

# Phylogenetic Tree of 50 Medicinal Plants in Shandong Province, China

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**Abstract:** Medicinal plants are a valuable resource for a variety of products which are important for human needs. The fundamental step in the process has always been the characterization of species. This is the reason which connects the phytopharmaceutical sciences with taxonomy and species identification. The DNA-based method of identification has revolutionized this field in taking the taxonomy to the molecular level. 50 medicinal plants were selected. Two nucleotide sequences of each collected plant were selected and 100 nucleotide sequences downloaded from the NCBI database. MEGA11 Software was used to analyze those nucleotide sequences, and then Maximum Likelihood tree was reconstructed to reveal genetic relationship of 50 medicinal plants. The phylogenetic tree showed that majority nucleotide sequences of 50 medicinal plants can be clustered on the same branch with the same plants of their network.

## 1 INTRODUCTION

Medicinal plants are a valuable resource for a variety of products that are important for human needs. With respect to medicinal plants, the use of plant-based materials dates back to ancient civilizations. There are several ancient written records that provide evidence regarding use of plant sources of remedies [1-2]. The knowledge from ancient systems of plant-based remedies has also been used by the modern pharmaceutical industry. There is thus an immense potential for discovery of new drugs from plants based on the ethno-medicinal data [3-4]. About one-third of currently available drugs come from natural products that have a plant origin [5]. Evaluation of these medicinally important chemicals is not an easy task. The fundamental step in the process has always been the characterization of species. This is the reason which connects the phytopharmaceutical sciences with taxonomy and species identification. The DNA-based method of identification has revolutionized this field in taking the taxonomy to the molecular level [6]. There are nearly 11,000 medicinal species in China [7]. In the study, 50 medicinal plants in Shandong Province were selected to explore the genetic relationship by maximum likelihood tree.

## 2 METHODS

The medicinal plant species list and therapeutic uses were compiled mainly from local pharmacopoeias. Besides the literature review, we conducted an in-depth

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analysis of the typical therapeutic uses of medicinal plants. According to the Flora of China, species names were standardized. Species-level groups were formed for infraspecific taxa. And then, 50 medicinal plants were collected. A total of 10 categories of medicinal plants were classified according to their traditional use as a remedy for diseases such as musculoskeletal disorder, skin disorder, hepatic disorder and circulatory disorder, etc. The reconstruction of the subfamily level system of the family selects the chloroplast genes *pasB*, *rbcl*, *matK*, *ycfl* and nuclear gene *Xdh* that are currently used to establish the high-level taxonomic hierarchy with a relatively slow evolution rate. When establishing a subfamily and genus-level system, the chloroplast genes *trnK*, *trnL-F* and nuclear gene ITS with a faster evolution rate were added. When downloading data, try to download gene fragments measured from the same specimen or from the same literature. Collect the screened species names and sequence numbers for the sub-genes to sort out the sampling table, and use the NCBI batch download function to download the original sequence in fasta format according to the sequence number. Two nucleotide sequences of each collected plant were selected and 100 nucleotide sequences downloaded from the NCBI database. In order to ensure that the sequences used are accurate and reliable, according to the maximum likelihood tree, check the suspicious species and analyze the reasons for the suspicious location of the species, further adjust the sampling, replace or delete the sequence, and repeat the screening process until there is no suspicious sequence. MEGA11 Software was used to analyze those

nucleotide sequences, and then maximum likelihood tree was reconstructed to reveal genetic relationship of the total of 50 medical plants.

### 3 RESULTS

#### 3.1 The basic characteristics of the collected 50 MEDICINAL plants

In the study, 50 medicinal plants were collected (Table1). According to the classification of drug action, the

collected medicinal plants were divided into 10 groups including Heat-clearing Drug(n=15), Expectorant Cough Suppressant and Anti-asthmatic Drug(n=12), Tonify Deficiency Drug(n=9), Relieving Exterior Disorder Drug(n=4), Discutient Drug, Cathartic Drug(n=3), Dispelling Internal Cold Drug(n=2), Digestant Drug(n=1), Sedative Drug(n=1) and Blood-activating Drug(n=1). Two sequences of each collected plant were selected. And then, 100 sequences downloaded from the NCBI database.

**Table1.** The basic characteristics of the collected 50 medicinal plants

Traditional Chinese Medicine (n=50)	Nucleotide Sequence	
<b>Heat-clearing Drug (n=15)</b>	GenBank	
<i>Pulsatilla chinensis</i>	KR611744.1	KR611745.1
<i>Andrographis paniculata</i>	MH142887.1	MH142898.1
<i>Fagopyrum dibotrys</i>	MK524275.1	MN966488.1
<i>Lonicera</i>	KY489892.1	KY489893.1
<i>Forsythia</i>	AF231823.1	AF231824.1
<i>Taraxacum mongolicum</i>	HQ436156.1	HQ436189.1
<i>Smilax glabra</i>	KJ130462.1	KJ130463.1
<i>Chrysanthemum indicum</i>	AB234770.1	AB234779.1
<i>Phellodendron chinense</i> var.	KT961097.1	KT961098.1
<i>Coptis chinensis</i>	AB163736.1	EU370101.1
<i>Scutellaria baicalensis</i>	MG269467.1	MG269468.1
<i>Sophora flavescens</i>	AB127029.1	MW961004.1
<i>Vincetoxicum</i>	KX364133.1	KX364134.1
<i>Artemisia annua</i>	KU555617.1	KU555618.1
<i>Bupleurum falcatum</i>	JN818036.1	JN818069.1
<b>Expectorant Cough Suppressant and Anti-asthmatic Drug (n=12)</b>	GenBank	
<i>Vincetoxicum glaucescens</i>	KX184263.1	MF004211.1
<i>Pinellia ternata</i>	AY725425.1	FJ638617.1
<i>Trichosanthes kirilowii</i>	AY584242.1	MH659988.1
<i>Dioscorea bulbifera</i>	KM043790.1	MH837858.1
<i>Platycodon grandiflorum</i>	FN397087.1	GQ436427.1
<i>Peucedanum decursivum</i>	U78411.1	U78471.1
<i>Arisaema consanguineum</i>	AB982816.1	AB982817.1
<i>Inula britannica</i>	EU385560.1	KY696297.1
<i>Ginkgo biloba</i>	AF313611.1	AB284317.1
<i>Stemona collinsae</i>	AB373226.1	AB429266.1
<i>Tussilago farfara</i>	EF537951.1	KY970765.1
<i>Aristolochia championii</i>	GU445336.1	U445349.1
<b>Tonify Deficiency Drug (n=9)</b>	GenBank	
<i>Atractylodes</i>	LC465409.1	LC465416.1
<i>Eleutherococcus senticosus</i>	AB080246.1	AB088017.1
<i>Glycyrrhiza glabra</i>	MN601954.1	EU531613.1
<i>Pseudostellaria heterophylla</i>	EF197884.1	EF197887.1
<i>Angelica</i>	MH188443.1	MH188441.1
<i>Rehmannia glutinosa</i>	KC977490.1	MK248733.1

Astragalus complanatus	KT163388.1	MH651352.1
Cynomorium songaricum	MH594764.1	MT587667.1
Lycium barbarum	KY570915.1	KY570934.1
<b>Relieving Exterior Disorder Drug (n=4)</b>		GenBank
Xanthium strumarium	KJ496389.1	KJ496390.1
Saposhnikovia divaricata	MK571406.1	MK571407.1
Ephedra	AB600681.1	AY599175.1
Magnolia sprengeri	AY158159.1	MH659418.1
<b>Discutient Drug (n=3)</b>		GenBank
Allium sativum	AF356825.1	AF356826.1
Acacia catechu	GQ435957.1	GQ436355.1
Strychnos nux-vomica	MZ505275.1	MZ749455.1
<b>Cathartic Drug (n=2)</b>		GenBank
Rheum officinale	AB115684.1	AB232456.1
Aloe	MG776035.1	MG776038.1
<b>Dispelling Internal Cold Drug (n=2)</b>		GenBank
Syzygium aromaticum	MH070007.1	MH070008.1
Zingiber officinale	HM567399.1	HM567401.1
<b>Digestant Drug (n=1)</b>		GenBank
Crataegus mollis	KP050186.1	KP050200.1
<b>Sedative Drug (n=1)</b>		GenBank
Polygala tenuifolia	DQ267099.1	HQ377319.1
<b>Blood-activating Drug (n=1)</b>		GenBank
Leonurus japonicus	MN712238.1	MT994239.1

### 3.2 Phylogenetic tree of the collected 50 medicinal plants

The downloaded 100 nucleotide sequences of 50 medicinal plants were analyzed by MEGA11 Software, and then Maximum Likelihood tree of the 50 medicinal plants was reconstructed to reveal genetic relationship (Figure 1). The result showed that 50 MEDICINAL plants cluster into two clades. Compared with

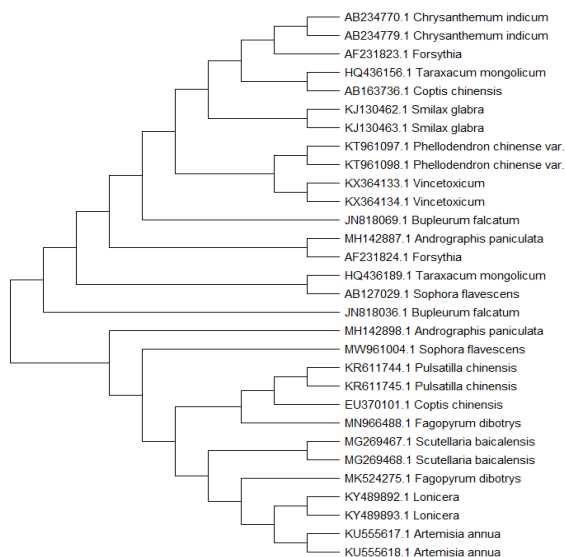
classification of drug action, the result of phylogenetic tree was the different classification result. And 2 sequences of the same plant may be divided into two clads, for example (AY599175.1 Ephedra and AB600681.1 Ephedra). The majority nucleotide sequences of 50 medicinal plants can be clustered on the same branch with the same plants of their network, and can be distinguished from their related plants.



**Fig1.** Phylogenetic tree of the 50 medicinal plants

### 3.3 Phylogenetic tree of 15 collected heat-clearing drugs

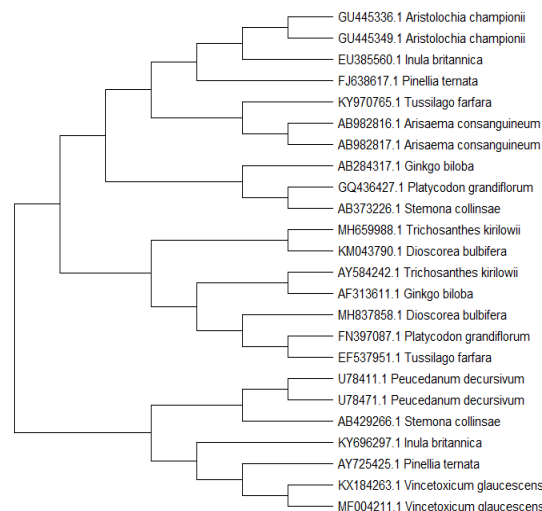
15 of 50 collected medicinal plants were divided into heat-clearing drugs which including *Pulsatilla chinensis*, *Andrographis paniculata*, *Fagopyrum dibotrys*, *Lonicera*, *Forsythia*, *Taraxacum mongolicum*, *Smilax glabra*, *Chrysanthemum indicum*, *Phellodendron chinense* var, *Coptis chinensis*, *Scutellaria baicalensis*, *Sophora flavescens*, *Vincetoxicum*, *Artemisia annua* and *Bupleurum falcatum*. Maximum Likelihood tree of the 15 plants was reconstructed to reveal genetic relationship (Figure 2). The result showed that 15 plants cluster into two clades. *Pulsatilla chinensis* was more closely related with *Fagopyrum dibotrys*, *Scutellaria baicalensis*, *Lonicera* and *Artemisia annua*. *Chrysanthemum indicum* was more closely related with *Forsythia*, *Taraxacum mongolicum*, *Smilax glabra*, *Phellodendron chinense* var, *Vincetoxicum*, and *Bupleurum falcatum*.



**Fig2.** Phylogenetic tree of 15 collected heat-clearing drugs

### 3.4 Phylogenetic tree of 12 expectorant cough suppressant and anti-asthmatic drug

12 of 50 collected medicinal plants were divided into expectorant cough suppressant and anti-asthmatic drug including *Vincetoxicum glaucescens*, *Pinellia ternate*, *Trichosanthes kirilowii*, *Dioscorea bulbifera*, *Platycodon grandiflorum*, *Peucedanum decursivum*, *Arisaema consanguineum*, *Inula Britannica*, *Ginkgo biloba*, *Stemona collinsae*, *Tussilago farfara* and *Aristolochia championii*. Maximum Likelihood tree of the 12 plants was reconstructed to reveal genetic relationship (Figure 3). The result showed that 12 plants cluster into two clades.

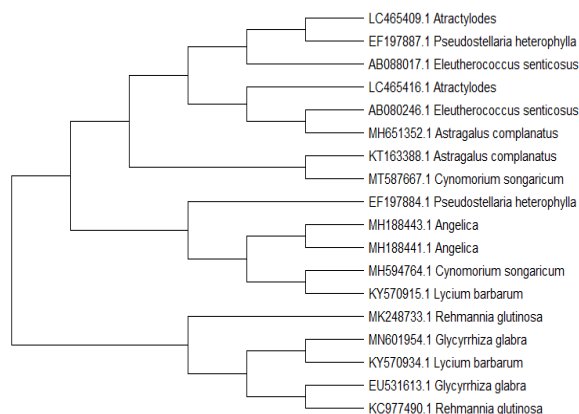


**Fig3.** Phylogenetic tree of 12 expectorant cough suppressant and anti-asthmatic drug

### 3.5 Phylogenetic tree of 9 tonify deficiency drug

9 of 50 collected medicinal plants were divided into Tonify Deficiency Drug including *Atractylodes*, *Eleutherococcus senticosus*, *Glycyrrhiza glabra*, *Pseudostellaria heterophylla*, *Angelica*, *Rehmannia glutinosa*, *Astragalus complanatus*, *Cynomorium songaricum* and *Lycium barbarum*.

Maximum Likelihood tree of the 9 plants was reconstructed to reveal genetic relationship (Figure 4). The result showed that 9 plants cluster into two clades. *Rehmannia glutinosa* was more closely related with *Glycyrrhiza glabra*. *Atractylodes* was more closely related with *Eleutherococcus senticosus*, *Pseudostellaria heterophylla*, *Angelica*, *Astragalus complanatus* and *Cynomorium songaricum*. Two sequences of *Lycium barbarum* were clustered into two clades.



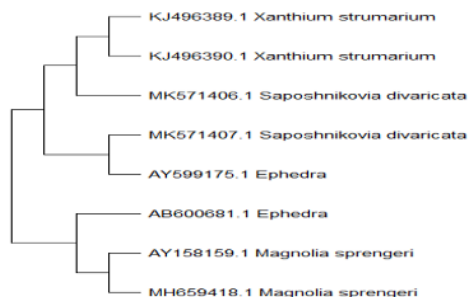
**Fig4.** Phylogenetic tree of 9 Tonify Deficiency Drug

### 3.6 Phylogenetic tree of 4 relieving exterior disorder drug

4 of 50 collected medicinal plants were divided into relieving exterior disorder drug including *Xanthium strumarium*, *Saposhnikovia divaricata*, *Ephedra* and *Magnolia sprengeri*. Maximum Likelihood tree of the 4

plants was reconstructed to reveal genetic relationship (Figure 5).

The result showed that 4 plants cluster into two clades ((*Xanthium strumarium*+ *Saposhnikovia divaricata*)+ *Magnolia sprengeri*). Two sequences of *Ephedra* were clustered into two clades.



**Fig5.** Phylogenetic tree of 4 relieving exterior disorder drug

## 4 DISCUSSION

The phylogenetic comparative methods were increasingly applied to analyze medicinal plants data with the main purpose of exploring the potential phylogenetic basis of traditional plant knowledge. In the study, phylogenetic tree was reconstructed to analyze the relationship of the collected medicinal plants in Shandong Province. In this study, we selected 50 medicinal plants because of their huge diversity, the accumulation of long-term experience, and significant preventive/curative effect. Previous studies tested the application of phylogenetics for exploring medicinal uses of plants but were restricted to the flora of specific regions and investigated only one or a few disease categories [8]. Hence, the phylogenetic exploration of medicinal plants at a large scale can help to screen or identify plants with high medicinal value. The collected medicinal plants were divided into 10 groups including Heat-clearing Drug, Expectorant Cough Suppressant and Anti-asthmatic Drug, Tonify Deficiency Drug, Relieving Exterior Disorder Drug, Discutient Drug, Cathartic Drug, Dispelling Internal Cold Drug, Digestant Drug, Sedative Drug and Blood-activating Drug. Maximum Likelihood tree showed that the majority nucleotide sequences of 50 medicinal plants can be clustered on the same branch with the same plants of their network, and can be distinguished from their related plants. Nucleotide sequences of minority medicinal plants were not clustered on the same branch. This may be related to sequencing differences or selected nucleotide sequences.

## 5 CONCLUSION

In the study, 50 medicinal plants were selected to analyze relationship. The phylogenetic tree showed that majority nucleotide sequences of 50 medicinal plants can be clustered on the same branch with the same plants of their network.

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