Exploring pharmaphylogeny from multiple perspectives: A case study on Lithospermeae

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Abstract

Lithospermeae, a tribe under the subfamily Boraginoidae, is a perennial herb containing about 470 species under 26 genera, mainly distributed in temperate and tropical regions. To gain a deeper understanding of the medicinal plants of Lithospermeae and better protect and develop plant medicinal resources, the phytochemistry, pharmacology, and traditional use of Lithospermeae with medicinal value were analyzed. Phylogenetic analysis was carried out based on the internal transcribed spacer (ITS) sequence. Through spatial analysis and the species distribution model, the spatial distribution pattern of Lithospermeae medicinal plants was analyzed. At the same time, the relevant targets and pathways involved in the pharmacological effects of commonly used medicinal plants were predicted using network pharmacology to further explore the genetic origin of Lithospermeae and enrich the pharmaphylogeny of medicinal plants. In this study, the chemical composition, traditional efficacy, modern pharmacological activity, geographical distribution model, molecular phylogeny, and network pharmacology of Lithospermeae were analyzed for the first time. Based on our findings, the pharmaphylogeny of Lithospermeae was preliminarily discussed, providing the scientific basis for basic research regarding Lithospermeae. At the same time, this study explored the relationship between the development of the regional medicinal plant industry and the protection of biodiversity. Furthermore, our findings provide direction and theoretical guidance for the study of the phylogenetic relationships in medicinal plants and the development of Lithospermeae medicinal plant resources.

Introduction

Pharmaphylogeny is a frontier subject that studies the relationship among medicinal plant genetics, chemical composition, and efficacy, which includes both pharmacological activity and traditional efficacy. Pharmaphylogeny as a field is interdisciplinary, and the research objects involve multidisciplinary fields, including plant phylogeny, plant taxonomy, plant chemistry, pharmacology, molecular systematics, and genomics, etc. Plants with similar genetic relationships have been emphasized to contain similar chemical or bioactive components, as well as similar therapeutic effects [1, 2]. In the early stage of the development of pharmaphylogeny, the cladistic systematics method mainly focused on morphological characteristics that relied on the previous work of species identification and character differentiation. However, the error concurred using this approach was large. In recent years, with the development of molecular biology, the relationship between plant morphology and molecular systematics has become the main approach used to study the systematic relationship between plants [3, 4]. Species under the same genus have been observed to have similar morphological characteristics and had no significant differences in molecular sequences, indicating that they are closely related to each other. At the same time, radiation evolution widely exists in plants [5]. The adaptive radiation and isolated differentiation of species under the same genus may originate from climate change, serving as the main driving force for radiation evolution in many plant lineages [6]. As such, climate change has the potential to lead to variations in the same species, including changes in chemical composition and biological activity [7]. Therefore, we introduce geographical distribution as a useful supplement to the pharmaphylogeny theory. Lithospermeae has a wide distribution of species and has a rich biodiversity. Notably, the species distribution data obtained in this study were different from those previously reported. As researchers provide more data in the Global Biodiversity Information Facility (GBIF) database, species distribution results can be enriched and improved. Through this, we hope to explore the association between climate and genetic relationships among species.

Lithospermeae, a tribe of Boraginoideae, includes 26 genera and approximately 470 species. It is typically in colors of corolla yellow, white, or bluish purple. Plants under this tribe have four-lobed ovaries, 4 ovules, two-lobed or unsplit styles, 1-4 column heads, and basally flat pistils. Its nuts are usually 4 in number, small, erect, smooth, tuberous, and marginless. The nut surface is located at the base, close to the top of the cotyledon. *Arnebia* Forssk., *Echium* L., *Lithospermum* L., *Onosma* L., *Alkanna* Tausch., *Stenosolenium* Turcz., and *Lobostemon* Lehm.constitute a closely related group of Lithospermeae with very similar plant morphology (http://www.iplant.cn/). Lithospermeae are widely distributed in temperate and tropical regions around the world [8]. Lithospermeae mainly includes *Lithospermum*, *Arnebia*, *Onosma*, *Echium*, *Stenosolenium* and others [8, 9]. The roots of Lithospermeae medicinal plants were traditionally used for fever palliation, scald burn relief, detoxification, detumescence, pain relief, indigestion relief and so on [10]. Lithospermeae plants contain an abundance of chemical constituents, including naphthoquinone, flavonoids, phenolic acids, and other bioactive substances [11-15]. Modern pharmacological studies have demonstrated that Lithospermeae plants generally have anti-inflammatory, antioxidant, antibacterial, antiviral, and antitumor activities [12, 16-18]. Due to the rich biological activities of Lithospermeae, it has gradually attracted people's attention and become a hot topic of research. However, the relationship among genera in Lithospermeae remains unclear. Moreover, most species have not yet been developed as medicinal plants, and limits the development and utilization of Lithospermeae.

In this paper, the pharmaphylogeny study of Lithospermeae plant groups was performed in combination with molecular phylogeny, geographical distribution, chemical composition, traditional applications, and pharmacological effects. Moreover, we used network pharmacology to predict the potential molecular correlation between the chemical composition of Lithospermeae medicinal plants and its anti-inflammatory, antitumor, and anti-anxiety effects. The study of pharmaphylogeny of Lithospermeae from multiple fields and disciplines provides novel ideas for the development of pharmaphylogeny and scientific evidence for the development of medicinal plants.

Results

Plant systematics of Lithospermeae

Arnebia, *Echium*, *Lithospermum*, *Onosma*, *Alkanna*, *Stenosolenium*, *Lobostemon* and so on , constitute a closely related group of Lithospermeae. Figure 1 illustrates the morphology of some medicinal plants of Lithospermeae.

In the phylogenetic tree, there are mainly four branches, with the first branch including *Onosma* and *Maharanga* A. DC. There are main notable differences between *Maharanga* and *Onosma*. *Maharanga* has a pot-shaped corolla, constricted throat, with its widest dimension in the lower part that expands along the five ribs. Grooves are formed between the ribs, and the anthers are only united at the base. On the other hand, *Onosma* has a simple bell-shaped corolla, with

its widest part in the upper or middle portion. Additionally, the base and the side of its anther are united [19]. Pollen morphology is a conservative characteristic of *Onosma* [19].

The second branch consists of *Echium*, *Lobostemon* and *Echium* begins as a cluster of panicles that gradually unites. It is obliquely funnel-shaped and is pubescent outside. The basal and lower leaves are strip-shaped oblanceolate, the base is gradually narrowed into a handle, and long, rough hairs are present on both sides. The leaves above the lower part of the stem are sessile and lanceolate. It is typically colored corolla purplish-blue. There are two different classifications of *Echium*, viewing it either as an independent tribe of Boraginoideae or an entity under Lithospermeae. Evolution-wise, the pollen of *Echium* is at a higher level than the three-groove pollen of *Onosma*. Based on the types of pollen pores, the authors believe that *Echium* has a classic three-groove pollen, supporting the classification of *Echium* under Lithospermeae [20]. *Lobostemon* is characterized by dry, papillary bifoliate stigma branches and a hairless appearance [4].

The third branch consists of *Alkanna*, *Arnebia*, *Stenosolenium*, *Lithodora* Griseb, *Lithospermum*, *Buglossoides* Moench, and *Glandora* D.C.Thomas, Weigend & Hilger. *Alkanna* is a perennial, dwarf emboli with obtuse to spoon-shaped basal leaves, erect pedicels, a golden corolla, and small nuts. It is milky white to pale yellow in color and three-lenticular, with ellipsoid-rotated pollen, equatorial and polar axis, and a smooth reflex. *Lithodora* has evident style polymorphisms. Pollen grains have two forms, with one above the anther (near female gametes) and the other below the anther (reverse female gametes). *Lithospermum* may be annual or perennial in their life cycle. It has alternate leaves, and flowers may be solitary, bracteate, or in terminal cymes. Its calyx is five-parted nearing the base and its fruits are slightly enlarged. Its corolla may be white, yellow, or violet, and may be actinomorphic, funnelform, or salverform. It has a throat with appendages or hair, 5 stamens, short filaments, and oblong anthers. *Buglossoides* is an annually occurring herb. Its root contains a purple substance, while its leaves are stipeless and oblanceolate to linear. Its corolla may appear discoid and are in colors of white, blue, or light blue. *Buglossoides* is a small genus originally based on *Lithospermum tenuiflorum* L.f. and comprises seven species native to the Old World, mostly concentrated in the Mediterranean region. *Glandora* is a perennial shrub with flowers on top of leafy branches. Flowers are linear and petal-shaped, forming tubes. There are five small stamens in the corolla tube. *Arnebia* is an annual or perennial herb with hard or pubescent hairs. Its leaves have an alternate arrangement while its flowers have both long and short stigmas, with few pedicels. In long stamens, stamens lie central to the corolla tube and are concealed. The styles are long and they have an extended throat. Meanwhile, in short stamens, stamens lie central to the corolla throat and the style is short. Finally, *Stenosolenium* is a perennial herb with slender roots. Leaves are inverted lanceolate or lanceolate. Its corolla may appear purple, cyanish purple, or white. It has a slender tube and a hairy ring base. Figure 2 illustrates the phylogenetic tree of Lithospermeae species.

Geographical distribution

The medicinal plants of Lithospermeae are mainly distributed between 61°14' N and 14°35' N. Except for those in tropical rainforests, cold climates, tropical deserts, other climatic areas have distribution and abundant resources. The spatial distribution pattern illustrates high diversity in the Mediterranean climate regions, mainly including the Mediterranean coast, the Black Sea coast, southern Europe, and the northern African coast. High diversity is also observed in a few areas in western Asia, the western coast of southern Africa, and southwest and southeast coasts of Australia. The climate in these regions is usually hot and dry during the summer, and rainy in the winter. Notably, Lithospermeae species diversity is decreased at low latitudes. *Alkanna* is mainly distributed along the Mediterranean coast and the western coast of North America. *Echium* is mainly distributed along the Mediterranean coast and the western region of North America. *Arnebia* is mainly distributed in Africa and Asia. *Lithospermum* is mainly distributed in Asia. *Onosma* is mainly distributed along the Mediterranean coast and Tibet of China. *Lithodora* is mainly distributed near the Mediterranean Sea. *Lobostemon* is located in the southwest corner of Australia. *Stenosolenium* is distributed in eastern Asia. Figure 3A shows the distribution range of Lithospermeae. And the mainly distribution map of each genus is shown in the attachment (Supplementary Figures S1-8).

From the perspective of suitable ecological zones, *Alkanna* is suitable for growing along the Mediterranean coast, central Asia, and western North America. *Echium* is suitable for growing along the Mediterranean coast, North America, southern South America, southwest Australia, and southeast Australia. *Arnebia* is suitable for growing in northern Africa and central Asia, along the Mediterranean coast. *Lithospermum* is suitable in eastern Asia and the Mediterranean coast, Europe, and North America. *Onosma* is more suitable on the Mediterranean coast. *Lithodora* is suitable for growing along the Mediterranean coast, along the western coast of North America and the southeast corner of Australia. *Lobostemon* is suitable to grow in North America, Europe, and eastern Asia. *Stenosolenium* is suitable for growing in eastern Asia and parts of North America. Figure 3B shows the suitability distribution of Lithospermeae medicinal plants. And the distribution trend of each genus is shown in the attachment.(Supplementary Figures S9-17)

Chemical constituents

Research on the plant components of Lithospermeae mainly focuses on *Arnebia*, *Echium*, *Lithospermum*, and *Onosma*, followed by *Alkanna*, *Stenosolenium*, and *Lobostemon*. And *Lithodora*, *Buglossoides*, and *Maharanga* have been rarely reported. The core components of Lithospermeae are purple naphthoquinones (e.g., shikonins, alkannans), pyrrolizidine alkaloids, and phenolic acids. The distribution of chemical constituents in each genus is summarized in Table 1. (The chemical structures file is attached. Supplementary Table S1)

Traditional application and associated pharmacological activity

Traditionally, Lithospermeae medicinal plants are mainly used for burns, diarrhea, abscess, fever, detoxification, swelling, promoting blood circulation, dispelling wind pain, and contraception. Clinically, they are mainly used for their anti-inflammatory, antibacterial, antitumor, antioxidant, and wound-healing effects. The summary of its traditional application and pharmacological effects in individual genus distribution is shown in Table 2.

Network pharmacology study on representative genera of Lithospermeae

Screening of the active components of Lithospermum, Arnebia, and Echium and subsequent prediction of their targets

Through DL and IG screening conditions, 25 active components of *Lithospermum*, 26 active components of *Arnebia*, and 16 active components of *Echium* were obtained (Table 3). Furthermore, 571 component targets of *Lithospermum*, 549 component targets of *Arnebia*, and 399 component targets of *Echium* were predicted by the Swiss Target Prediction database.

Obtaining disease targets

In OMIM and GeneCards databases, disease targets were searched using the keywords “cancer” , “inflammation” , and “anxiety disorder”. Targets with scores≥ 5 were selected as potential targets. After merging the results of the two databases and deleting duplicates, 2976 cancer targets, 569 inflammatory targets, and 2102 anxiety disorder targets were obtained. Using the Venny 2.1.0 online platform, the intersection of component targets and disease targets was obtained. A total of 247 intersecting targets of *Lithospermum* and cancer, 98 intersecting targets of *Arnebia* and inflammation, and 156 intersecting targets of *Echium* and anxiety disorder were obtained.

KEGG pathway analysis and construction of “active ingredient-target-pathway” network

The Metascape database was used to analyze the KEGG pathway of 247 core targets of the anticancer effects of *Lithospermum*, 98 core targets of the anti-inflammatory effects of *Arnebia*, and 156 core targets of the anti-anxiety effects of *Echium*. The first 20 most significant pathways upon visual analysis are shown in Figures 4-6B. The results showed that the signaling pathways closely related to the anticancer effect of *Lithospermum* mainly included pathways involving microRNAs in cancer and so on. The signaling pathways closely related to the anti-inflammatory effect of Arnebia mainly include the NOD-like receptor and MAPK signaling pathways. The signaling pathways closely related to the anti-anxiety effect of *Echium* mainly include the serotonergic synapse and neuroactive ligand-receptor interaction pathways. The “active ingredient-target-pathway” network was further constructed. The results are shown in Figures 4-6A.

Discussion

Lithospermeae, originating from the Mediterranean and Western Asia, has a long history of medicinal use. Previous studies have found that the four most diverse genera of Lithospermeae changed during Miocene, with *Alkanna* and *Onosma* showing considerable diversity, *Lithospermum* further dispersing into North America, South America, and Africa, and *Echium* dispersing into Macaronesia [21]. Lithospermeae has the largest diversity in semi-arid to arid habitats and has an obvious overall diversity center in western Eurasia and the Mediterranean Basin [8], consistent with our results. Lithospermeae is rich in red shikonin naphthoquinones, and the naphthoquinones in most genera are shikonin or akanin. The main nucleus of shikonin is 5,8-dihydroxy1,4-naphtoquinone and has an isohexenyl side chain, and the difference of its configuration comes from the chiral carbon atom on the side chain [22]. These compounds have good biological activity and are the main substances in the pharmacological effects of Lithospermeae. It has been well-established that the chemical constituents of plants are closely related to their efficacy and pharmacological activity. Different groups of medicinal plants and different parts of the same medicinal plant may have different varying traditional efficacy.

The molecular phylogeny, geographical distribution, chemical composition, traditional application, and pharmacological effects of Lithospermeae were explored with respect to its pharmaphylogeny. According to the genetic theory, Lithospermeae plants are divided into three groups. Group one consists mainly of *Onosma*, followed by *Maharanga*, group two consists of *Lobostemon* and *Echium*, and group three consists of *Alkanna*, *Arnebia*, *Stenosolenium*, *Lithodora*, *Lithospermum*, *Buglossoides*, and *Glandora*.

Group 1: *Onosma* and *Maharanga*. While only a few studies on *Maharanga* , Maharanga has been used in traditional applications forconstipation [10], with antibacterial and antiviral pharmacological activities [23]. *Onosma* was mainly distributed on the Mediterranean coast and Tibet of China. The roots of *Onosma* were often reported to have been used as medicine for clearing heat and cooling blood, detoxification, treatment of rashes, and respiratory diseases [10]. The petals of *Onosma* were used for heart disease and rheumatic diseases. The main chemical constituents of *Onosma* are shown in Table 1, and its unique chemical components make its pharmacological activity remarkable. *Onosma* contains alkannins, shikonins, flavonoids, ferulic acids, and vanillic acids, which have been responsible for anti-inflammatory, wound healing, analgesic, and antibacterial activities(Table1-2). Cypriots commonly used *Onosma fruticosa* Sm. for respiratory diseases [10]. The activity of the crude extract of *Onosma bracteatum* has shown promising results for asthma [24]

Group 2: *Echium* and *Lobostemon*. *Echium* is mainly distributed along the Mediterranean coast and in western North America. *Echium* is often used as whole herbs. It can be seen from Table 2 that in the traditional application, it is used to treat rheumatic pain, abscess, respiratory tract infection, trauma, exterior relief, and diuresis. Particularly, its petals are used for antianxiety, sedation, and colds relief [10]. Table 1 shows that the *Echium* mainly contains shikonins, phenolic acids, and pyrrolizidine alkaloids. Chemical components of *Echium* were notably associated with its traditional applications and pharmacological effects. Shikonins are often used for their anti-inflammatory, antibacterial, antitumor, wound healing effects and so on [25]. Phenolic acids have certain anticancer properties and are known to promote blood circulation [26]. The activity of the crude extract of *Echium amoenum* Fischer. has been found to have anti-anxiety effects [27, 28], which was consistent with its traditional medicinal use. In previous studies, shikonin has been found to have a protective effect on the nervous system [29], but the active ingredients responsible for its anti-anxiety effects are not clear. In this paper, we use network pharmacology to analyze the molecular association between the chemical composition and anti-anxiety effects of *Echium* to explain its overall mechanism of action. In addition to shikonin, results of network pharmacology revealed the presence of caffeic acid, isobutyrylshikonin, and other compounds involved in the serotonergic pathway through targets such as monoamine oxidase B and APP. Compounds such as acetylshikonin and 2-methyl-n-butyrylshikonin regulate neuroactive ligand-receptor interaction pathways through targets such as ADORA1 and ADORA2A, allowing exertion of anti-anxiety effects (Figure 4A-B).

Lobostemon is located in the southwest corner of Australia and mainly used for bacteriosis, ulcers, and wounds in traditional applications [10]. The chemical constituents of *Lobostemon* were mainly phenolic acids, flavonoids, and alkaloids. Clinically, it is mainly used for its anti-HIV and wound healing effects, etc [17, 30]. At present, there are only a limited number of reports on *Lobostemon* and more studies are needed to better explore its pharmaphylogeny.

Group 3: Alkanna, *Arnebia*, *Stenosolenium*, *Lithodora*, *Lithospermum, Buglossoides* and *Glandora*. Group 3 was further divided into three parts. *Alkanna* and *Lithodora* were first discussed, followed by *Lithospermum*, *Buglossoides*, and *Glandora*, and finally, *Arnebia* and *Stenosolenium*.

Part 1: *Alkanna* and *Lithodora. Lithodora* is mainly distributed on the Mediterranean coast and has been traditionally used for blood purification, chilblains, colds, cough, enteritis, fever, high blood pressure, and liver protection [10]. However, there are limited studies on its chemical constituents, with only aliphatic and ester compounds being reported (Table 1).

Alkanna is mainly distributed along the Mediterranean coast and the western coast of North America. In traditional applications, its roots are often used to treat diarrhea, abscess, wounds, burns, and sore throat, etc [10]. It mainly consists of quinone compounds, but phenolic acids, alkaloids, and flavonoids have also been found in *Alkanna*. Pharmacological studies have shown that in addition to analgesic, antibacterial, anticancer, antibacterial, anti-inflammatory, antioxidant, and wound healing effects, it also has benefits against nociceptive pain and cutaneous leishmaniasis (Table 2).

Part 2: *Lithospermum ,Buglossoides* and *Glandora. Lithospermum erythrorhizon* Sieb. et Zucc. is one of the commonly used Chinese herbal medicines [31]. *Lithospermum* diversity notably changed during the Middle Ages, with further dispersals of *Lithospermum* into North America, South America, and Africa were observed [8]. In this study, it was found that *Lithospermum* was geographically limited to Asia. The results are inconsistent with previous reports, which may be due to the small sample size and the need to increase the sample information for further analysis. In addition to the common functions of Lithospermeae of clearing heat and detoxification, traditional applications for coughing, anti-inflammatory, promoting blood circulation, analgesia, and tonification for weakness have also been reported. It has been used as a diuretic and contraceptive in Europe and Indian, and its fruit is also known to assist in digestion [10]. *Lithospermum* mainly contains shikonins, alkannins, and pyrrolizidine alkaloids (Table 1). *Lithospermum* plants have antibacterial, anti-inflammatory, and antithrombotic effects, as well as the ability to palliate skin diseases (Table 2). At the same time, crude extracts of *Lithospermum erythrorhizon* have been reported to assist in weight loss [32]. *Lithospermum ruderale*, mainly native to India, has also exhibited anti-gonadotropic activity [33]. From Figure 1, *Lithospermum canescens* and *Lithospermum ruderale* can be seen to have similar sequences, but *Lithospermum canescens* has not been reported to have a contraceptive effect [10, 33]. This highlights how genetic background is not the only determinant for the pharmacologic effects of medicinal plants. The potential of environmental factors in changing plant constituents merits further study. In previous studies, shikonin and its derivatives were observed to inhibit the growth of tumor cells by activating miR-17-5p/PTEN/Akt, STAT3, PI3K/Akt, ROS, among other pathways [25]. Through network pharmacology, we identified the signaling pathways closely related to the anticancer effect of *Lithospermum*. These pathways had the most enrichment targets, far more than others, and were mainly regulated by anhydroalkannin, arnebifuranone, and other compounds by regulating MMP1, CDK2, EGF2. These findings provide a direction for *Lithospermum* as an antitumor drug (Figure 5A-B).

Buglossoides and *Glandora* are relatively less studied. *Buglossoides* is produced in Asia, Europe, and North Africa. It is traditionally used for weight loss and has the biological activity of antioxidant [10]. Aliphatic and ester compounds have been identified in chemical studies. *Glandora* is native to North Africa and South Europe. It is worth noting that the synonym of *Glandora diffusa* (Lag.) D. C. Thomas. in *Glandora* is *Lithospermum diffusum* Lag. and *Lithodora diffusa* (Lag.) I. M. Johnst. [34, 35]. In traditional applications, *Glandora* is used for its diuretic, depurative, and antihypertensive effects. It contains phenolic acids, flavonoids, and fatty acids, etc, known to have a certain anticancer effect.(Table 1-2)

Part 2: *Arnebia* and *Stenosolenium. Arnebia* is mainly distributed in northern Africa and western Asia. *Arnebia guttata* Bge. and *Arnebia euchroma* (Royle) Johnst. are the source of authentic Chinese medicinal materials of 'Zicao' [31]. In traditional applications, it is often used for heat clearance, detoxification, burns, heart disease, skin diseases, and blood circulation promotion, etc [10]. *Arnebia* contains a large number of quinone compounds, mainly shikonins and alkannins. It is commonly used for its anti-inflammatory, antibacterial, antioxidant, anticancer, and antithrombotic effects (Table1-2). In addition to shikonin, polysaccharides in certain species have also been observed to exert antitumor effects. The crude extract has been found to inhibit the growth of MCF-7 cells and reduce the level of estrogen and progesterone in mice to achieve its anti-reproductive effect [36]. This seems to be consistent with the contraceptive effect of *Lithospermum*. The *Arnebia* species is often used for a variety of inflammatory conditions, such as arthritis, colitis, and dermatitis and so on [25]. Through network pharmacology, *β*-santalol, osthol, arnebifuranone in species of *Arnebia* were identified to regulate the NOD-like receptor signaling pathway through MAPK8, XIAP, MAPK14, and TNF targets. Isovalerylshikonin, isovalerylalkannin, and arnebifuranone et al. regulate the MAPK signaling pathway through EGFR, MAPK8, and AKT1 targets, exerting anti-inflammatory effects. Previous studies on the anti-inflammatory effect of *Arnebia* have identified MAPKs, TNF, and AKT as targets and MAPK as the signaling pathway. In this study, *Arnebia* was also found to regulate NOD-like receptor signaling pathways through XIAP targets. Although requiring further verification, this provides a new idea for harnessing the anti-inflammatory effects of *Arnebia* (Figure 6A-B).

Stenosolenium is mainly distributed in eastern Asia. In traditional applications, the whole herb was used for dispelling wind, removing dampness, and managing joint pain. Meanwhile, the root has the effects of clearing heat, cooling blood, stopping bleeding, and relieving cough [10]. *Stenosolenium* contains quinones, phenolic acids, and pyrrolizidine alkaloids (Table1). Further research is needed to better explore the pharmaphylogeny of *Stenosolenium*.

Alkanna, *Lithospermum*, *Arnebia*, *Echium*, and *Onosma* are the main sources of medicinal plants of Lithospermeae. According to palynology research, *Echium* and *Onosma* have a close genetic relationship. The chemical constituents of *Echium* and *Onosma* have similar traditional efficacy and pharmacological effects, suggesting their close relationship, which is consistent with the conclusion based on the ITS sequence. *Lithospermum* and *Arnebia* also have many similar characteristics as the main sources of medicinal 'Zicao'. For instance, they have similar efficacy in traditional applications (Table 2). In the current study, they were found to have a contraceptive effect. Their chemical constituents are mainly quinones, and their growth areas are similar, mainly in Asia.

During the phylogenetic process, *Lithospermum* and *Arnebia* originate from the same evolutionary branch. Therefore, the consistency of plant efficacy between different groups suggests a subsequent relationship between the two in terms of chemical composition and phylogenetic development. This has important practical value for finding new drug sources by exploring the genetic relationship between plants.

At present, there are only a few studies on *Lobostemon.* However, according to the surface characteristics of the stigma and the sinkage of the stem tip, *Lobostemon* has been reported to be similar to *Echium*[4]. *Lithodora* and *Glandora* are perennial shrubs with flowers on top of leafy branches. The flowers of the two genera are both linear and petal-shaped, forming tubes. They have five small stamens in the corolla tube. *Glandora* is a species that evolved from *Lithodora* [35, 37]. Therefore, it is speculated that *Lithodora* and *Glandora* have a close genetic relationship. *Buglossoides arvense* (L.) I. M. Johnst. originally belonged to *Lithospermum* plant, and the ecological suitability and geographical locations of the two genera were also similar. According to the existing data, it is speculated that *Buglossoides* may have a close relationship with *Lithospermum*.

This paper summarizes the chemical constituents, traditional applications, and pharmacological effects of Lithospermeae medicinal plants. Moreover, this paper reports on the genetic relationship among Lithospermeae through molecular phylogeny, species distribution model, and network pharmacology. At present, studies on Lithospermeae mainly focus on taxonomy and systematic position relationship, and preliminary studies on chemical constituents and pharmacological activities have demonstrated the rich diversity and activity potential of Lithospermeae. Our study explored the intrinsic correlation of the medicinal value of Lithospermeae. Based on previous pharmaphylogeny studies, we included a species distribution model and ecological adaptability evaluation to provide research basics for the future cultivation of Lithospermeae species. At the same time, network pharmacology was added to predict the targets and pathways of related diseases. This study enriches the pharmaphylogeny theory, helps to better understand and excavate the medicinal value of Lithospermeae plants, and provides a reference for further research on the utilization of Lithospermeae plants. Unfortunately, the medicinal value of 470 species of Lithospermeae plants has not attracted much attention and in-depth research for a long time, and only a few species have been highlighted for their chemical and pharmacological activities. Moreover, the internal relationship among Lithospermeae medicinal plants remains unclear. These areas are worthy of further in-depth studies in the future.

Methods

Plant systematics of Lithospermeae

Search for morphological characteristics of plants through http://www.iplant.cn/frps, CNKI, Pubmed and Google scholar. (The data collected from 1975 to 2022.)

Phylogenetic studies mainly reflect the evolutionary relationship between species by constructing phylogenetic tree among species, so as to provide a basis for exploring the evolution of related organisms. Molecular systematics is to use DNA to construct phylogenetic tree. It is generally believed that the posterior probability greater than 90% indicates that branches are significantly supported. The ITS sequences of nucleotide were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/) and analyzed by MEGA11 software. The phylogenetic tree was constructed by Test Neighbor-Joining Tree method. No. of Bootstrap Replications was set to 1000, Substitutions Type was Nucleotide, Model/Method selected Kimura 2-parameter model, Site Coverage Cutoff (%) was 90.

Geographical distribution

The geographical distribution points of Lithospermeae, 8 genera, 30 species, 219890 collected from the GBIF database are saved as. csv format in the order of longitude and latitude. At the same time, 19 environmental factors and 1 altitude factor worldwide were transformed into. asc format in ArcGIS. The distribution points of 219890 Lithospermeae species and 20 environmental variables were imported into MaxEnt software. The MaxEnt model selects Jackknife method to evaluate the contribution of variables, and uses crossvalidate method to run 10 times. The output mode of the model is 'Logisitc' to make the predicted results closer to the probability of Lithospermeae species distribution, and the other parameters are the default parameters. The predicted value of the model is between 0 and 1, and the ASCII format file is output. This file is imported into ArcGIS software, and the file in. tif format is output. The sdmtool tool is used to classify the species distribution based on the default threshold of 0.5, and the potential distribution areas of each species of Lithospermeae in the world are obtained. Finally, the potential distribution grid data of each species are added to obtain the global potential distribution areas of each genus and whole family of Lithospermeae.

Chemical constituents, traditional applications and pharmacological activities

The chemical constituents, traditional applications and pharmacological activities of Lithospermeae was summarized and sorted through CNKI, Pubmed, Google scholar, Wanfangdata and Library of congress. The collected Lithospermeae data were analyzed.(The data collected from 1975 to 2022.)

Network pharmacology

Prediction of potential targets of drug components

We transformed each chemical component identified above into standard SMILES through PubChem, and screened the above chemical components with SwissADME data platform. The screening conditions were drug likeness (DL) (at least three yes in five items) and high gastrointestinal absorption (GI). Subsequently, these SMILES were imported into Swiss Target Prediction to predict targets of compounds. Species were selected as Homo sapiens with probability >0 as the screening condition (Table 3).

Disease targets prediction

We selected the common genera of Lithospermeae and treated the disease. In GeneCards database (http://www.genecards.org/), Online Mendelian Inheritance in Man (OMIM, http://www.omim.org/) databases, disease targets were searched by keywords such as cancer, inflammation and anxiety disorder, and targets with score ≥ 5 were selected as disease targets. At the same time, the intersection targets of *Lithospermum* target and cancer target, *Arnebia* target and inflammation target, *Echium* target and anxiety disorder target were screened by Venny platform.

KEGG pathway analysis

Based on Metascape 3.5 database, GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis were performed on the intersection targets of *Lithospermum* and cancer, *Arnebia* and inflammation, *Echium* and anxiety disorder (Figure 4-6A). The key biological functions and signaling pathways of *Lithospermum* anti-cancer, *Arnebia* anti-inflammatory and *Echium* anti-anxiety were obtained with $P < 0.05$. And the data were visualized using the microbiotic cloud platform. Finally, the “active ingredient – core target – pathway” association network was constructed (Figure 4-6B).

Declarations

Data availability

All data generated or analysed during this study are included in this article and its supplementary information files.

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Author contributions

ML, BG designed the research. YY, XW, BQ, GW, BZ, MZ, YL and SL performed the research and analyzed the data. YY and ML wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript, read, and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

References

1. He, R. P., Jin, Z., Ma, R. Y, Hu, F. D., Dai, J. Y. Network pharmacology unveils spleen-fortifying effect of Codonopsis Radix on different gastric diseases based on theory of "same treatment for different diseases" in traditional Chinese medicine. 2021.
2. Chen, S. B., Peng, Y., Chen, S. L. Sustainable utilization of traditional chinese medicine resources- Pharmaphylogeny. Modemization of Traditional Chinese Medicine and Materia Medica. **7**, 7. (2005)
3. Kikuchi, S., Mimura, M., Naruhashi, N., Setsuko, S., Suzuki, W. Phylogenetic inferences using nuclear ribosomal ITS and chloroplast sequences provide insights into the biogeographic origins, diversification timescales and trait evolution ofRubusin the Japanese Archipelago. Plant Systematics and Evolution **308**(3). https://doi.org/10.1007/s00606-022-01810-6 (2022)
4. Buys, M. H. The stigma and style of Lobostemon (Boraginaceae) and their systematic implications. South African Journal of Botany https://doi.org/10.1016/S0254-6299(15)31090-5 (2001)
5. Kelly, C., Barker, N. P., Villet, M. H., Broadley, D. G. Phylogeny, biogeography and classification of the snake superfamily Elapoidea: a rapid radiation in the late Eocene. Cladistics **25**(1). https://doi.org/10.1111/j.1096-0031.2008.00237.x (2009)
6. Nge, F. J,, Biffin, E., Thiele, K. R., Waycott, M. Reticulate Evolution, Ancient Chloroplast Haplotypes, and Rapid Radiation of the Australian Plant Genus Adenanthos (Proteaceae). Frontiers in Ecology and Evolution **8**. https://doi.org/10.3389/fevo.2020.616741 (2021)
7. Yu, X., Xiao, J., Chen, S., Yu, Y., Liu, R. Metabolite signatures of diverse Camellia sinensis tea populations. Nature Communications **11**(1), 5586. (2020)
8. Chacón, J., Luebert, F., Selvi, F., Cecchi, L., Weigend, M. Phylogeny and historical biogeography of Lithospermeae (Boraginaceae): Disentangling the possible causes of Miocene diversifications. Molecular Phylogenetics and Evolution **141**,106626. https://doi.org/10.1016/j.ympev.2019.106626 (2019)
9. Juliana, C., Federico, L., Maximilian, W. Biogeographic Events Are Not Correlated with Diaspore Dispersal Modes in Boraginaceae. Frontiers in Ecology and Evolution **5**. (2017)
10. Jiang, J. W. World Dictionary of Medicinal Plants. *China Medical Science Press*. (2015)
11. Ahmad, L., He, Y., Semotiuk, A. J., Liu, Q. R., Hao, J. C. Survey of pyrrolizidine alkaloids in the tribe Lithospermeae (Boraginaceae) from Pan-Himalaya and their chemotaxonomic significance. Biochemical Systematics and Ecology **81**,49–57. https://doi.org/10.1016/j.bse.2018.09.002 (2018)
12. Arumugam, R., Sarikurkcu, C., Ozer, M. S. Comparison of methanolic extracts of Doronicum orientale and Echium angustifolium in terms of chemical composition and antioxidant activities. Biocatalysis and Agricultural Biotechnology **33**,101984. https://doi.org/10.1016/j.bcab.2021.101984 (2021)

13. Bai, X. R., Zhang, N., Ren, K., Xu, J. P., Li, Z. Y., Zhang, C. H. Chemical constituents from Stenosolenium saxatile (Pall.) Turcz. (Boraginaceae). Biochemical Systematics & Ecology **74**,30–32. https://doi.org/10.1016/j.bse.2017.08.001 (2017)
14. Zhu, X. C., Skoneczny., Weidenhamer, J. D., Mwendwa. Identification and localization of bioactive naphthoquinones in the roots and rhizosphere of Paterson's curse (Echium plantagineum), a noxious invader. J EXP BOT **67**, 3777–3788. https://doi.org/10.1093/jxb/erw182 (2016)
15. Vranová, E., Coman, D., Gruissem, W. Network Analysis of the MVA and MEP Pathways for Isoprenoid Synthesis. Annual Review of Plant Biology **64**(1), 665. (2013)
16. Chawuke, P., Berg, N., Fouche, G., Maharaj, V., Alexandre, K. B. Lobostemon trigonus (Thunb.) H. Buek, a medicinal plant from South Africa as a potential natural microbicide against HIV-1. Journal of Ethnopharmacology **277**(1):114222. https://doi.org/10.1016/j.jep.2021.114222 (2021)
17. Harnett, S. M, Oosthuizen, V., Venter, M. Anti-HIV activities of organic and aqueous extracts of Sutherlandia frutescens and Lobostemon trigonus. Journal of Ethnopharmacology 96, 113–119. https://doi.org/10.1016/j.jep.2004.08.038 (2005)
18. Sankawa., Ebizuka., Miyazaki., Isomura., Otsuka. Antitumor activity of shikonin and its derivatives. *Chemical & pharmaceutical bulletin*. (1977)
19. Jian, C. N., Yi, X., Yu, L. Z. A comparative palynological study on the maharanga and onosma (Boraginaceae). Acta Phytotaxonomica Sinica. 10.1039/C3TC32244K (1995)
20. Liu, J. X., Li, J. Y., Zhang, Y. L., Ning, J. C. Pollen morphology of the tribe Lithospermeae of Boraginoideae in China and its taxonomic significance. Plant Systematics & Evolution **290**, 75–83. https://doi.org/10.1007/s00606-010-0350-x (2010)
21. Selvi, F., Papini, A., Hilger, H. H, Bigazzi, M., Nardi, E. The phylogenetic relationships of Cynoglottis (Boraginaceae- Borageneae) inferred from ITS, 5.8S and trn L sequences. *Plant Systematics & Evolution* **246**(3–4). https://doi.org/10.1007/s00606-004-0151-1 (2004)
22. *Angewandte Chemie International Edition*. https://doi.org/10.1002/(SICI)1521-3773(19990201)38:3<270::AID-ANIE270>3.0 (1999)
23. Rajbhandari, M., Schoepke, T. H., Mentel, R., Lindequist, U. Antibacterial and antiviral naphthazarins from Maharanga bicolor. Pharmazie **62**(8), 633–635. https://doi.org/10.1016/S1726-4901(09)70125-X (2007)
24. Patel, K. G., Detroja, J. R., Shah, T. A, Patel, K.V., Gandhi, T. R. Evaluation of the effect of Onosma bracteatum Wall (Boraginaceae) using experimental allergic and inflammatory models. Global Journal of Pharmacology **5**(1), 40–49. (2011)
25. Wang, X., Yang, M. K., Han, H. W., Wen, Z. L., Lu, G. H., Qi, J. L., Lin, H. Y., Yang, Y. H. Shikonin - Natural Products of Traditional Chinese Medicine: Biosynthesis, Genetic Regulation, Structural Modification and Pharmaceutical Function. Scientia Sinica Vitae. **52**, 26. (2022)
26. Hamaideh, K., Elelimat, T., Afifi, F., Kasabri, V. Phytochemical Screening and Pharmacological Activities of Echium judaeum Lacaita Extracts Growing Wild in Jordan. (2018)
27. Sayyah, M., Boostani, H., Pakseresht, S., Malaieri, A. Efficacy of aqueous extract of Echium amoenum in treatment of obsessive-compulsive disorder. Progress in Neuropsychopharmacology & Biological Psychiatry **33**(8), 1513–1516. https://doi.org/10.1016/j.pnpbp.2009.08.021 (2009)
28. Shafaghi, B., Naderi, N., Tahmasb, L., Kamalinejad, M. Anxiolytic Effect of Echium amoenum L. in Mice. Iranian journal of pharmaceutical research (IJPR) **1**(1),37–41. (2002)
29. Jayasooriya., Tharanga, R., Lee., Kyoung, T., Kang., Chang, H. Isobutyrylshikonin inhibits lipopolysaccharide-induced nitric oxide and prostaglandin E2 production in BV2 microglial cells by suppressing the PI3K/Akt-mediated nuclear transcription factor-κB pathway. Nutrition Research **34**(12),1111–1119. https://doi.org/10.1016/j.nutres.2014.10.002 (2014)
30. Swarts, A., Matsiliza-Mlathi, B., Kleynhans, R. Rooting and survival of Lobostemon fruticosus (L) H. Buek stem cuttings as affected by season, media and cutting position. South African Journal of Botany **119**, 80–85. https://doi.org/10.1016/j.sajb.2018.08.019 (2018)
31. Wang, Y. P., Basic, Clinical, Medicine. China Standardization **04**, 132–135. (2015)
32. Nam, C., Hwang, J. S., Kim, M. J., Choi, Y. W., Han, K. G., Kang, J. K. Single- and Repeat-dose Oral Toxicity Studies of Lithospermum erythrorhizon Extract in Dogs. Toxicological Research. https://doi.org/10.5487/tr.2015.31.1.077 (2015)
33. Findley, W. E., Jacobs, B. R. The antigonadotropic activity of Lithospermum ruderale I. The lack of steroid-like activity at the receptor level. Contraception **21**(2), 199–205. https://doi.org/10.1016/0010-7824(80)90132-8 (1980)
34. Cecchi., Lorenzo., Coppi., Andrea., Hilger., Hartmut. Non-monophyly of Buglossoides (Boraginaceae: Lithospermeae): Phylogenetic and morphological evidence for the expansion of Glandora and reappraisal of Aegonychon. *Taxon*.(2014)
35. Ferrero, V., Chapela, I., Arroyo, J., Navarro, L. Reciprocal style polymorphisms are not easily categorised: the case of heterostyly in Lithodora and Glandora (Boraginaceae). *Plant Biology*. https://doi.org/10.1111/j.1438-8677.2009.00307.x (2010)
36. Xiong, W., Gang, L., Zhou, L., Yun, Z., Yang, W. In vitro and in vivo antitumor effects of acetylshikonin isolated from Arnebia euchroma (Royle) Johnst (Ruanzicao) cell suspension cultures. Chinese Medicine, **4**(1), 14. https://doi.org/10.1186/1749-8546-4-14 (2009)
37. Aedo, C. I. Distribución de Lithodora Griseb. en la Cornisa Cantábrica. Munibe Ciencias Naturales 41–42. (1989)
38. Aljanaby, A. Antibacterial activity of an aqueous extracts of Alkanna tinctoria roots against drug resistant aerobic pathogenic bacteria isolated from patients with burns infections. https://doi.org/10.15275/rusomj 2018.
39. Bame, J., Graf, T., Junio, H., Bussey, R., Jarmusch, S., El-Elimat, T. Sarothrin from Alkanna orientalis Is an Antimicrobial Agent and Efflux Pump Inhibitor. Planta Medica **79**, 327–329. (2013)
40. Elsohly, H. N., El-Feraly, F. S., Joshi, A. S., Walker, L. A. Antiviral Flavonoids from Alkanna orientalis. Planta Medica **63**(4):384–384. (1997)
41. Jinyvarghese, K., Karpe, S. T., Kulkarni, S. R. Immunostimulant activity of Adhatoda vasica, Lawsonia inermis and Alkanna tinctoria. Indian Drugs **42**(6):345–352. (2005)

42. Khan, U. A., Rahman, H., Qasim, M., Hussain, A., Azizllah, A., Murad, W. Alkanna tinctoria leaves extracts: a prospective remedy against multidrug resistant human pathogenic bacteria. Bmc Complementary & Alternative Medicine **15**(1), 1–6. https://doi.org/10.1186/s12906-015-0646-z (2015)
43. Mohammed, M. S., Mohamed, A. A., Basudan, O., Tahir, K., Osman, W. Evaluation of Antipyretic, Antinocieptive and Sedative Effects of Tribulus Terrestris, Mimosa Pigra and Alkanna Tinctoria Methanolic Extracts. https://doi.org/10.31254/phyto.2016.5303 2016.
44. Ozer, M. S., Sarikurkcu, C., Tepe, B., Can. S. Essential oil composition and antioxidant activities of alkanet (Alkanna tinctoria subsp. tinctoria). Food Science & Biotechnology **19**(5), 1177–1183. https://doi.org/10.1007/s10068-010-0168-x (2010)
45. Papageorgiou, V. P. Wound healing properties of naphthaquinone pigments from Alkanna tinctoria. Experientia **34**(11), 1499–1501. https://doi.org/10.1007/BF01932375 (1978)
46. Papageorgiou, V. P., Mellidis, A. S., Sagredos, A. N. Study on the antibiotic fraction of Alkanna tinctoria Tausch. (1980)
47. Yildirim, H., Şenol, S. Alkanna malatyana (Boraginaceae), a new species from East Anatolia, Turkey. Phytotaxa **164**(2), 124–132. https://doi.org/10.11646/phytotaxa.164.2.6 (2014)
48. Yousefi, R., Ghaffarifar, F., Asl, A. The Effect of Alkanna tincturia and Peganum harmala Extracts on Leishmania major (MRHO/IR/75/ER) in vitro. Iranian Journal of Parasitology **4**(1). https://doi.org/10.1016/j.ijpara.2008.06.014
49. Showkat, A. G., Asima, J. B. Radical scavenging and antibacterial activity of Arnebia benthamii methanol extract - ScienceDirect. Asian Pacific Journal of Tropical Medicine **5**(10), 766–772. https://doi.org/10.1016/S1995-7645(12)60140-0 (2012)
50. Cao, H., Zhang, W., Liu, D., Hou, M., Shao, M. Identification, in vitro evaluation and modeling studies of the constituents from the roots of Arnebia euchroma for antitumor activity and STAT3 inhibition. Bioorganic Chemistry **96**, 103655. https://doi.org/10.1016/j.bioorg.2020.103655 (2020)
51. Chen, F., Xu, F., Zhang, Z., Upton. Anti-inflammatory effects of shikonin in human periodontal ligament cells. Pharmaceutical Biology. https://doi.org/10.1080/13880209.2018.1506482 (2018)
52. Doulah, A. H., Neisi, N., Zekavati, R., Farjam M. H. Antibacterial, antifungal and antioxidant activity of four species from Arnebia genus growing wild in Iran. Iranian Journal of Science & Technology Transaction A Science **170**(A2), 1009–1014.(2014)
53. He, J. M., Sun, S. C., Sun, Z. L, Chen, J. T., Mu, Q. Isovalerylshikonin, a new resistance-modifying agent from Arnebia euchroma, supresses antibiotic resistance of drug-resistant Staphylococcus aureus. International Journal of Antimicrobial Agents **53**, S0924857918302474-. https://doi.org/10.1016/j.ijantimicag.2018.08.021 (2018)
54. Kashiwada, Y., Nishizawa, M., Yamagishi, T., Tanaka, T., Nonaka, G. I., Cosentino, L. M. Anti-AIDS agents, 18. Sodium and potassium salts of caffeic acid tetramers from Arnebia euchroma as anti-HIV agents. Journal of Natural Products **58**(3), 392–400. https://doi.org/10.1021/np50117a007 (1995)
55. Katoch, M. A. Streptomyces spiroverticillatus from the rhizospheric soil of Arnebia euchroma: its antimicrobial and anticancer potential. (2013)
56. Kumar, A., Shashni, S., Kumar, P., Pant, D., Verma, R. K. Phytochemical constituents, distributions and traditional usages of Arnebia euchroma: A review. Journal of Ethnopharmacology **271**(6),113896. https://doi.org/10.1016/j.jep.2021.113896 (2021)
57. Li, H. M., Tang, Y. L., Zhang, Z. H., Liu, C. J., Xia, X. S. Compounds from Arnebia euchroma and Their Related Anti-HCV and Antibacterial Activities. Planta Medica **78**(1), 39–45. https://doi.org/10.1055/s-0031-1280266 (2011)
58. Ma, Y., Cong, W., Huang, H., Liang, S., Mai, A. H., Kurt, B. Identification of fukinolic acid from Cimicifuga heracleifolia and its derivatives as novel antiviral compounds against enterovirus A71 infection. International Journal of Antimicrobial Agents S092485791830205X-. https://doi.org/10.1016/j.ijantimicag.2018.07.014 (2018)
59. Rafiei, M. H., Jafarzadeh, H., Mollaei, A., Nasiri, E. Effect of Arnebia Euchroma Root Ointment on Surgical Wound Healing in Wistar Rats. Journal of Mazandaran University of Medical Sciences **30**(187), 28–37. (2020)
60. Abed, A., Vaseghi, G., Jafari, E., Fattahian, E., Abed, M. Echium Amoenum Fisch. Et Mey: A Review on its Pharmacological and Medicinal Properties. (2014)
61. Abolhassani, M. Antiviral activity of borage (Echium amoenum). Archives of Medical Science Ams **6**(3), 366. https://doi.org/10.5114/aoms.2010.14256 (2010)
62. Azizi, H., Ghafari, S., Ghods, R., Shojaii, A., Ghafarzadeh, J. A review study on pharmacological activities, chemical constituents, and traditional uses of Echium amoenum. Pharmacognosy Reviews **12**(24), 208. https://doi.org/10.4103/phrev.phrev_13_18 (2018)
63. Bozorgi, H., Motaghi, E., Chizari, Z., Taghian, M. Anxiolytic and anticonvulsant properties of Anthocyanin extract of Echium amoenum in mice. Online Journal of Veterinary Research.(2013)
64. Brown, A., Burdon, J. J. Multilocus Diversity in an Outbreeding Weed, Echium piantagineum L. Australian Journal of Biological Sciences **36**(6), 503–510. https://doi.org/10.1071/BI9830503 (1983)
65. Corbet, S. A., Unwin, D. M., Pr?S-Jones, O. E. Humidity, nectar and insect visits to flowers, with special reference to Crataegus, Tilia and Echium. Ecological Entomology **4**(1), 9–22. https://doi.org/10.1111/j.1365-2311.1979.tb00557.x (2010)
66. El-Rokh, A. R., Negm, A., El-Shamy, M., El-Gindy, M., Abdel-Mogib, M. Sucrose diester of aryldihydronaphthalene-type lignans from Echium angustifolium Mill. and their antitumor activity. Phytochemistry **149**, 155–160. (2018)
67. Eruygur, N., Y?Lmaz, G., Kutsal, O., Yücel, G., üstün, O. Bioassay-guided isolation of wound healing active compounds from Echium species growing in Turkey. Journal of Ethnopharmacology 370–376. https://doi.org/10.1016/j.jep.2016.02.045 (2016)
68. Hamidi, E. M., Khaksari, M., Hojabri, K. The effects of aqueous extracts of Echium amoenum and citrus aurantiflia on blood pressure and heart rate before and after phynelephrine injection in rat. Journal of Kerman University of Medical Sciences **18**(4), 349–357. (2011)
69. Leila S, Vahid YB, Farzeen T. Improving effects of Echium amoenum aqueous extract on rat model of Alzheimer's disease. Journal of Integrative Neuroence **17**, 1–9. https://doi.org/10.3233/JIN-180093 (2018)

70. Mohammadi, S., Piri, K. H., Mohammadi, S., Piri, K. H. Antifungal Effects of Two Medicinal Plant Native to Iran. (2014)
71. Shariatifar, N., Fathabad, A. E., Madihi, S. Antibacterial activity of aqueous and ethanolic extracts of Echium amoenum on food- borne pathogens. (2016)
72. Adrian., Lupescu., Rosi., Bissinger., Kashif., Jilani. In Vitro Induction of Erythrocyte Phosphatidylserine Translocation by the Natural Naphthoquinone Shikonin. Toxins. https://doi.org/10.3390/toxins6051559 (2014)
73. Amiri, Z. M., Tanideh, N., Seddighi, A., Mokhtari, M., Mehrabani, D. The Effect of Lithospermum officinale, Silver Sulfadiazine and Alpha Ointments in Healing of Burn Wound Injuries in Rat. World Journal of Plastic Surgery **6**(3), 313–318. (2017)
74. Cohen, J. I. Continuous characters in phylogenetic analyses: Patterns of corolla tube length evolution in Lithospermum L. (Boraginaceae). *Biological Journal of the Linnean Society* **2**, 442–457. https://doi.org/10.1111/j.1095-8312.2012.01938.x (2012)
75. Esmaeil, K., Amir, M., Mohsen, F., Jaleh, G., Faezeh, G., Kamahldin, H. Pyrrolizidine Alkaloids-Free Extract from the Cell Culture of Lithospermum officinale with High Antioxidant Capacity. Applied Biochemistry & Biotechnology. https://doi.org/10.1007/s12010-018-2830-3 (2018)
76. Ji-Hyun, Bae. Antimicrobial Effect of Lithospermum erythrorhizon Extracts on the Food-borne Pathogens. Korean journal of food science and technology **36**(5), 823–827. (2004)
77. Liu, H., Wei, W., Tang, J., Lu, M. A., Wang, J., Ting, Y. U. Experimental Study on the Antifertility Effects of Self-made Siwu Lithospermum Decoction in Female Rat. China Health Standard Management. (2018)
78. Rajasekar, S., Da. J. P., Park, C., Park. S., Park, Y. H., Sun, T. K. In vitro and in vivo anticancer effects of Lithospermum erythrorhizon extract on B16F10 murine melanoma. Journal of Ethnopharmacology **144**(2), 335–345. https://doi.org/10.1016/j.jep.2012.09.017 (2012)
79. Rui-Rui, H. E., Liu, Y. G., Wei, M. J., Chen, X., Zhan, X. W., Wang, Y. X. Study on antitumor activities and its mechanisms of Lithospermum components. The Chinese Journal of Clinical Pharmacology. (2012)
80. Sourgens, f. F. W., Gumbinger, h. G., Kemper, f. H. Anti-hormonal effects of plant-extracts - tsh-suppressing and prolactin-suppressing properties of lithospermum-officinale and other plants. Planta med. (1982)
81. Zhang, Y., Han, H., Sun, L., Qiu, H., Yang, Y. Antiviral activity of shikonin ester derivative PMM-034 against enterovirus 71 in vitro. Brazilian Journal of Medical and Biological Research **50**(10). https://doi.org/10.1590/1414-431X20176586 (2017)
82. Zhao, X. M., Deng, W., Ying, L, I., Wang, G. L., Fei, H. R., Zhang, Y. Experimental Study on the Anti-inflammatory and Antibacterial Effects of Different Extracts from Lithospermum erythrorhizon. Lishizhen Medicine and Materia Medica Research. (2008)
83. Ahmad, B., Ali, N., Bashir, S., Chou, D., Hary, M. I., Khan, I. Parasiticidal, antifungal and antibacterial activities of Onosma griffithii Vatke. African Journal of Biotechnology **8**(19), 5084–5087. (2009)
84. Ahmad, V., Kousar, F., Khan, A., Zubair, M., Iqbal, S., Tareen, R. A New Ketone and a Known Anticancer Triterpenoid from the Leaves of Onosma limitaneum. Helvetica Chimica Acta **88**(2):-. https://doi.org/10.1002/hlca.200590013 (2005)
85. Asghar, M., Islam, M., Saeed, H., Imtiaz, F., Iqtedar, M. Investigations on Onosma Hispidum wall root extracts for in-vitro antidiabetic, proliferative and cytotoxic effects. Journal of Animal and Plant Sciences **28**(5), 1339–1347. (2018)
86. Kretschmer, N., Rinner, B., Deutsch, A., Lohberger, B., Bauer R. Naphthoquinones from Onosma paniculata Induce Cell-Cycle Arrest and Apoptosis in Melanoma Cells. Journal of Natural Products **75**(5), 865–869. https://doi.org/10.1021/np2006499 (2012)
87. Kumar, N., Gupta, A. K, Prakash, D., Kumar, P. Hypoglycemic activity of Onosma hispidum (Ratanjot). International Journal of Diabetes in Developing Countries **30**(4):213–216. https://doi.org/10.4103/0973-3930.70862 (2010)
88. Lang, C., Yong, X., Yang, Z., Li, Y., Li, H., Chen, X. Shikonofuran E plays an anti-inflammatory role by down-regulating MAPK and NF-κB signaling pathways in lipopolysaccharide-stimulated RAW264.7 macrophages. Journal of Natural Medicines 1–8. https://doi.org/10.1007/s11418-018-1238-2 (2018)
89. Moghaddam, P. Z. Antibacterial and antioxidant activities of root extract of Onosma dichroanthum Boiss. in north of Iran. African Journal of Microbiology Research **6**(8). https://doi.org/10.5897/AJMR11.1225 (2012)
90. Redzic, A., Redzic, S., Sejdic, N. Genotoxic effects of aquatic extract of endemic plant onosma stellulata waldst. & Kit. (Boraginaceae). African Journal of Traditional Complementary & Alternative Medicines.(2009)
91. Safavi, F., Farimani, M. M., Golalipour, M., Leung, P. C, Lau, B. S. Investigations on the wound healing properties of Onosma dichroantha Boiss root extracts. South African Journal of Botany **125**, 344–352. https://doi.org/10.1016/j.sajb.2019.08.005 (2019)
92. Scapin, M. Isolamento e caratterizzazione di composti antiproliferativi da Onosma visianii Clem.
93. Pilar, D. M., Armesto-Baztan, S., Díaz-Losada, E. A study of variation in the pollen spectra of honeys sampled from the Baixa Limia-Serra do Xurés Nature Reserve in north-west Spain. Grana Palynologica **45**(2), 137–145. https://doi.org/10.1080/00173130600708537 (2006)
94. Surh., Young, J. Cancer chemoprevention with dietary phytochemicals. Nature Reviews Cancer **3**(10), 768–780. (2003)

Tables

Table 1. Distribution of Chemical Species in Lithospermeae(Note + represents 1-5 compounds)

Genus	Quinones					Phenolic acids				Pyrrolizidine alkaloids	Flavonoids	Alip and con
	Shikonins	Alkannans	shikonin dimer	Furans	Others	Caffeic acid dimer	Caffeic acid trimer	Caffeic acid tetramers	Others			
Alkanna	+	++			+	+				+	+	
Arnebia	+++++	+++++	+	+	+++++	+		+	+	++	+	+
Echium	+++	+				+			++	+++	+	+
Lithospermum	++++	++	+	++++	+	+	+		+	++	+	
Onosma	+++	++		+	+	+			++	+++	++	+
Stenosolenium	+	+				+	+		+	+		
Lithodora												+
Buglossoides												+
Lobostemon						+	+		+	+	++	+
Maharanga		+										
Glandora						+	++	+	+		++	+++

Table 2.Traditional applications and biological activities of Lithospermeae. plants

Genus	Traditional applications	Biological activity	References
Alkanna	diarrhea	analgesia	10, 38-48
	abscess	antibacterial	
	wounds	anticancer	
	burns	antimicrobial	
	sore throat	anti-inflammatory	
		anti-nociceptive	
		antioxidant	
		antiviral	
		cutaneous leishmaniasis	
		wound healing	
		antipyretic	
		sedative effects	
Arnebia	antipyretic	antiAIDS	10, 49-59
	bactericidal	antibacterial	
	boils	antibiotic	
	burns	anticancer	
	clearing heat and detoxifying	antidermatophytic	
	colic pain	antifungal	
	cooling blood	anti-inflammatory	
	detoxify	antimicrobial	
	detumescence	antioxidant	
	fever	antithrombotic	
	headache	antitumor	
	heart disease	antiviral	
	helminthiasis	wound healing	
	promoting blood circulation	anti-HCV	
	rash		
	skin diseases		
	stomach pain		
	wounds		
Echium	abscess	acute and chronic constipation	10, 60-71
	antianxiety	analgesic	
	contusion	antianxiety	
	cooling blood	antibacterial	
	diuresis	anticonvulsant	
	diuretic	antifungal	
	expectorant	anti-inflammatory	
	hand crack	antimicrobial	
	lubricant	antioxidant	
	protective agent	antitumor	
	rheumatic pain	antiviral	
	sedation	anxiolytic	
	trauma	delayaging	
	trauma	hemostasis	

		hypolipidemia	
		insecticidal	
		lower blood Pressure	
		prevention of Alzheimer's disease	
		wound healing	
		demulcent	
Lithospermum	analgesia	angiostatic	10, 33, 72-82
	bronchitis	antibacterial	
	clearing heat and detoxifying.	anticancer	
	contraception	antifertility	
	cooling blood	antigonadotropic	
	cough	antihormonal	
	detumescence	anti-inflammatory	
	diminishing inflammation	antimicrobial	
	dispelling wind	antioxidant	
	diuretics	antithrombotic	
	dyspepsia	antitumor	
	fever	antiviral	
	fracture	immunostimulatory	
	gastric distention	lymphatic tuberculosis	
	hematemesis	pulmonary tuberculosis	
	hemostasis	wound healing	
	joint pain		
	promoting blood circulation		
	rash		
	relieving pain		
	sore		
	swelling		
	traumatic		
	weakness		
Onosma	abdominal	antibacterial	10, 83-92
	alexipharmic eye diseases	anticancer	
	anthelmintic	antifungal	
	blood disorders	anti-inflammatory	
	bronchitis	antimicrobial	
	burns	antioxidant	
	clearing heat and detoxifying.	antiproliferativie	
	cooling blood	antithrombosis	
	foot ulcers	antitumor	
	heart disease	hypoglycemic	
	itch	respiratory diseases	
	laxative	tetranychusurticae	
	lung cancer	wound healing	
	pain	antidiabetic	
	promoting blood circulation		

	rash		
	respiratory diseases		
	rheumatism		
Stenosolenium	clearing heat		10
	cold		
	cooling blood		
	cough		
	dehumidification		
	dispelling wind		
	hematemesis		
	joint pain		
	lung heat		
	respiratory diseases		
	stopping bleeding		
Lithodora	blood purification		10
	chilblain		
	cold		
	cough		
	enteritis		
	fever		
	flu		
	hypertension		
	liver protection		
	pneumonia		
	rheumatism		
	sedation		
	stomachache		
Buglossoides	weight loss	antioxidant	10, 34
Lobostemon	bacteriosis	antiHIV	10, 17, 30
	ulcer	wound healing	
	wounds		
Maharanga	constipation	antibacterial	10, 23
		antiviral	
Glandora	diuretic	anticancer	93, 94
	depurative		
	anti-hypertensive		

Table 3. *Echium Lithospermum* and *Arnebia*, screened active compounds

Compounds	GI	DL	Source
1,8-Cineole	high	3yes	*Lithospermum.*, *Arnebia*
2-methyl-n-butyryl shikonin	high	5yes	*Echium*
4-hydroxybenzoic acid	high	3yes	*Echium*
7-tigloylretronecine	high	5yes	*Lithospermum*, *Arnebia*
acetylshikonin	high	5yes	*Echium*, *Lithospermum*
alkannan	high	5yes	*Echium*
angelylalkannin	high	5yes	*Lithospermum*, *Arnebia*
anhydroalkannin	high	5yes	*Lithospermum*, *Arnebia*
arnebifuranone	high	5yes	*Lithospermum*, *Arnebia*
arnebinone	high	5yes	*Lithospermum*, *Arnebia*
caffeic acid	high	4yes	*Echium*
cyanidin	high	5yes	*Echium*
deoxyalkannin	high	5yes	*Lithospermum*, *Arnebia*
deoxyshikonin	high	5yes	*Arnebia*
echimidine	high	5yes	*Lithospermum*, *Arnebia*
geranylbutyrate	high	5yes	*Lithospermum*, *Arnebia*
glabridin	high	5yes	*Lithospermum*, *Arnebia*
hydrocaffeic acid	high	4yes	*Echium*
isobutyrylshikonin	high	5yes	*Echium*, *Lithospermum*, *Arnebia*
isovalerylalkannin	high	5yes	*Lithospermum*, *Arnebia*
isovalerylshikonin	high	5yes	*Echium*, *Lithospermum*, *Arnebia*
jolkinolide E	high	5yes	*Lithospermum*, *Arnebia*
leptanthine	high	4yes	*Echium*
lycopsamine	high	4yes	*Lithospermum*, *Arnebia*
osthol	high	5yes	*Lithospermum*, *Arnebia*
p-coumaric acid	high	4yes	*Echium*
p-hydroxybenzoic acid	high	3yes	*Echium*
pinoresinol	high	5yes	*Echium*
propionylalkannin	high	5yes	*Lithospermum*, *Arnebia*
propionylshikonin	high	5yes	*Echium*, *Lithospermum*, *Arnebia*
shikonofuran A	high	5yes	*Lithospermum*, *Arnebia*
shikonofuran E	high	4yes	*Lithospermum*, *Arnebia*
supinine	high	5yes	*Lithospermum*, *Arnebia*
syringic acid	high	4 yes	*Echium*
teracrylshikonin	high	4yes	*Lithospermum*, *Arnebia*
vanillic acid	high	4yes	*Echium*, *Lithospermum*, *Arnebia*
β-hydroxyisovalerate	high	3yes	*Arnebia*
β-Santalol	high	4yes	*Lithospermum*, *Arnebia*

Figures

Figure 1

Morphology of some medicinal plants of Lithospermeae. (A: *Arnebia decumbens* (Vent.) Coss. et Kral., B: *Onosma exsertum* Hemsl., C: *Onosma confertum* W. W. Smith., D: *Onosma paniculatum* Bur. et Franch., E: *Lithospermum erythrorhizon* Sieb. et Zucc., F: *Echium vulgare* L., G: *Arnebia guttata* Bge., H: *Stenosolenium saxatile* (Pallas) Turczaninow.)

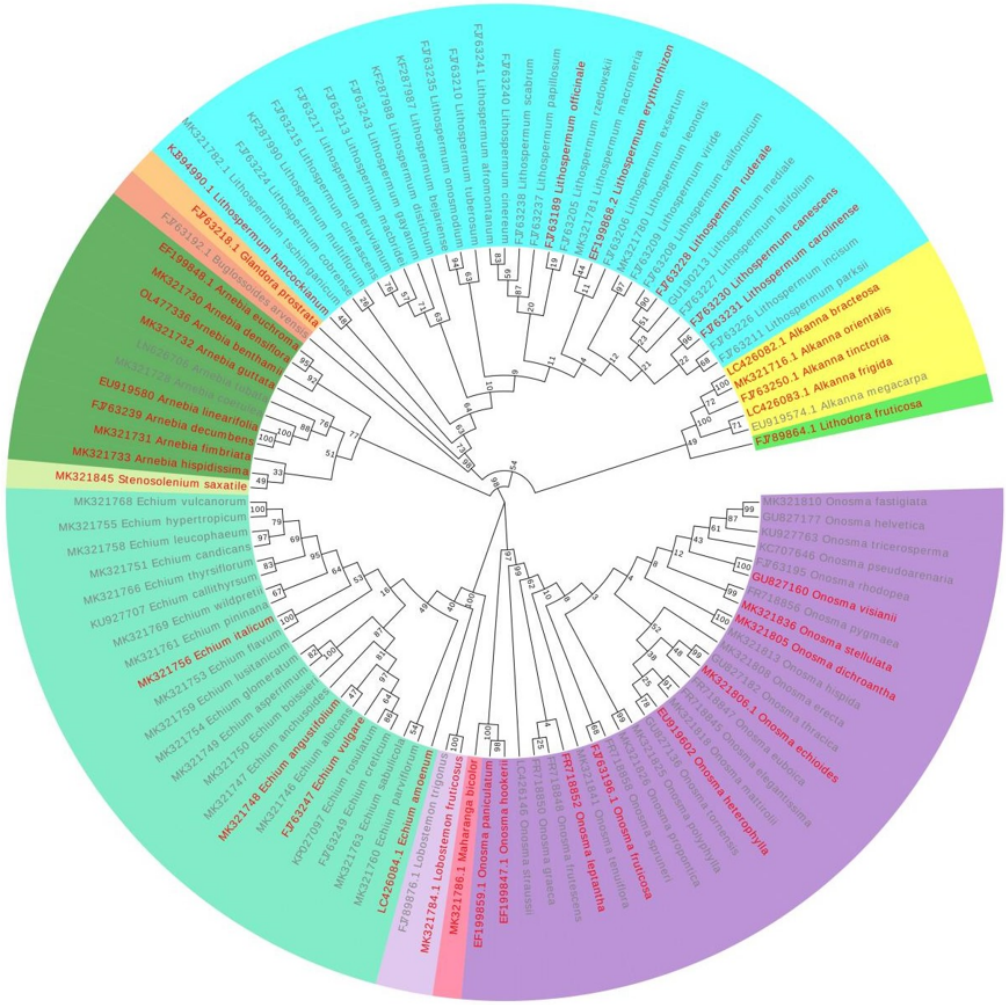
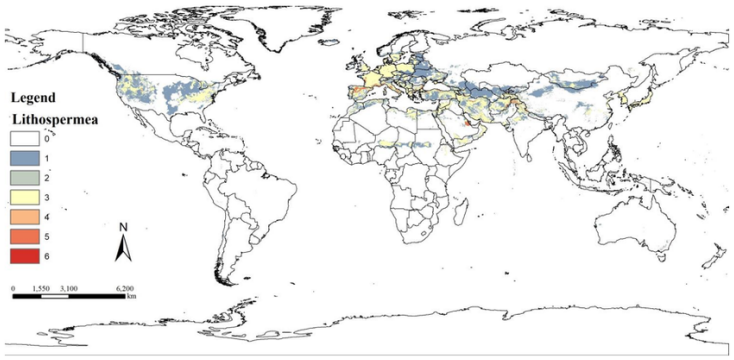
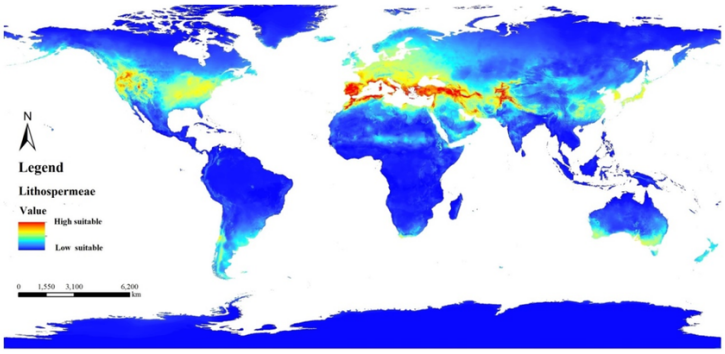


Figure 2

Phylogenetic tree of Lithospermeae species(The red font is a medicinal plant of Lithospermeae)



(A)



(B)

Figure 3

A. Distribution map of medicinal plants of Lithospermeae(genuses of medicinal plants of Lithospermeae, the superposition of main distribution areas is at least 0 and at most 6 generas)

B. Species adaptability distribution map of Lithospermeae

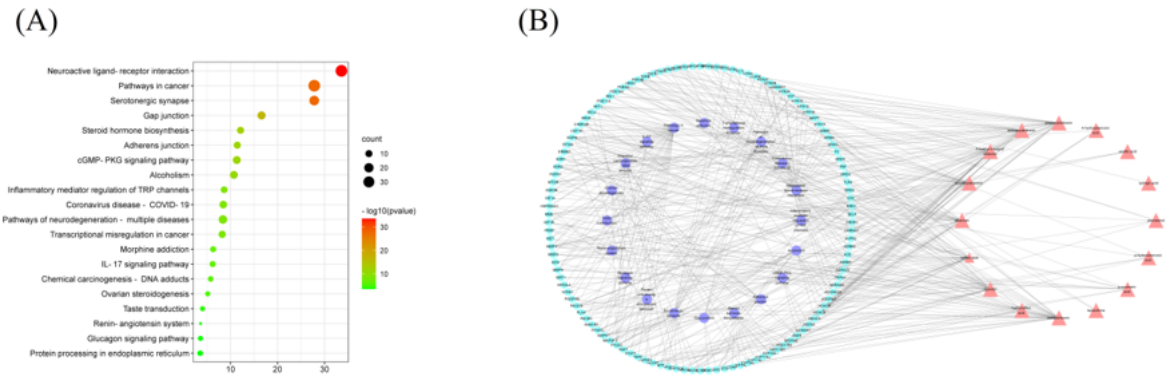


Figure 4

A. Analysis of the anti-anxiety KEGG pathway in *Echium*; **B**. Anti-anxiety Chemical Composition - Target - Pathway Network of *Echium*

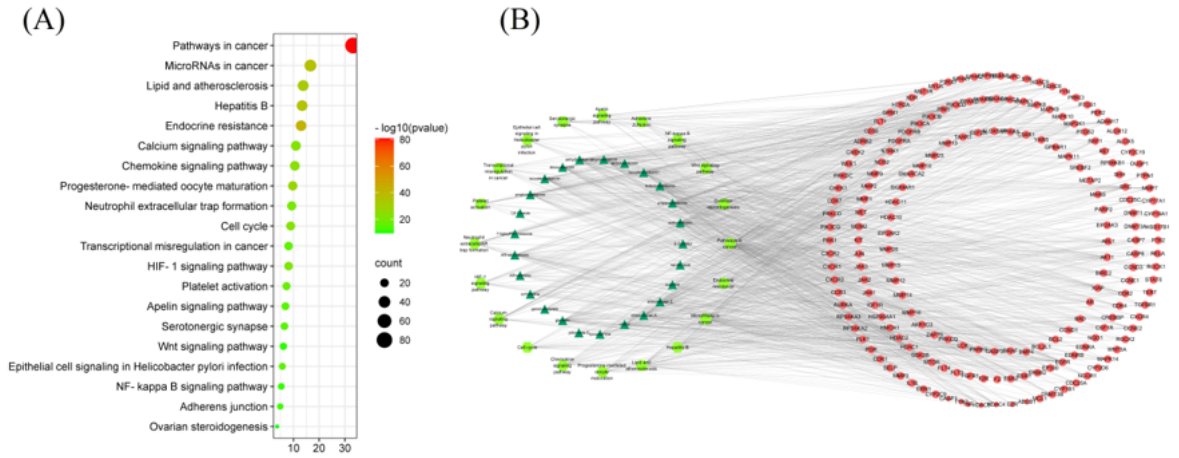


Figure 5

A. Analysis of the anti-cancer KEGG pathway in *Lithospermum*; **B**. Anticancer Chemical Composition - Target - Pathway Network of *Lithospermum*

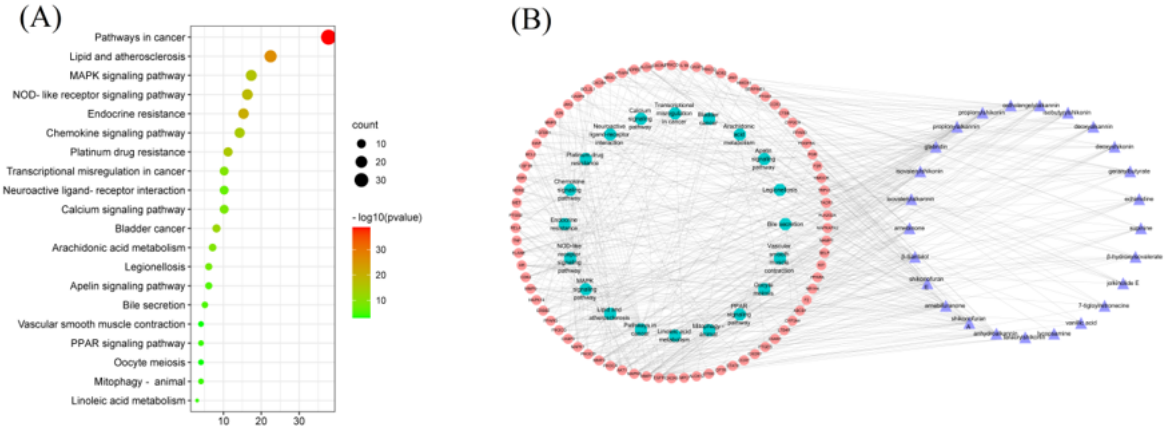


Figure 6

A. Analysis of the anti-inflammatory KEGG pathway in *Arnebia*; **B**. Anti-inflammatory Chemical Composition - Target - Pathway Network of *Arnebia*

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