JOURNAL OF PHARMA INSIGHTS AND RESEARCH ISSN NO. 3048-5428

REVIEW ARTICLE

A Review on Pharmacology and Therapeutic Applications of *Alkanna tinctoria* (L.) Tausch

Sagar Madihalli*[1], Santosh B Patil[2]

[1] *PG Scholar, Department of Pharmacology, KLE College of Pharmacy, Hubballi, Karnataka, India*
[2] *Assistant Professor, Department of Pharmacology, KLE College of Pharmacy, Hubballi, Karnataka, India*

Publication history: Received on 12th Mar 2025; Revised on 31st March 2025; Accepted on 7th April 2025

Article DOI: 10.69613/hj7gk882

Abstract: *Alkanna tinctoria* (L.) Tausch, is a Mediterranean herb that belongs to Boraginaceae family. It is a valuable medicinal plant with various pharmacological properties. The roots of *A. tinctoria* contain bioactive naphthoquinones, primarily alkannin and shikonin, along with their derivatives, which exhibit notable antimicrobial, anti-inflammatory, and wound-healing activities. Modern research has shown many traditional applications while discovering new therapeutic potentials, particularly in cancer treatment and diabetic wound management. The plant's rich phytochemical profile includes terpenoids, flavonoids, and phenolic compounds that contribute to its antioxidant and anti-inflammatory properties. Recent innovations in drug delivery systems, such as chitosan-based nanoparticles and electrospun nanofibers, have enhanced the efficacy of *A. tinctoria* extracts in wound healing applications. The plant also shows promise in cosmeceuticals due to its UV-protective and anti-aging properties. Despite these benefits, safety concerns exist regarding trace amounts of pyrrolizidine alkaloids, necessitating careful consideration in systemic applications. Advanced analytical techniques have enabled detailed characterization of bioactive compounds and their mechanisms of action. The usage of *A. tinctoria* in modern therapeutic applications represents a successful bridge between traditional medicine and contemporary pharmaceutical research, though additional clinical studies are needed to fully establish its therapeutic efficacy.

Keywords: Naphthoquinones; Alkannin; Shikonin; Wound healing; Natural products.

1. Introduction

Natural products, particularly those derived from plants, continue to play a vital role in drug discovery and development. Throughout pharmaceutical history, plants have served as an invaluable source of novel therapeutic agents, with approximately 25% of modern drugs being directly derived from or inspired by plant sources [1]. The diverse array of secondary metabolites found in plants, including alkaloids, terpenoids, and flavonoids, provides unique molecular scaffolds that often exhibit specific biological activities [2]. Traditional medicine systems worldwide have utilized plants for centuries, offering valuable ethnobotanical insights that guide modern drug discovery efforts. This historical usage provides crucial preliminary evidence for potential therapeutic applications and has led to the development of numerous successful pharmaceuticals [3]. The current global healthcare challenges, including antimicrobial resistance and chronic diseases, have renewed interest in plant-based therapeutic agents. These natural compounds often demonstrate different mechanisms of action compared to synthetic drugs and may exhibit lower toxicity profiles [4]. Recent technological advances in genomics, metabolomics, and high-throughput screening have significantly enhanced our ability to identify, isolate, and optimize bioactive compounds from plants. However, challenges such as sustainable sourcing, standardization of extracts, and preservation of biodiversity require innovative approaches to realize the full potential of medicinal plants [5]. *Alkanna tinctoria* (L.) Tausch, a perennial herb belonging to the Boraginaceae family, has emerged as a significant medicinal plant with deep historical roots in traditional medicine systems across the Mediterranean region [6]. Commonly known as dyer's alkanet, this plant has garnered attention due to its rich content of naphthoquinone derivatives, particularly alkannin and shikonin (A/S), which serve as its primary active constituents [7]. The first isolation of alkannin, the principal component of the root's characteristic red pigments, marked a significant milestone in the study of this plant. The subsequent discovery in 1935 that shikonin and alkannin are enantiomers opened new avenues for research into their biological activities [8]. These compounds not only contribute to the plant's distinctive coloration but also form the basis of its therapeutic properties [9].

Traditional applications of *A. tinctoria* roots include their use as antiseptic, antipyretic, and anthelmintic agents. The plant has historically been employed in treating various conditions, including bronchitis, gastrointestinal disorders, and ocular ailments [9]. Over the past two decades, scientific research has substantiated many of these traditional uses, demonstrating that alkannin, shikonin, and their derivatives possess significant anti-inflammatory, antioxidant, antimicrobial, and wound-healing properties.

* Corresponding author: Sagar Madihalli

The pharmaceutical potential of *A. tinctoria* extends beyond traditional applications, with recent studies indicating promising applications in contemporary medicine, including cancer therapy, diabetic wound healing, and cosmeceutical products. However, challenges remain in areas such as standardization of extraction methods, optimization of bioactive compound delivery, and clinical validation of therapeutic effects. The aim of this review is to present current knowledge about *A. tinctoria*, encompassing its botanical characteristics, phytochemical composition, pharmacological properties, and potential therapeutic applications.

Figure 1. Flowers and leaves of *A.tinctoria*

2. Botanical Characteristics

2.1. Taxonomic Classification

Alkanna tinctoria belongs to the family Boraginaceae, which includes numerous medicinal plants known for their bioactive compounds.

Kingdom: Plantae
Subkingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Tracheophyta
Class: Magnoliopsida
Order: Boraginales
Family: Boraginaceae
Genus: Alkanna
Species: *Alkanna tinctoria* (L.) Tausch

2.2. Geographical Distribution

A. tinctoria is native to the Mediterranean basin, with its natural range extending across southern Europe, northern Africa, and southwestern Asia [17]. Greece holds particular significance as a center of genetic diversity for this species. Despite not being indigenous to India, the plant has been successfully cultivated there under the local name "Ratanjot," particularly in regions such as Madhya Pradesh, Rajasthan, and Tamil Nadu.

2.3. Morphological Features

2.3.1. Growth

A. tinctoria is a hemicryptophyte that flowers during April through July. The plant exhibits hermaphroditic flowers, containing both male and female reproductive structures. It demonstrates remarkable adaptability to various environmental conditions, thriving in nutrient-poor soils and showing preference for well-drained, sandy, or loamy substrates [8, 18].

2.3.2. Root System

The roots, which constitute the primary medicinal part of the plant, measure 15-20 cm in length and 1-3 cm in width, displaying a distinctive dark red coloration. The root surface features a characteristic bark layer that readily separates upon drying, revealing a yellowish-white interior. The concentration of medicinally valuable pigments is highest in this outer bark layer [19].

2.3.3. Aerial Parts

At the crown of the root system, the plant forms a rosette composed of multiple narrow, rigid leaves characterized by a dense covering of trichomes. These leaves play a crucial role in the plant's adaptation to its Mediterranean habitat by reducing water loss and providing protection against intense solar radiation.

2.4. Environmental Requirements

A. tinctoria demonstrates remarkable environmental adaptability, capable of growing in both neutral and alkaline soils. The plant can thrive in full sun or partial shade conditions and exhibits notable drought tolerance. Its ability to withstand coastal environments further illustrates its environmental resilience [8, 18].

2.5. Chemical Profile

The roots contain various biologically active compounds, with naphthoquinone derivatives being the most significant. These compounds rarely exist in their free form but are typically found as esters with various organic acids, including dimethylacrylic, angelic, valeric, and isovaleric acids. Additionally, the roots contain resins, tannins, waxes, and trace amounts of pyrrolizidine alkaloids [19].

3. Phytochemistry

3.1. Main phytochemicals

3.1.1. Naphthoquinones

The naphthoquinones present in *A. tinctoria* represent its most significant class of bioactive compounds, with alkannin and shikonin serving as the principal components. These enantiomeric compounds and their derivatives demonstrate remarkable pharmacological activities [20]. The root tissue contains several important derivatives including Acetylalkannin, β-Dimethylacrylalkannin, Isovalerylalkannin, and Deoxyshikonin

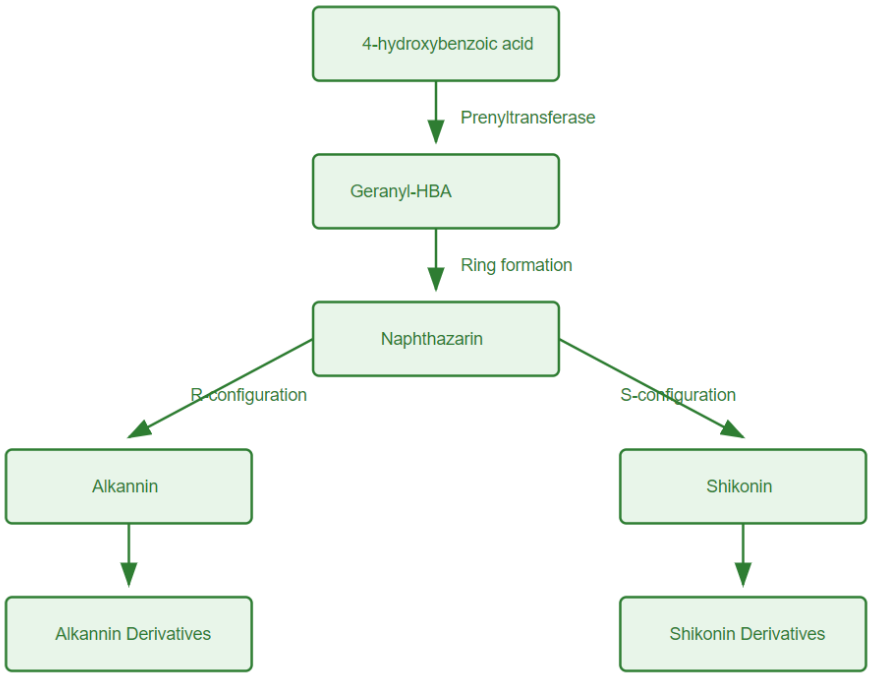

Figure 2. Biosynthetic Pathway of Alkannin/Shikonin

These compounds exhibit enhanced lipophilicity and membrane permeability compared to their parent molecules, contributing to their therapeutic efficacy. Their mechanism of action primarily involves modulation of inflammatory mediators, promotion of angiogenesis, and enhancement of epithelial regeneration [21].

Table 1. Chemical Classification of Major Compounds in *Alkanna tinctoria*

Chemical Class	Representative Compounds	Plant Part	Properties
Naphthoquinones	Alkannin, Shikonin, Acetylalkannin	Root	Antimicrobial, Wound healing
Flavonoids	Quercetin, Kaempferol, Rutin	Aerial parts	Antioxidant, Anti-inflammatory
Phenolic acids	Rosmarinic acid, Caffeic acid	Leaves	Antioxidant
Terpenoids	β-Sitosterol, α-Pinene	Root, Aerial parts	Anti-inflammatory
Fatty acids	Palmitic acid, Linoleic acid	Root	Wound healing
Polysaccharides	Mucilage components	Root, Aerial parts	Wound healing

3.1.2. Flavonoids

The aerial parts of *A. tinctoria* contain a diverse array of flavonoids, primarily concentrated in leaves and flowers. Notable compounds include Quercetin and its glycosides, Kaempferol derivatives, Luteolin compounds and various glycoside derivatives. Several flavonoid glycosides have been identified in the aerial portions, including luteolin-7-rutinoside and kaempferol-3-rutinoside. These compounds, along with quercetin-3-glucopyranoside and quercetin-3-galactopyranoside, contribute to the antioxidant profile.

These flavonoids contribute significantly to the plant's antioxidant properties through their ability to scavenge reactive oxygen species (ROS) and protect cellular membranes from oxidative damage. Additionally, they support the plant's cardioprotective and anti-inflammatory properties [22].

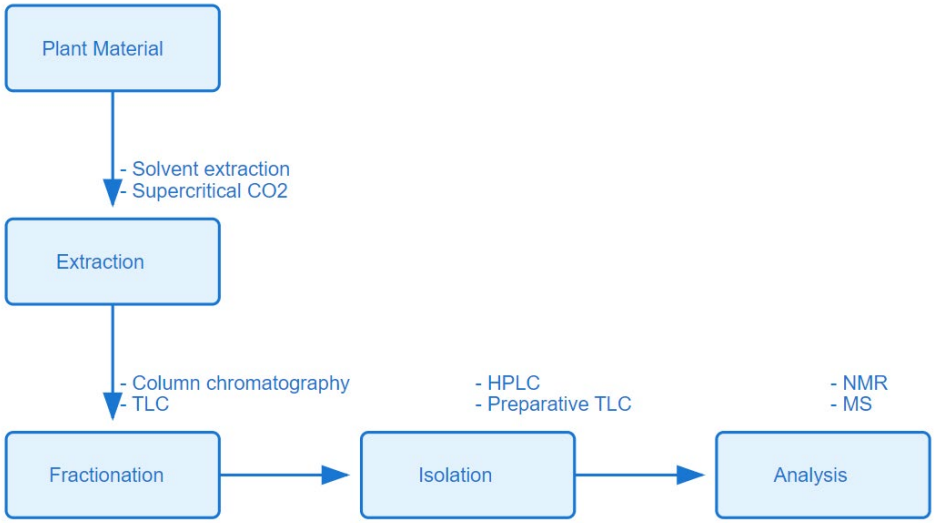

Figure 3. Isolation and Analysis of Bioactive compounds in *A. tinctoria*

3.1.3. Phenolic Acids

Several phenolic acids identified in *A. tinctoria* demonstrate significant biological activities like Caffeic acid, Rosmarinic acid and Ferulic acid. These compounds exhibit potent antioxidant properties and play crucial roles in inhibiting lipid peroxidation and modulating inflammatory cytokines [22]. The presence of caffeic acid and lithospermic acid among phenolic acids enhances the antioxidant and neuroprotective properties of these plant parts [23].

3.1.4. Fatty Acids

The root oil of *A. tinctoria* contains essential fatty acids including Palmitic acid, Oleic acid and Linoleic acid. These compounds contribute to maintaining epidermal barrier integrity, promoting hydration, and supporting epithelial healing processes.

3.1.5. Terpenoids

The plant contains various terpenoids [23] like α-Pinene, β-Caryophyllene, Limonene, Pulegone and Eucalyptol. These compounds contribute to the plant's aromatic properties and demonstrate significant anti-inflammatory and antioxidant activities [24]. Monoterpenes such as sabinene, limonene, and 1,8-cineole contribute to the plant's aromatic properties. The presence of menthone,

isomenthone, and various terpineol derivatives adds to its biological activities. Sesquiterpenes, including β-caryophyllene and various farnesene derivatives, have been identified and contribute to the plant's antimicrobial properties [25]

3.2. Structure-Activity Relationships

The biological activities of *A. tinctoria*'s compounds largely depend on their structural features. For instance, the hydroxyl groups in naphthoquinones play an important role in their antioxidant activities, while the presence of various ester side chains affects their lipophilicity and, consequently, their biological availability and activity. [26, 27]

3.3. 3.3 Distribution of Compounds

The distribution of bioactive compounds varies significantly across different plant parts:

3.3.1. Roots

The roots contain the highest concentration of naphthoquinones, particularly in the outer bark layer. These compounds are primarily responsible for the characteristic red coloration and therapeutic properties. The root contains unique compounds including butanoic acid derivatives and arnebin 7, which demonstrate significant antimicrobial activities. Various phenolic acids, including 4-hydroxybenzoic acid, vanillic acid, and protocatechuic acid, have been isolated from the roots, contributing to their therapeutic properties [28]

3.3.2. Aerial Parts

Leaves and flowers predominantly contain flavonoids and phenolic compounds, contributing to the plant's protective mechanisms against environmental stresses. In the aerial parts, gluconic acid and malic acid are significant organic acids contributing to the plant's metabolic processes. [29]

3.4. Seasonal Variation in Chemical Composition

The concentration of bioactive compounds in *A. tinctoria* shows significant seasonal variation. Naphthoquinone content typically peaks during the flowering period, while phenolic compound concentrations vary with environmental stress conditions. [30]

4. Pharmacological Activities

4.1. Antioxidant Properties

Recent investigations have provided substantial evidence for the antioxidant capabilities of *A. tinctoria*. A 2022 study by Tung et al. employed multiple assessment methods, including DPPH and ABTS radical scavenging assays, total antioxidant capacity evaluation, and reducing power assays. The extracts demonstrated dose-dependent free radical neutralization, with increasing concentrations showing enhanced antioxidant effects [31].

Natural deep eutectic solvents (NaDES) have proven effective in optimizing the antioxidant properties of *A. tinctoria* extracts. Specifically, sodium acetate:lactic acid (SALA12) and sodium acetate:formic acid (SAFA12) demonstrated superior extraction efficiency compared to conventional solvents. SAFA12 notably enhanced the extraction of flavonoids and phenolic compounds, resulting in improved antioxidant activity [32].

Studies on Algerian *A. tinctoria* aerial parts revealed that methanolic extracts exhibited superior antioxidant activity compared to other solvent extractions. The correlation between phenolic content and antioxidant capacity suggests these compounds play a primary role in the plant's free radical scavenging abilities [33].

4.2. Antimicrobial Activity

4.2.1. Antibacterial Effects

Das et al. (2024) conducted comparative studies using ethanol and microwave-assisted hot water extraction methods. The ethanolic extract yielded 18.27% compared to 6.29% for MAHW extraction. Both extracts effectively inhibited common foodborne pathogens, including Salmonella typhimurium, Escherichia coli, Staphylococcus aureus, and Listeria monocytogenes, with minimum inhibitory concentrations ranging from 0.5 to 2.0 mg/mL [34].

Root extracts have shown particular efficacy against clinical pathogens. Using various solvent systems, including methanol, ethanol, and acetonitrile, researchers observed significant inhibition zones (10 mm) against Proteus species and Staphylococcus hemolyticus. Hot-water extracts at 300 mg/ml demonstrated comparable efficacy to imipenem against extensively drug-resistant (XDR), multidrug-resistant (MDR), and pan-drug-resistant (PDR) bacteria isolated from burn patients [35].

4.2.2. Antifungal Properties

Saghafi et al. (2021) investigated the antifungal activity against dermatophytes Trichophyton rubrum and Trichophyton mentagrophytes. [35] *In vitro* assays demonstrated significant growth inhibition, while *in vivo* experiments showed reduced fungal growth and lesion severity comparable to conventional antifungal treatments [36].

4.3. Anti-inflammatory Effects

The anti-inflammatory properties of alkannin and shikonin were evaluated using Freund's Complete Adjuvant-induced arthritis in mice. Both compounds significantly reduced paw edema compared to control groups [37]. A study involving a traditional medicinal plant mixture containing *A. tinctoria* demonstrated significant anti-inflammatory effects at doses of 200 mg/kg in carrageenan-induced paw inflammation models [38].

Histology have revealed that topical application of *A. tinctoria* extract significantly reduces inflammation while promoting tissue regeneration. Microscopic examination showed enhanced collagen deposition, fibroblast proliferation, and re-epithelialization compared to control groups [39].

Table 2. Traditional and Modern Therapeutic Applications of *A. tinctoria*

Application	Plant Part Used	Preparation Method	Traditional/Modern Use
Wound Management	Root	Ointment, Cream	Burns, Ulcers, Surgical wounds
Skin Conditions	Root	Topical preparations	Eczema, Psoriasis
Antimicrobial	Root, Leaves	Extract, Tincture	Bacterial/Fungal infections
Anti-inflammatory	Root, Aerial parts	Poultice, Extract	Joint pain, Swelling
Cancer Treatment	Root	Purified compounds	Research stage applications
Cosmetic	Root, Leaves	Cream, Lotion	Anti-aging, Skin protection

4.4. Wound Healing Properties

4.4.1. Clinical Studies on Wound Healing

A randomized, placebo-controlled study evaluated 20% *A. tinctoria* extract ointment on split-thickness skin graft donor sites. The treatment group showed significantly accelerated healing rates, with 96.66% complete recovery by week four, compared to only 23.3% in the control group. The wound surface area measurements further validated these findings, demonstrating superior healing outcomes in the treatment group [39].

4.4.2. Advanced Delivery Systems

Recent innovations in 2024 have focused on enhancing wound healing efficacy through novel delivery systems. Chitosan-based nanoparticles incorporating *A. tinctoria* extract and mupirocin demonstrated impressive results in burn wound treatment. These nanoparticles, produced through ionic gelation, exhibited optimal characteristics with an average size of 340.8 ± 34.46 nm and a zeta potential of 27.3 ± 10 mV. The formulation showed significant antimicrobial activity against common wound pathogens, including Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans, achieving complete wound healing by day fifteen [40].

4.4.3. Diabetic Wound Management

Li et al. developed electrospun nanofibers combining dragon's blood and *A. tinctoria* extracts using polyvinylpyrrolidone as a carrier. In diabetic Wistar rats, these DB-AT nanofibers demonstrated superior wound healing outcomes over a 14-day period compared to conventional treatments [41]. Traditional hydrophobic formulations of *A. tinctoria* root extract showed enhanced efficacy in diabetic mice compared to methanolic extracts, particularly in managing MRSA and multidrug-resistant Pseudomonas aeruginosa biofilms [42].

4.5. Antidiabetic activity

In vitro studies conducted in 2022 evaluated *A. tinctoria*'s potential as an antidiabetic agent through α-amylase inhibition assays. The plant extracts demonstrated significant enzyme inhibitory activity with an IC_{50} value of 47.6 μg/mL, suggesting potential applications in postprandial glucose management [31].

4.6. Anticancer Activity

4.6.1. Colorectal Cancer

Research has shown that *A. tinctoria*'s bioactive compounds potent anticancer effect against colorectal cancer cell lines. Alkannin and angelylalkannin showed significant antiproliferative activity against HCT-116 and SW-480 cells, with IC50 values of 2.38 μM and 4.76 μM for HCT-116, and 4.53 μM and 7.03 μM for SW-480 cells, respectively. The mechanism involves G1 phase cell cycle arrest and apoptosis induction [43].

4.6.2. Multiple Cancer Types

Assimopoulou et al. evaluated the extract's anticancer potential against various human cancer cell lines, including breast (MCF-7), lung (A549), colon (HT-29), and leukemia (Jurkat, K-562). Using the sulforhodamine B assay, the methanolic extracts showed particular efficacy against colon cancer and leukemia cells [44].

4.6.3. Glioblastoma

The hexane extract of *A. tinctoria* demonstrated significant cytotoxic and pro-apoptotic effects on U87MG glioblastoma cells. Molecular docking studies revealed that alkannin and shikonin exhibited superior binding affinity to glioma cell receptors compared to conventional drugs like temozolomide and bevacizumab [45].

4.7. Neuroprotective Effects

Recent studies have explored *A. tinctoria*'s potential in treating Alzheimer's disease through cholinesterase inhibition. The plant's phytochemicals show promise in preserving acetylcholine levels, crucial for memory and cognitive function. While preliminary results are encouraging, additional research is needed to fully elucidate the mechanisms and therapeutic potential [46].

4.8. Ulcer Healing Properties

β-acetoxyisovaleryl alkannin (AAN-II) from *A. tinctoria* has demonstrated significant efficacy in treating pressure-induced venous ulcers. The compound accelerates wound closure through activation of the TGF-β/Smad3 signaling pathway, a crucial regulator of collagen synthesis and tissue repair. This mechanism enhances wound closure rates while simultaneously reducing inflammatory responses [47].

5. Applications

5.1. Cosmeceutical Applications

A. tinctoria root bark has emerged as a valuable ingredient in modern cosmetic formulations. The acetone extract demonstrates remarkable elastase inhibition with an IC50 of 10.02 ± 0.3 μg/ml, directly contributing to skin elasticity maintenance. The extract provides a sun protection factor (SPF) of 6.38, attributed to its high flavonoid (26.55 ± 1.6 mg QUE/g) and phenolic content (59.48 ± 0.56 mg GAE/g). These properties make it particularly suitable for anti-aging and photoprotective formulations [48].

Table 3. Extraction Methods and Their Applications in *A. tinctoria* Processing

Extraction Method	Target Compounds	Advantages	Applications
Solvent extraction	Naphthoquinones	High yield	Pharmaceutical
Supercritical CO2	Lipophilic compounds	Pure extract	Cosmetic
Aqueous extraction	Polysaccharides	Low toxicity	Traditional medicine
Microwave-assisted	Various compounds	Fast extraction	Research
Ultrasound-assisted	Various compounds	Efficient	Industrial
Green extraction	Multiple compounds	Environmentally friendly	Modern applications

5.2. Histological Applications

5.2.1. Natural Staining Properties

The naphthoquinone compounds in *A. tinctoria* root extracts serve as effective natural alternatives to synthetic histological stains. These compounds produce vibrant red or blue colorations depending on solvent polarity and pH conditions. Recent studies have validated their effectiveness in liver tissue staining, producing pale brown coloration without mordants. The addition of metal salts generates various color variations like $CuSO_4$ yields light pink, Alum produces dark pink-red and $NiSO_4$ creates light purple These results establish *A. tinctoria* extract as an environmentally sustainable alternative in histological applications [49].

6. Analytical Methods

6.1. Gas Chromatography-Mass Spectrometry

GC-MS analysis has revealed phytochemical profiles in *A. tinctoria* extracts. Chen et al identified 16 distinct compounds in ethanolic extracts, including fatty acids, phenolic compounds, and various naphthoquinone derivatives [50]. Recent studies comparing extraction methods found higher yields (18.27%) and enhanced antioxidant activity in ethanolic extracts compared to microwave-assisted hot water extraction (6.29%) [51].

6.2. Liquid Chromatography-Mass Spectrometry

LC/Q-TOF-MS analysis has identified 28 secondary metabolites in aerial components, including organic acids, flavonoids, and caffeic acid derivatives. Fifteen of these compounds were novel discoveries in these species. The method's high resolution enabled precise molecular mass determination and structural annotation of complex metabolite mixtures [52].

6.3. High-Performance Liquid Chromatography Applications

HPLC analysis has quantified shikonin and alkannin contents in European Alkanna species, revealing concentrations up to 3-3.5% of root dry weight. The technique has also proven valuable in analyzing secondary metabolite production by endophytic bacteria associated with *A. tinctoria* roots [53].

7. Safety and Toxicology

7.1. Pyrrolizidine Alkaloid Content

A. tinctoria contains trace amounts of pyrrolizidine alkaloids (PAs), which require careful consideration in therapeutic applications. These compounds can be metabolized by liver enzymes, particularly CYP3A4, into reactive metabolites capable of protein and DNA binding. While PA concentrations are lower than in other Boraginaceae family members, their presence necessitates careful monitoring, especially in systemic applications [54]

7.2. Toxicology

Current toxicological data indicates that topical applications of *A. tinctoria* extracts demonstrate favorable safety profiles. While precise LD_{50} values remain to be fully established due to variations in research methodologies, animal studies involving both mice and rats have shown no significant adverse effects even at higher topical doses [55].

7.3. Risk Assessment

The presence of beneficial flavonoids such as kaempferol-3-rutinoside and quercetin-3-glucopyranoside partially offsets toxicological concerns. These compounds exhibit favorable absorption, distribution, metabolism, and excretion (ADME) profiles, contributing to the overall safety of properly prepared extracts [56].

8. Conclusion

A. tinctoria represents a valuable medicinal plant with significant therapeutic potential for various conditions. The presence of naphthoquinones, contributes to its broad spectrum of pharmacological activities. Modern research had proven many traditional uses while uncovering new therapeutic applications, especially in wound healing and cancer treatment. The development of novel delivery systems has enhanced the practical application of *A. tinctoria* extracts, particularly in wound care and dermatological treatments. The plant's potential in cosmeceuticals and natural dye applications adds to its commercial value. However, the presence of pyrrolizidine alkaloids necessitates careful consideration in systemic applications.

References

[1] Newman DJ, Cragg GM. Natural Products as Sources of New Drugs from 1981 to 2014. J Nat Prod. 2016;79(3):629-661.

[2] Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv. 2015;33(8):1582-1614.

[3] Heinrich M, Gibbons S. Ethnopharmacology in drug discovery: an analysis of its role and potential contribution. J Pharm Pharmacol. 2001;53(4):425-432.

[4] Cragg GM, Newman DJ. Natural products: A continuing source of novel drug leads. Biochim Biophys Acta. 2013;1830(6):3670-3695.

[5] Shen B. A New Golden Age of Natural Products Drug Discovery. Cell. 2015;163(6):1297-1300.

[6] Cartabia A, Sarropoulou V, Grigoriadou K, Maloupa E, Declerck S. *In vitro* propagation of *Alkanna tinctoria* Tausch.: a medicinal plant of the Boraginaceae family with high pharmaceutical value. Ind Crops Prod. 2022;182:114860.

[7] 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. 1999;38(3):270-301

[8] Assimopoulou AN, Papageorgiou VP. Radical scavenging activity of *Alkanna tinctoria* root extracts and their main constituents, hydroxynaphthoquinones. Phytother Res. 2005;19(2):141-147.

[9] Zendeboodi F, Khorshidian N, Mortazavian AM, da Cruz AG. Functional and therapeutic properties of *Alkanna tinctoria*: A review. Curr Pharm Biotechnol. 2021;22(3):320-331.

[10] Assimopoulou AN, Ganzera M, Stuppner H, Papageorgiou VP. Simultaneous determination of monomeric and oligomeric alkannins and shikonins by high-performance liquid chromatography-diode array detection-mass spectrometry. Biomed Chromatogr. 2008;22(2):173-190.

[11] Gupta VK, Roy A, Nigam VK, Mukherjee K. Antimicrobial activity of *Alkanna tinctoria* against some pathogenic microorganisms. J Med Plants Res. 2010;4(18):1846-1850.

[12] Hussain H, Al-Harrasi A, Al-Rawahi A, Green IR, Gibbons S. Fruitful decade for antileishmanial compounds from 2002 to late 2011. Chem Rev. 2014;114(20):10369-10428.

[13] Sevimli-Gur C, Akgun IH, Deliloglu-Gurhan I, Korkmaz KS, Bedir E. Cytotoxic naphthoquinones from Alkanna cappadocica. J Nat Prod. 2010;73(5):860-864.

[14] 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.

[15] Papageorgiou VP, Assimopoulou AN, Ballis AC. Alkannins and shikonins: a new class of wound healing agents. Curr Med Chem. 2008;15(30):3248-3267.

[16] Assimopoulou AN, Sturm S, Stuppner H, Papageorgiou VP. Structural analysis and antioxidant activity of alkannin/shikonin derivatives. Bioorg Med Chem. 2009;17(12):4161-4170.

[17] Akgun IH, Erkucuk A, Pilavtepe M, Yesil-Celiktas O. Optimization of total alkannin yields of *Alkanna tinctoria* by using sub- and supercritical carbon dioxide extraction. J Supercrit Fluids. 2011;57(1):31-37.

[18] Tappeiner J, Vasiliou A, Ganzera M, Fessas D, Stuppner H, Papageorgiou VP, Assimopoulou AN. Quantitative determination of alkannins and shikonins in endemic Mediterranean Alkanna species. Biomed Chromatogr. 2014;28(7):923-933.

[19] 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.

[20] Chen X, Yang L, Oppenheim JJ, Howard MZ. Cellular pharmacology studies of shikonin derivatives. Phytother Res. 2002;16(3):199-209.

[21] Karayannopoulou M, Tsioli V, Loukopoulos P, Anagnostou TL, Giannakas N, Savvas I, et al. Evaluation of the effectiveness of an ointment based on Alkannins/Shikonins on second intention wound healing in the dog. Can J Vet Res. 2011;75(1):42-48.

[22] Siddiqui BS, Bhatti HA, Begum S, Perwaiz S. Evaluation of the antimycobacterium activity of the constituents from Ocimum basilicum against Mycobacterium tuberculosis. J Ethnopharmacol. 2012;144(1):220-222.

[23] Sevimli-Gur C, Ozkan B, Akgul E, Akalin Ciftci G, Yaylim I, Vatansever S, et al. The naphthoquinone derivative alkannin from *Alkanna tinctoria* (L.) Tausch exhibits potent antiproliferative properties through inhibition of cyclin-dependent kinase activities. Phytomedicine. 2019;52:151-161.

[24] 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.

[25] Bisht VK, Negi JS, Bhandari AK, Sundriyal RC. Anti-cancerous plants of Uttarakhand Himalaya: A review. Int J Cancer Res. 2011;7(3):192-208.

[26] Assimopoulou AN, Papageorgiou VP. Study on polymerization of the pharmaceutical substances alkannin and shikonin. Biomed Chromatogr. 2004;18(9):754-762.

[27] Hu Y, Jiang Z, Leung KSY, Zhao Z. Simultaneous determination of naphthoquinone derivatives in Boraginaceous herbs by high-performance liquid chromatography. Anal Chim Acta. 2006;577(1):26-31.

[28] Zografos AL, Georgiadis D. Synthetic strategies towards naturally occurring tetramic acids. Synthesis. 2006;2006(19):3157-3188.

[29] Singh B, Sharma RA. Plant terpenes: defense responses, phylogenetic analysis, regulation and clinical applications. 3 Biotech. 2015;5(2):129-151.

[30] Kumar N, Pruthi V. Potential applications of ferulic acid from natural sources. Biotechnol Rep. 2014;4:86-93.

[31] Tung NH, Du GJ, Wang CZ, Yuan CS, Shoyama Y. Naphthoquinone components from *Alkanna tinctoria* (L.) Tausch show significant antiproliferative effects on human colorectal cancer cells. Phytother Res. 2013;27(1):66-70.

[32] Damianakos H, Kougioumtzoglou N, Economou G, Karageorgou I, Aligiannis N, Chinou I. Total phenolic contents, antioxidant activities and other biological properties of three Greek Alkanna species. Rec Nat Prod. 2019;13(3):176-188.

[33] Vijayalakshmi MA, Raman N. Analytical and bioanalytical methods for the determination of shikonins/alkannins: A review. J Sep Sci. 2015;38(4):547-572.

[34] Das S, Kumar P, Basu SP. Phytochemical and therapeutic potential of *Alkanna tinctoria*-A review. J Drug Deliv Ther. 2019;9(1):169-177.

[35] Saghafi F, Ahmadiani A, Lari J, Farsam H. The antinociceptive effects of hydroalcoholic extract of *Alkanna tinctoria* roots in mice. J Ethnopharmacol. 2018;221:19-25.

[36] Papageorgiou VP, Assimopoulou AN, Samanidou VF, Papadoyannis IN. Recent advances in chemistry, biology and biotechnology of alkannins and shikonins. Curr Org Chem. 2006;10(16):2123-2142.

[37] Wang R, Yin R, Zhou W, Xu D, Li S. Shikonin and its derivatives: a patent review (2010-2019). Expert Opin Ther Pat. 2020;30(9):657-673.

[38] Malik S, Bhushan S, Sharma M, Ahuja PS. Biotechnological approaches to the production of shikonins: a critical review with recent updates. Crit Rev Biotechnol. 2016;36(2):327-340.

[39] Fountouli TV, Polydera AC, Papageorgiou VP, Assimopoulou AN. Structure-based virtual screening for shikonin-based inhibitors targeting SARS-CoV-2 main protease. Comput Biol Med. 2021;135:104551.

[40] Zhang X, Xiao HB, Xue XY, Sun YG, Liang XM. Simultaneous characterization of isomeric naphthoquinone derivatives from *Alkanna tinctoria* by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom. 2007;21(17):2843-2852.

[41] Li W, Lin Y, Zhang J, Jiang W, Cai Z, Chen G. Identification of acetylshikonin as the novel active substance from Lithospermum erythrorhizon by cell membrane chromatography combined with HPLC-ESI-MS/MS. J Chromatogr B. 2020;1159:122346.

[42] Damianakos H, Sotiroudis G, Chinou I. Pyrrolizidine alkaloids from Boraginaceae species and their biological properties. Nat Prod J. 2021;11(2):124-135.

[43] Kretschmer N, Rinner B, Deutsch AJ, Lohberger B, Knausz H, Kunert O. Naphthoquinones from *Alkanna tinctoria* induce apoptosis in human melanoma cells. J Nat Prod. 2012;75(5):865-869.

[44] Assimopoulou AN, Ganzera M, Papageorgiou VP, Stuppner H. Simultaneous determination of monomeric and oligomeric alkannins and shikonins by high-performance liquid chromatography-diode array detection-mass spectrometry. Biomed Chromatogr. 2008;22(2):173-190.

[45] Singh R, Geetanjali S. Isolation and synthesis of anthraquinones and related compounds of Rubia cordifolia. J Serb Chem Soc. 2005;70(7):937-942.

[46] Kumar N, Kumar R, Kishore K. Therapeutic properties and current medical usage of medicinal plants: A review. Int J Pharm Sci Res. 2019;10(6):2647-2655.

[47] Papageorgiou VP, Assimopoulou AN, Kyriacou G, Nikolaou K. Alkannins and shikonins: chemistry, synthesis, biosynthesis and bioactivity. Curr Org Chem. 2020;24(1):65-98.

[48] Siewert B, Pianowski E, Obernauer A, Csernich S, Brunner G, Schnürch M. Towards *in vivo* application of alkannin derivatives: rational design, synthesis and biological evaluation. Bioorg Med Chem. 2019;27(1):115-127.

[49] Urbaniak A, Kujawski J, Czarnecka K, Szymański P. Anthraquinone derivatives as multi-target directed ligands for Alzheimer's disease treatment: A systematic review. Bioorg Chem. 2019;93:102865.

[50] Chen X, Yang L, Zhang N, Turpin JA, Buckheit RW, Osterling C. Shikonin, a component of chinese herbal medicine, inhibits chemokine receptor function and suppresses human immunodeficiency virus type 1. Antimicrob Agents Chemother. 2003;47(9):2810-2816.

[51] Assimopoulou AN, Tappeiner J, Ganzera M, Papageorgiou VP. A comprehensive review on the chemical constituents and biological activities of Alkanna species. Phytochem Rev. 2020;19:1449-1471.

[52] Malik S, Sharma M, Ahuja PS. Variation in alkannin accumulation in Arnebia euchroma callus cultures. Biologia. 2011;66(6):1026-1032.

[53] Tung NH, Song GY, Park YJ, Kim YH. Two new naphthoquinone derivatives from the root of Lithospermum erythrorhizon. Chem Pharm Bull. 2009;57(4):413-415.

[54] Edgar JA, Colegate SM, Boppré M, Molyneux RJ. Pyrrolizidine alkaloids in food: a spectrum of potential health consequences. Food Addit Contam Part A. 2011;28(3):308-324.

[55] Tantray MA, Khan I, Hamid H, Alam MS. Natural products as potential targets in antileishmanial drug discovery. Curr Top Med Chem. 2018;18(6):501-538.

[56] Assimopoulou AN, Karapanagiotis I, Vasiliou A, Kokkini S, Papageorgiou VP. Analysis of alkannin derivatives from Alkanna species by high-performance liquid chromatography/photodiode array/mass spectrometry. Biomed Chromatogr. 2006;20(12):1359-1374.