-silico* studies revealed that flavonoid derivatives, i.e. kaempferol-3-rutinoside and quercetin-3-glucopyranoside, exhibited considerable inhibitory effects on AChE, with docking scores of -13.233 and -12.566 kcal/mol respectively. Furthermore, docking scores of -11.827 and -11.529 kcal/mol on BuChE was observed with both the flavonoid derivatives. (Yousefi R et al., 2009).

3.4 Anti-Diabetic Activity

The alpha-amylase inhibitory activity was used to evaluate antidiabetic potential of *Alkanna tinctoria* roots methanol extract. The plant extract showed significant inhibition of alpha-amylase, suggesting its potential use in managing diabetes by reducing the breakdown of starches into sugars. The concentration of plant samples prepared from stock solution of plant extract are 25,50,75, and 100 $\mu\text{g/ml}$ and study reveal that 100 $\mu\text{g/ml}$ dose show highest inhibition rate. (Alireza Yazdinezhad et al., 2013).

3.5 Anti-Parasitic Activity

In-vitro anti-parasitic activity of *Alkanna tinctoria* roots and stems extract was evaluated against *Leishmania major* and can be applied as new treatment for cutaneous leishmaniasis. The results reveal that *Alkanna tinctoria* plant extract inhibit the growth of *Promastigote* parasites at concentration of 200 $\mu\text{g/ml}$ (IC_{50} value) and may exert cytotoxic effect

with macrophages viability of 90% (20 µg/ml), 86% (40 µg/ml), 82% (100 µg/ml) and 80% (200 µg/ml) after 60 h of adding extracts. (MERCİMEK TAKCI H A et al., 2019).

3.6 Anti-Microbial Activity

By integrating into microbial cell walls, shikonins and its related compounds alter the composition and functionality of the lipid bilayer of microbial cellular outer membrane. Anti-multi drug resistant bacterial activity of aqueous and ethanol extracts of *Alkanna tinctoria* leaves evaluated by well diffusion method concludes that the MIC and MBC of aqueous extract was 12.5 mg/ml and >100 mg/ml and of ethanol extract was 25 mg/ml and >100 mg/ml against *Staphylococcus aureus* show bactericidal effect. The ethanol extract of *Alkanna tinctoria* roots also exhibits antimicrobial activity with higher inhibitory effect against gram positive bacteria like *Listeria monocytogenes* and *Staphylococcus aureus* with 0.049–0.098 mg/mL as MIC and 0.098–0.195 mg/mL as MBC. (Gholami M et al., 2023; Emad W et al., 2024).

Naphthoquinones isolated from acetonitrile extract of *Alkanna tinctoria* roots, categorized as DNA-topoisomerases inhibitors possess antimicrobial activity against 2 clinical microorganisms i.e. *Proteus bacilli* (14 mm) and *Staphylococcus haemolyticus* (10mm). (Khan R A et al., 2022).

3.7 Anti- bacterial Activity:

In another studies, electrospun nanofibers (NFs) based on polyvinyl pyrrolidone (PVP) were co-loaded with both Dragon's Blood-*Alkanna tinctoria*, showed effective growth inhibition for both *P. aeruginosa* and *S. aureus* in contrast to control NFs. The extract of Dragon's Blood, *Alkanna tinctoria* and Dragon's Blood-*Alkanna tinctoria* showed significant antibacterial activity at the concentrations of 0.5 and 1.0% w/v against *P. aeruginosa* and *S. aureus*. Furthermore, the investigated NFs showed antibacterial activity against *P. aeruginosa* and *S. aureus* at the weight of 0.5% w/v from the NFs. NFs demonstrated potent antibacterial activity, with higher inhibition zones compared to individual extracts. The minimum inhibitory concentration (MIC) values were significantly lower for the combined DB-AT NFs, indicating enhanced antibacterial effectiveness. (Yang X et al., 2021).

3.8 Wound Healing Activity

The *in vivo* study designed to evaluate wound healing abilities using ethanolic extract of *Alkanna tinctoria* roots and Dragons Blood. Both AT and DB are coloaded with electrospun nanofibers made of polyvinyl pyrrolidone which aims to enhance wound healing property. The extract loading capacity of AT and DB into nanofibers was (51.3 and 53.2 µg/mg) and in coloaded nanofibers was (49.6 and 57.2 µg/mg). After 14 days of clinical investigation, the results explained that DB NFs, (93.15 ± 4.6%), AT NFs (92.51 ± 7.0%) and DB-AT NFs (97.20 ± 3.6%) exhibited high healing rates in contrast to healing on 7th day by DB NFs, (37.23 ± 6.7%), AT NFs (52.07 ± 3.5%) and DB-AT NFs (61.14 ± 2.3%) for respectively. (Alireza Yazdinezhad et al., 2013).

3.9 Skin Burn Activity

In an experimental study, the burn wounds healing effect was evaluate on human skin with formulation containing beeswax, olive oil and *Alkanna tinctoria*. In sample size of 64 patients, prepared dressing material was applied on 31 experimental groups and wounds healing effect was compared with 33 clinic's routine dressing control groups. The mean epithelization initiation time found to be 3.00±0.85 days in experimental group and 6.90±1.77days in clinic control group. (Naz S et al., 2006).

Ointment containing chloroform extract of *Alkanna orientalis* roots was applied topically on group of 60 patients in a clinical trial (pilot-scale studies) for evaluation of wound healing effect. Comparison was made with conventional treatment group daily for 4 weeks and the outcomes concludes that the recovery percentage was 26.7% for control and 60% for treatment group indicating promising wound healing treatment for skin burn with ointment containing chloroform extract of *Alkanna orientalis* roots. (Gümüş K, and Özlü Z K., 2017).

3.10 Anti- Aging Activity

Alkanna tinctoria roots extract possess protection effect against UV radiations on the human skin, thereby exhibiting skin rejuvenating properties such as anti-aging, melanin reduction and sun protection effects in erythema. The o/w emulsion extract of *Alkanna tinctoria* containing 48.04 ± 0.0033 mg GAE/g of phenolics and 8.64 ± 0.0046 mg QE/g of flavonoids along with carbopol-940 as gelling agent, was evaluated in the form of emulgel for its antiaging effect. After 12 weeks isolation period, it was applied for evaluation of human facial parameters such as skin pigmentation, sebum, redness and skin elasticity. The studies further reveal about considerably effectiveness of *Alkanna tinctoria* roots extract loaded emulgel in reducing sebum, small pores, and big pores ($p \leq 0.05$) while maximizing skin hydration and suppleness in compared with control emulgel. (Ganie S A et al., 2014).

3.11 Anti- Ulcer Activity

Alkannin based formulations containing extract of *Alkanna tinctoria* possess anti-ulcer activity. Research investigation concludes about the synergistic role of beta-acetoxyisovaleryl alkannin (AAN-II) and TGF- β exhibiting better effect on ulcer healing during pressure -induced venous ulcers in a rabbit model through activation of TGF- β /Smad 3 signalling pathway. AAN-II promotes ulcer healing activity by suppressing the development of inflammation and promoting fibroblast proliferation and secretion of proangiogenic factors. (Lazarzarah E et al., 2023).

3.12 Wound Healing in Skin Graft Activity

Randomised placebo-controlled clinical trial was conducted to investigate the wound healing property of *Alkanna tinctoria* (L.) Tausch (Boraginaceae) at donor site after removal of the skin graft. Topically *A. tinctoria* extract ointment (20%) was applied on 30 patients in each category randomly along with standard dressing base ointment daily in group of 60 patients. The percentage of patients in the *A. tinctoria* group exhibited better (96.66%) wound healing property at 2nd and 4th weeks in comparison to patients getting conventional treatment (50%) using the Bates-Jenson assessment tool. This clinical investigation reveals that *Alkanna tinctoria* accelerates healing property after skin graft harvesting. (Salimikia I et al., 2015).

3.13 Hypolipidemic Activity

Hypolipidemic action with aqueous extract of *Alkanna strigosa* aerial parts was seen in alloxan-induced diabetic rats. After 30 days of treatment, it was concluded that there was significant reduction in serum cholesterol, triglycerides, and LDL cholesterol and accompanied by an increase in HDL cholesterol in normal rats. (Ullah Wazir N et al., 2023).

3.14 Anti-Cancer Activity

Cytotoxicity studies on HuGu (normal fibroblast) and (AGS) human gastric cancer cell line by sulphorhodamine B assay and an MTT assay reveals that methanol and ethyl acetate extracts of *Alkanna tinctoria* exhibited the most significant dose dependent effects on cell line cultures with IC₅₀ values of 0.8 mg/mL for AGS cells and 3.2 mg/mL for HuGu cells.

The expression of HER2 was significantly downregulated ($p = 0.0383$) in AGS cells, while HSP90 α was upregulated ($p = 0.0011$). Apoptosis was confirmed using flow cytometry, showing 23.3% apoptotic and 13.3% necrotic cells at 0.8 mg/mL concentration. (AlMotawa R Y et al., 2024; Mita G et al., 1994).

In another cytotoxic experimental study, *Alkanna orientalis*, it was revealed that IC₅₀ values of bioactives isolated from flowers for A549 (184.18 μ M) and HepG-2 (33.57 μ M) was high in comparison to IC₅₀ values of leaves for A549 (75.14 μ M) and HepG-2 (25.35 μ M) in MTT assay. (Kheiri A et al., 2017).

3.15 Pharmaceutical additive

Formulation studies of foods and beverages reveals that red colour pigment containing alkannin and its derivatives obtained from *Alkanna tinctoria* root bark extract act as natural colouring agent. Root cultures (RC₁) medium produced 289.73 μ g/g fresh weight of pigment production whereas in RC₂ medium, the production was slightly lower 196.17 μ g/g fresh weight. Furthermore, the culture medium produces pigmentation rate of 61.54 μ g/g fresh weight in RC₁ and 30.19 μ g/g fresh weight in RC₂, after 25 days of incubation. (Shahzad Khan M et al., 2023).

4. CONCLUSION

Alkanna tinctoria, commonly known as Ratanjot, has demonstrated significant pharmacological potential due to its rich composition of bioactive compounds, particularly alkannin and shikonins. These compounds exhibit strong antioxidative, anti-inflammatory, and neuroprotective properties, making the plant a promising candidate for managing neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and vascular dementia. By activating the Nrf2/ARE signalling pathway, *Alkanna tinctoria* enhances the production of key antioxidant enzymes like superoxide dismutase and catalase, effectively neutralizing oxidative stress, a major contributor to neuronal damage. Additionally, its ability to suppress NF- κ B and MAPK pathways reduces inflammatory cytokine levels, further protecting neurons from degeneration.

Beyond its neurological benefits, *Alkanna tinctoria* has been found to possess antibacterial, antifungal, anti-cancer, wound-healing, and anti-ulcer properties. The plant's antimicrobial activity is particularly noteworthy, as it has demonstrated efficacy against multiple bacterial and fungal pathogens. Moreover, its role in enhancing endothelial nitric oxide synthase leads to improved cerebral blood flow, which is essential for vascular dementia prevention.

Despite its promising pharmacological effects, the transition from preclinical studies to clinical applications requires further investigation. Standardization of extraction methods, determination of optimal dosages, and comprehensive safety evaluations are necessary to ensure its efficacy and minimize potential side effects. Sustainable cultivation and extraction methods should also be developed to maintain the availability and quality of this medicinal herb. Further the research on enantiomeric forms of shikonin and alkannin influence their biological activities and potency are ongoing.

In conclusion, *Alkanna tinctoria* presents a compelling case for use in neurodegenerative disease management and beyond. However, rigorous clinical trials are essential to validate its therapeutic potential and establish it as a mainstream natural remedy. If successfully translated into clinical practice, it could offer a natural, cost-effective alternative for treating a wide range of chronic diseases, improving healthcare outcomes worldwide.

FUNDING SOURCE

This review did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict or Declaration of Interest Statement

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.

5. REFERENCES

1. Alshamar HA, Dapson RW. Using extract from alkanet (*Alkanna tinctoria*) as a source of both a red lipid stain and a blue counterstain for histology. *Biotech Histochem*, 2023; 98(8): 554-560. doi:10.1080/10520295.2023.2271397.
2. Rana S, Chauhan P. Spices that heal: Review on untapped potential of lesser-known spices as immunity booster during COVID-19 pandemic. *Ann Phytomed*, 2022; 11: 7-11.
3. Arampatzis AS, Tsave O, Kirchweiger B, Zwirchmayr J, Papageorgiou VP, Rollinger JM, Assimopoulou AN. Expanding the biological properties of alkannins and shikonins: Their impact on adipogenesis and life expectancy in nematodes. *Front Pharmacol*, 2022; 13: 909285. doi:10.3389/fphar.2022.909285.
4. Kourounakis AP, Assimopoulou AN, Papageorgiou VP, Gavalas A, Kourounakis PN. Alkannin and shikonin: Effect on free radical processes and on inflammation - a preliminary pharmacochemical investigation. *Arch Pharm (Weinheim)*, 2002; 335(6): 262-266. doi:10.1002/1521-4184(200208)335:6<262:AID-ARDP262>3.0.CO;2-Y.
5. Saraf S, Parihar RS. Sushruta: The first plastic surgeon in 600 B.C. 2006.
6. Khan UA, Rahman H, Qasim M, Hussain A, Azizillah A, Murad W, Khan Z, Anees M, Adnan M. *Alkanna tinctoria* leaves extracts: A prospective remedy against multidrug resistant human pathogenic bacteria. *BMC Complement Altern Med*, 2015; 15: 127. doi:10.1186/s12906-015-0646-z.
7. Dwivedi C, Chandrakar K. Indian herbal medicines used for treatment of dementia: An overview. *Int J Pharm Sci Res*. 2014; 1(9): 553-71. doi:10.13040/IJPSR.0975-8232.
8. Pluhár Z, Bernáth J, Hermándy-Berencz J. Introduction of alkanet (*Alkanna tinctoria* (L.) Tausch), a traditional dye plant into cultivation. *Int J Horti Sci*. 2001. doi:10.31421/IJHS/7/2/264.
9. Gadhave DG, Sugandhi VV, Jha SK, Nangare SN, Gupta G, Singh SK, Dua K, Cho H, Hansbro PM, Paudel KR. Neurodegenerative disorders: Mechanisms of degeneration and therapeutic approaches with their clinical relevance. *Ageing Res Rev*, 2024; 102357. doi:10.1016/j.arr.2024.102357.
10. Lamptey RNL, Chaulagain B, Trivedi R, Gothwal A, Layek B, Singh J. A review of the common neurodegenerative disorders: Current therapeutic approaches and the potential role of nanotherapeutics. *Int J Mol Sci*, 2022; 23(3): 1851. doi:10.3390/ijms23031851.
11. Wilson DM, Cookson MR, Van Den Bosch L, Zetterberg H, Holtzman DM, Dewachter I. Hallmarks of neurodegenerative diseases. *Cell*, 2023; 186(4): 693-714. doi:10.1016/j.cell.2022.12.032.
12. Ganos C, Zengin G, Chinou I, Aligiannis N, Graikou K. Phytochemical profiling and biological assessment of the aerial parts from three Mediterranean *Alkanna* species (*A. orientalis*, *A. tinctoria*, *A. kotschyana*) in the Boraginaceae family. *Plants (Basel)*, 2024; 13(2): 278. doi:10.3390/plants13020278.
13. Shahzad Khan M, Mazher M, Adnan Q, Iqbal Z, Shaukat U, Lashari HM, Quddoos A, Khan M, Sana M, Khurram S, Khurshid U, Akhtar N, Mehmood Khan F, Rehman M. Formulation and characterization of *Alkanna tinctoria*

- loaded emulgel and its in vitro and in vivo evaluation as a cosmeceutical product. J Popul Ther Clin Pharmacol. 2023. doi:10.53555/jptcp.v30i18.3300.
14. Das R, Habiba SU, Dash R, Seo Y, Woo J. Unveiling the potentiality of shikonin derivatives inhibiting SARS-CoV-2 main protease by molecular dynamic simulation studies. Int J Mol Sci., 2023; 24(4): 3100. doi:10.3390/ijms24043100.
 15. Zannou O, Koca I. Optimization and stabilization of the antioxidant properties from alkanet (*Alkanna tinctoria*) with natural deep eutectic solvents. Arab J Chem. 2020. doi:10.1016/j.arabjc.2020.06.002.
 16. Ordoudi SA, Tsermentseli SK, Nenadis N, Assimopoulou AN, Tsimidou MZ, Papageorgiou VP. Structure-radical scavenging activity relationship of alkannin/shikonin derivatives. Food Chem, 2011; 124(1): 171-176. doi:10.1016/j.foodchem.2010.06.004.
 17. Yadav S, Sharma A, Nayik GA, Cooper R, Bhardwaj G, Sohal HS, Mutreja V, Kaur R, Areche FO, AlOudat M, Shaikh AM, Kovács B, Mohamed Ahmed AE. Review of shikonin and derivatives: Isolation, chemistry, biosynthesis, pharmacology and toxicology. Front Pharmacol, 2022; 13: 905755. doi:10.3389/fphar.2022.905755.
 18. Assimopoulou AN, Sturm S, Stuppner H, Papageorgiou VP. Preparative isolation of alkannin/shikonin derivatives by high-speed counter-current chromatography. Planta Med, 2008; 74(9): PC51. doi:10.1055/s-0028-1084569.
 19. Papageorgiou VP, Assimopoulou AN, Couladouros EA, Hepworth D, Nicolaou KC. The chemistry and biology of alkannin, shikonin, and related naphthazarin natural products. Angew Chem Int Ed Engl, 1999; 38(3): 270-301. doi:10.1002/(SICI)1521-3773(19990201)38:3<270::AID-ANIE270>3.0.CO;2-0.
 20. Assimopoulou AN, Papageorgiou VP. Study on the enantiomeric ratio of the pharmaceutical substances alkannin and shikonin. Biomed Chromatogr, 2004; 18(10): 791-799. doi:10.1002/bmc.390.
 21. Assimopoulou AN, Papageorgiou VP. Radical scavenging activity of *Alkanna tinctoria* root extracts and their main constituents, hydroxynaphthoquinones. Phytother Res, 2005; 19(2): 141-147.
 22. Wang F, Yao X, Zhang Y, Tang J. Synthesis, biological function and evaluation of shikonin in cancer therapy. Fitoterapia, 2019; 134: 329-339.
 23. Oshikiri H, Li H, Manabe M, Yamamoto H, Yazaki K, Takanashi K. Comparative analysis of shikonin and alkannin acyltransferases reveals their functional conservation in Boraginaceae. Plant Cell Physiol, 2024; 65(3): 362-371. doi:10.1021/acs.orglett.1c00360.
 24. Kontogiannopoulos KN, Tsermentseli SK, Assimopoulou AN, Papageorgiou VP. Sterically stabilized liposomes as a potent carrier for shikonin. J Liposome Res, 2014; 24(3): 230-240. doi:10.3109/08982104.2014.891233.
 25. Kumari P, Singh V, Kant V, Ahuja M. Current status of 1,4-naphthoquinones and their derivatives for wound healing. Eur J Med Chem Rep, 2024; 100194. doi:10.1016/j.ejmcr.2024.100194.
 26. Kaur K, Sharma R, Singh A, Attri S, Arora S, Kaur S, et al. Pharmacological and analytical aspects of alkannin/shikonin and their derivatives: An update from 2008 to 2022. Chin Med. 2022. doi:10.1016/j.chmed.2022.08.001.
 27. Depeursinge A, Racocanu D, Iavindrasana J, Cohen G, Platon A, Poletti PA, et al. Fusing visual and clinical information for lung tissue classification in HRCT data. Artif Intell Med, 2010; 50(1): 13-21. doi:10.1016/j.artmed.2010.04.006.
 28. Jaradat NA, Zaid AN, Hussen F, Issa L, Altamimi M, Fuqaha B, et al. Phytoconstituents, antioxidant, sun protection and skin anti-wrinkle effects using four solvent fractions of the root bark of the traditional plant *Alkanna tinctoria* (L.). Plants (Basel), 2018. doi:10.3390/plants13020278.

29. Ganos C, Zengin G, Chinou I, Aliannidis N, Graikou K. Phytochemical profiling and biological assessment of the aerial parts from three Mediterranean *Alkanna* species (*A. orientalis*, *A. tinctoria*, *A. kotschyana*) in the Boraginaceae family. *Plants (Basel)*, 2024; 13(2): 278. doi:10.3390/plants13020278.
30. Das A, Biswas S, Satyaprakash K, Bhattacharya D, Nanda PK, Patra G, et al. Ratanjot (*Alkanna tinctoria* L.) root extract, rich in antioxidants, exhibits strong antimicrobial activity against foodborne pathogens and is a potential food preservative. *Foods*, 2024; 13(14): 2254. doi:10.3390/foods13142254.
31. Öberg AI, Yassin K, Csikasz RI, Dehvari N, Shabalina IG, Hutchinson DS, et al. Shikonin increases glucose uptake in skeletal muscle cells and improves plasma glucose levels in diabetic Goto-Kakizaki rats. *PLoS One*, 2011; 6(8): e22510. doi:10.1371/journal.pone.0022510.
32. Saeed M, Shoaib A, Tasleem M, Al-Shammary A, Kausar MA, El Asmar Z, et al. Role of alkannin in the therapeutic targeting of protein-tyrosine phosphatase 1B and aldose reductase in type 2 diabetes: An in silico and in vitro evaluation. *Molecules*, 2024; 26(13): 3996. doi:10.3390/molecules26133996.
33. Assimopoulou AN, Boskou D, Papageorgiou VP. Antioxidant activities of alkannin, shikonin and *Alkanna tinctoria* root extracts in oil substrates. *Food Chem*, 2004; 87(3): 433-438. doi:10.1016/j.foodchem.2003.12.017.
34. Cheng YW, Chang CY, Lin KL, Hu CM, Lin CH, Kang JJ. Shikonin derivatives inhibited LPS-induced NOS in RAW 264.7 cells via downregulation of MAPK/NF- κ B signaling. *J Ethnopharmacol*, 2008; 120(2): 264-271. doi:10.1016/j.jep.2008.09.002.
35. Zannou O, Koca I. Optimization and stabilization of the antioxidant properties from alkanet (*Alkanna tinctoria*) with natural deep eutectic solvents. *Arab J Chem*. 2020. doi:10.1016/j.arabjc.2020.06.002.
36. Alshamar HA, Dapson RW. Using extract from alkanet (*Alkanna tinctoria*) as a source of both a red lipid stain and a blue counterstain for histology. *Biotech Histochem*, 2023; 98(8): 554-560. doi:10.1080/10520295.2023.2271397.
37. Assimopoulou AN, Boskou D, Papageorgiou VP. Antioxidant activities of alkannin, shikonin and *Alkanna tinctoria* root extracts in oil substrates. *Food Chem*, 2004; 87(3): 433-438. doi:10.1016/j.foodchem.2003.12.017.
38. Nikolova M, Aneva I, Zhelev P, Semerdjieva I, Zheljazkov VD, Vladimirov V, et al. Metabolic profiles, genetic diversity, and genome size of Bulgarian population of *Alkanna tinctoria*. *Plants (Basel)*, 2022; 12(1): 111. doi:10.3390/plants12010111.
39. Yadav S, Sharma A, Nayik GA, Cooper R, Bhardwaj G, Sohal HS, et al. Review of shikonin and derivatives: Isolation, chemistry, biosynthesis, pharmacology and toxicology. *Front Pharmacol*, 2022; 13: 905755. doi:10.3389/fphar.2022.905755.
40. Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, et al. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Front Microbiol*, 2018; 9: 1639. doi:10.3389/fmicb.2018.01639.
41. Nakanishi K, Li H, Ichino T, Tatsumi K, Osakabe K, Watanabe B, et al. Peroxisomal 4-coumaroyl-CoA ligases participate in shikonin production in *Lithospermum erythrorhizon*. *Plant Physiol*, 2024. doi:10.1093/plphys/kiad157.
42. Khan UA, Rahman H, Qasim M, Hussain A, Azizullah A, Murad W, et al. *Alkanna tinctoria* leaves extracts: A prospective remedy against multidrug resistant human pathogenic bacteria. *BMC Complement Altern Med*, 2015; 15: 127. doi:10.1186/s12906-015-0646-z.

43. Assimopoulou AN, Sturm S, Stuppner H, Papageorgiou VP. Preparative isolation and purification of alkannin/shikonin derivatives from natural products by high-speed counter-current chromatography. *Biomed Chromatogr*, 2009; 23(2): 182-198. doi:10.1002/bmc.1101.
44. Papageorgiou VP, Assimopoulou AN, Couladouros EA, Hepworth D, Nicolaou KC. The chemistry and biology of alkannin, shikonin, and related naphthazarin natural products. *Angew Chem Int Ed Engl*, 1999; 38(3): 270-301. doi:10.1002/(SICI)1521-3773(19990201)38:3<270::AID-ANIE270>3.0.CO;2-0.
45. Kheiri A, Amini S, Javidan AN, Saghaei MM, Khorasani G. The effects of *Alkanna tinctoria* Tausch on split-thickness skin graft donor site management: A randomized, blinded placebo-controlled trial. *BMC Complement Altern Med*, 2017; 17: 421. doi:10.1186/s12906-017-1741-0.
46. Shang X, Ni Z, Shi G. Shikonin inhibits inflammation and chondrocyte apoptosis by regulation of the PI3K/Akt signaling pathway in a rat model of osteoarthritis. *Exp Ther Med*. 2016. doi:10.3892/etm.2016.3642.
47. Kumar N, Singh A, Sharma DK, Kishore K. In-vitro anti-inflammatory and antioxidant activity of *Onosma hispidum* (Ratanjot) roots. *Asian J Pharm Clin Res*, 2018; 11(4). doi:10.22159/ajpcr.2018.v11i4.23583.
48. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*, 1999; 299: 152-178. doi:10.1016/S0076-6879(99)99017-1.
49. Tufa T, Damianakos H, Zengin G, Graikou K, Chinou I. Antioxidant and enzyme inhibitory activities of disodium rabdosiin isolated from *Alkanna sfikasiana* Tan, Vold and Strid. *S Afr J Bot*, 2019; 120: 174-178. doi:10.1016/j.sajb.2018.04.005.
50. Salimikia I, Yazdinezhad A, Golfakhrabadi F, Esfahani HR. In vitro antioxidant and free radical scavenging activity of four *Alkanna* species growing in Iran. *Anc Sci Life*, 2015; 34(4): 201-206. doi:10.4103/0974-8490.147218.
51. Kumar N, Singh A, Sharma DK, Kishore K. In-vitro anti-inflammatory and antioxidant activity of *Onosma hispidum* (Ratanjot) roots. *Asian J Pharm Clin Res*, 2018; 11(4). doi:10.22159/ajpcr.2018.v11i4.23583.
52. Khan RA, Awan AK, Ahmed M, Alkreathy H, Gul A, Khan AM, et al. In-vitro antioxidant, antidiabetic activities and phytotoxic profile of *Alkanna tinctoria* (Boraginaceae). *Pak J Pharm Sci.*, 2022.
53. Yousefi R, Ghaffarifar F, Asl AD. The effect of *Alkanna tinctoria* and *Peganum harmala* extracts on *Leishmania major* (MRHO/IR/75/ER) in vitro. *Iran J Parasitol*, 2009.
54. Yazdinezhad A, Monsef-Esfahani H, Ghahremani MH. Effect of *Alkanna frigida* extracts on 3T3 fibroblast cell proliferation. *Int J Pharm Biol Sci*, 2013; 3(4): 212-215.
55. Mercimek Takci HA, Turkmen FU, Anlas FC, Ustun Alkan F, Bakirhan P, Demir C, et al. Antimicrobial activity and cytotoxicity of *Alkanna tinctoria* (L.) Tausch root extracts. *KSU J Agric Nat*, 2019. doi:10.31466/kfbd.566276.
56. Gholami M, Elyasigorji Z, Amoli AD, Farzaneh P. Effects of *Alkanna bracteosa* extract on the expression level of HSP90 α and HER2 genes in human gastric cancer cell line. *Physiol Mol Biol Plants*, 2023. doi:10.1007/s13596-022-00657-z.
57. Emad W, Abo Elmaaty S, El-DougDoug NK, Nasr-Eldin M. Anticancer potential of *Alkanna orientalis*: Unveiling a medicinal plant's promise. *Benha J Appl Sci*, 2024. doi:10.21608/bjas.2024.289006.1427.
58. Khan RA, Awan AK, Ahmed M, Alkreathy H, Gul A, Khan AM, et al. In-vitro antioxidant, antidiabetic activities and phytotoxic profile of *Alkanna tinctoria* (Boraginaceae). *Pak J Pharm Sci.* 2022.

59. Yang X, Fan W, Huang R, Liu G. β -acetoxyisovaleryl alkannin (AAN-II) from *Alkanna tinctoria* promotes the healing of pressure-induced venous ulcers in a rabbit model through the activation of TGF- β /Smad3 signaling. *Cell Biosci*, 2021; 11: 127. doi:10.1186/s11658-021-00278-5.
60. Naz S, Ahmad S, Ajaz Rasool S, Asad Sayeed S, Siddiqi R. Antibacterial activity directed isolation of compounds from *Onosma hispidum*. *Microbiol Res*, 2006; 161(1): 43-48. doi:10.1016/j.micres.2005.05.001.
61. Gümüş K, Özlü ZK. The effect of a beeswax, olive oil and *Alkanna tinctoria* (L.) Tausch mixture on burn injuries: An experimental study with a control group. *Complement Ther Med*, 2017; 34: 66-73. doi:10.1016/j.ctim.2017.08.001.
62. Ganie SA, Dar TA, Hamid R, Zargar O, Abeer SU, Masood A, et al. In vitro antioxidant and cytotoxic activities of *Arnebia benthamii* (Wall ex. G. Don): A critically endangered medicinal plant of Kashmir Valley. *Biomed Res Int*, 2014; 2014: 792574. doi:10.1155/2014/792574.
63. Lazarzareh E, Davami B, Valizadeh H, Shahsavarinia K, Nazemiyeh H, Khodaie L, et al. Formulation and clinical evaluation of a topical dosage form of *Alkanna orientalis* root extract for management of pressure lesions: A pilot cross-sectional clinical trial. *Pharm Sci*. 2023. doi:10.34172/PS.2022.41.
64. Salimikia I, Yazdinezhad A, Golfakhrabadi F, Esfahani HR. In vitro antioxidant and free radical scavenging activity of four *Alkanna* species growing in Iran. *Anc Sci Life*, 2015; 34(4): 201-206. doi:10.4103/0974-8490.147218.
65. Ullah Wazir N, Amir Khan I, Javed A, Khan T, Jabbar A. *Onosma hispidum* L. extract reverses hyperlipidemia, hypertension, and associated vascular dysfunction in rats. *Saudi J Biol Sci*, 2023; 30(8): 103712. doi:10.1016/j.sjbs.2023.103712.
66. AlMotawa RY, Alhamid G, Badran MM, Orfali R, Alomrani AH, Tawfik EA, et al. Co-delivery of Dragon's Blood and *Alkanna tinctoria* extracts using electrospun nanofibers: In vitro and in vivo wound healing evaluation in diabetic rat model. *Pharmaceutics*, 2024; 16(6): 704. doi:10.3390/pharmaceutics16060704.
67. Mita G, Gerardi C, Miceli A, Bollini R, De Leo P. Pigment production from in vitro cultures of *Alkanna tinctoria* Tausch. *Plant Cell Tissue Organ Cult*, 1994; 37: 193-196. doi:10.1007/BF00234148.