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Lycoris, the Basis of the Galanthamine Industry in China

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Commentary

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ABSTRACT

Alzherimer's disease is an age-related dementia with an estimated population of 44 million sufferers worldwide in 2013 and a number is expected to be 135 million in 2050. The patients exhibit a progressive decline in intellect including memory, learning, orientation, language, comprehension and judgement; they therefore need considerable care which has resulted in a heavy burden both on the health service and the patient family.

Although the mechanism of Alzherimer's disease is not very clear, clinical trials suggested that Galanthamine, one of the acetylcholinesterase inhibitors, can be used to slow the progression of the disease and mitigate many of the symptoms, and has a long-term positive effect on brain functional activities and cognitive stabilization. This in turn can improve quality of life, and allow patients to live more independently for longer.

Galanthamine, an alkaloid compound, is obtained from the plants which belong to the family of Amaryllidaceae like Lycoris, daffodil and snowflake. This article however aims to provide a brief overview of the galanthamine industry in China using Lycoris.

This article introduces the biology of Lycoris, resources and its distribution in China. Plant cultivation and propagation practices are reviewed and the alkaloids of Lycoris and galanthamine extraction techniques are summarized.

INTRODUCTION

Alzheimer's disease (AD) is an age-related dementia **(Table 1)** which is about 50-75% of all dementia cases. It changes the chemistry and histology of the brain which result in a progressive decline in intellect including memory, learning, orientation, language, comprehension and judgement **(Table 2)** [1,2].

Table 1. Consensus estimate of population prevalence of late onset dementia [1]

Age (years)	Female (%)	Male (%)	Total (%)
65–69	1.0	1.5	1.3
70–74	2.4	3.1	2.9
75–79	6.5	5.1	5.9
80–84	13.3	10.2	12.2
85–89	22.2	16.7	20.3
90–94	29.6	27.5	28.6
95+	34.4	30.0	32.5

The overall dementia population is large and growing at a considerable rate. In 2013, there were about 44 million dementia sufferers worldwide while there were 6.6 million in China, 5.5 million in America and over 830,000 in the UK about two thirds from AD. A number is predicted to be 135 million in 2050 worldwide with more than one new case of dementia in the world every seven seconds [2,4,5]. The patients need considerable care which has resulted in a heavy burden both on the health service and the patient family. It has been estimated that the yearly cost of dementia patients is about US$604 billion USD worldwide. In America, an annual cost of each patient is about $33,000, approximately three times the comparable costs for an older person without AD. While in the UK, an average of yearly cost of each dementia patient is £29,746 and the total cost is above £23 billion per annum [6].

Table 2. Stages and symptoms of Alzheimer's disease (AD) [3]

Mild: Early - stage AD	Moderate: Mid - stage AD	Severe: Late - stage AD
Mood swings	Confusion	Helplessness
Impaired mental ability	Uncontrolled feelings	Repetitive actions
Memory loss	Reduction in verbal communication	Rigidity of body
Changes to language	Delusional misjudgements	Increase in falls
Altered perception	Withdrawal	Loss of speech
Loss of abstract thinking	Sleep disturbance and agitation	Incontinence
Loss of judgement	Agitation	
	Mood changes - aggression	

Although the mechanism of AD is still not very clear, one of the main hypotheses has been suggested that cholinergic deficits are consistently detected in the brains of patent with AD, therefore, reducing the amount of acetylcholine (ACh) degradation in the brain will slow down even reverse the progression of the disease. The clinical trials suggested that although a class of drugs, called acetylcholinesterase (AChE) inhibitors cannot "cure" the disease, they do slow the progression of the disease and mitigate many of the symptoms. This in turn can improve quality of life, and allow patients to live more independently for longer [3,7]. Galanthamine, an alkaloid compound, is one of the AChE inhibitors which is being used in the treatment of Alzheimer's disease. It has dual mechanism against Alzheimer's disease. Apart from the function of AChE inhibition, it also allosterically modulates nicotinic ACh receptors, increasing the stimulatory effect of Ach. This dual action makes galanthamine a more attractive treatment than other currently available drugs [8].

Galanthamine is marketed in the UK as Reminyl. Its generic name, Galantamine hydrobromide (HBr) is known chemically as (4aS,6R,8aS)-4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro[3a,3,2-ef][2]benzazepin-6-ol hydrobromide. It is a white to almost white powder. There are mainly two types of products in the market, i.e. in prolonged release capsules with 8, 16 or 24 mg, and in colour coded tablets of 4 mg (yellow), 8 mg (dark pink) and 12 mg (red) equivalent of free galanthamine base [3]. There is also Dihydrogalanthamine hydrobromide injection with 6 or 12 mg/ml equivalent of free galanthamine base available in Chinese market [5]. Since galanthamine was gained FDA approval for clinical use in the early to mid-stage of Alzheimer's disease in February 2001 it has been launched in more than 70 countries around the world [5]. Apart from AD treatment, galantamine is also used in the treatment of poliomyelitis and other neurological diseases [5,9]. Therefore the demand for the compound has significantly increased.

Galanthamine is mainly produced from plants although the chemical synthesis has been successfully achieved. It is currently being extracted from daffodils (Narcissus in central and west Europe), Leucojum (in East Europe), Lycoris (in China) and Ungernia Victoria (in Uzbekistan and Kazakhstan) [9,10]. Another possible plant is Phaedranassa negistrophylla (synonyms: Rauhia multiflora Ravenna 1969) (in Peru) which could have 7.4% of galanthamine of dry based materials [11]. Yang et al. [12] reported that there are some other potential resources, such as Haemanthus albiflos, Hymenocallis littoralis and Crinum could also be used for galanthamine extraction. They, however, are not yet available for agricultural practices due to the limitation of resources and research studies. Lycoris is one of the important resources of plant materials to secure the supply chain for the pharmaceutical industry to produce sustainable and scalable galanthamine and other alkaloids. Therefore, this article aims to provide a brief overview of the galanthamine industry in China using Lycoris as raw materials which has been used as ornamental plant and one of the Traditional Chinese Medicines (de tumescence) for about 1,500 years [13,14].

LYCORIS BIOLOGY, RESOURCES AND DISTRIBUTION IN CHINA

Lycoris is a genus of about 20 species of perennial flowering and bulb producing plants which belongs to the family of Amaryllidaceae, formerly often treated in the family of Liliaceae. They are native to eastern and southern Asia in eastern and southern China, Japan, southern Korea, northern Vietnam, northern Laos, northern Thailand, northern Burma, Nepal, northern Pakistan, Afghanistan, and eastern Iran [15,16]. Chinese people often use the flowers as decorations in festivals or celebrations. Since when the flowers of Lycoris bloom, their leaves would have fallen; and when their leaves grow, the flowers would have wilted; Lycoris therefore got several beautiful names such as Magic Lily, Surprise Lily, Spider Lily etc. [14]. There are 18 species found in China, and 12 of them native to China. There are 13 species in JiangSu, 9 in Zhejiang, 7 in Anhui, and there are only 2-3 species distributed in other provinces in China. *L. radiata* and *L. aurea* are the most widely distributed species in China [17,18]. Detailed information about the description and distribution of the species are available online. http://web.archive.org/web/20010423083358/http://members.ozemail.com.au/~davcooke/lycoris.htm

The leaves are long and slender, about 30-60 cm long and 0.5-2 cm broad. The scape is erect, 30-70 cm tall, bearing a terminal umbel of four to eight flowers, which could be white, yellow, orange, or red. The flowers occur in two types, those very long, filamentous stamens two or three times as long as the tepals (e.g. *Lycoris radiata*), and those with shorter stamens not much longer than the tepals (e.g. *Lycoris sanguinea*). The fruit is a three-valved capsule containing several black subglobose seeds, and thousand seed weight is about 376 g. Many of the species are sterile, reproducing only vegetatively, and are probably of hybrid origin [14,17-20].

CULTIVATION AND PROPAGATION PRACTICES

Although ZhongShan Botanical Garden in NangJing first introduced *Lycoris aurea* as a horticultural crop in 1950s, only in recent years did other regions, such as JiangSu, JiangXi; Beijing, ShangHai, XiaMen, XiAn and SiChuan etc., start to study Lycoris cultivation techniques. Therefore, most of the harvest from the plants is still in the wild [18,19,21].

In practice, planting date will depend on the areas either in late September (warmer areas) or in March or early April (cooler areas). The rate of planting depends on the purposes of the planting. If it is for propagation and using year one bulblet (nursery garden), the planting rate could be up to 900 bulbs/m^2, while for flower cropping the optimum density is about 25 plants/m^2 [22]. It was suggested that the density of plants in cultivation for bulb production should be between 12-16 plants/m^2. Planting depth depends on the size of the bulb in which the neck of the bulb has to be above the soil surface. The soils should be well drained without standing water as the plant cannot grow in waterlogged conditions [22]. Bulbs are planted by hand as there is no machine for planting or lifting available in China [23].

Since both biomass and galanthamine level of Lycoris were enhanced under partial shade conditions (ca 35% shade) compared with no shade and heavily shade, some trees, such as, populous, Bischofis polycarpa (Level.) Airy Shau, Koelreuteria paniculate Laxm, Elaeocarpus sylvestri Poir and Alnus cremastogyne Burk were selected for producing these microclimate conditions [21]. And the optimum spacing for trees is 4 m x 4 m while the spacing for Lycoris is 10 cm x 15 cm [5]. This finding is supported by Yuan et al. [24] who reported that microclimate significantly affect the level of galanthamine.

The effects of fertilizers on plant growth and development were investigated in recent years. Nitrogen and potassium significantly increased bulb fresh weight 88% and 84.4% respectively [5]. Based on a pot trial, K significantly enhanced both plant growth (biomass and number of new bulbs) and galanthamine level, and suggested that 0.9 g/kg (K/soil) of potassium was the optimum level. It was shown however when K application above 1.2 g/kg (K/soil) both plant growth and galanthamine level were decreased (Figure 1). Nitrogen and phosphate fertilizers also significantly improved the biomass yield but not on the level of galanthamine [5,25]. However, high input of urea (>0.5 kg/m^{-2}) significantly reduced the biomass yield [26].

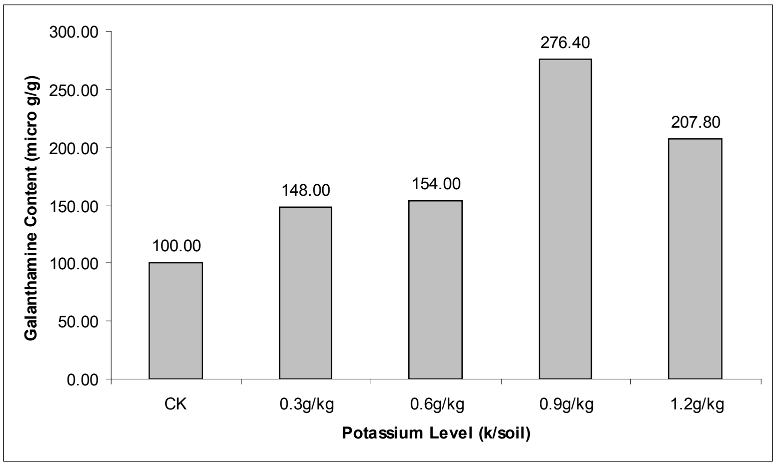

Figure 1. Galanthamine content of *L radiata* with different levels of potassium

Yield and the size of bulb were dramatically increased after four years of planting, particularly the years 3 and 4. Moisture content of the bulbs on another hand was significantly reduced **(Table 3)** [5].

Table 3. Yield and size of bulb of *L. radiata* in different years after 4 years of planting

Year of Growth	Bulb Diameter (cm)	Bulb Height (cm)	Fresh Weight (g)	Dry Weight (g)	Moisture Content (%)
1	1.26	2.34	2.89	0.63	78.20
2	2.64	3.39	15.17	4.61	69.61
3	3.63	4.30	33.56	13.76	59.00
4	4.16	4.38	44.33	23.55	46.88

It has been reported that five years after planting, the content of galanthamine reached peak level and it was about 20 fold of that of the first year **(Figure 2)**. From year three to year four, one year growth cycle, the peak level of galanthamine was the middle of the dormant period **(Figure 3)**. **Figure 3** also suggested that galanthamine content increased from 25.56 µg/g to 51.63 µg/g from year 3 to year 4 [5]. However, it was reported that the alkaloid content is different in plants planted in different areas due to differences in phenology [20].

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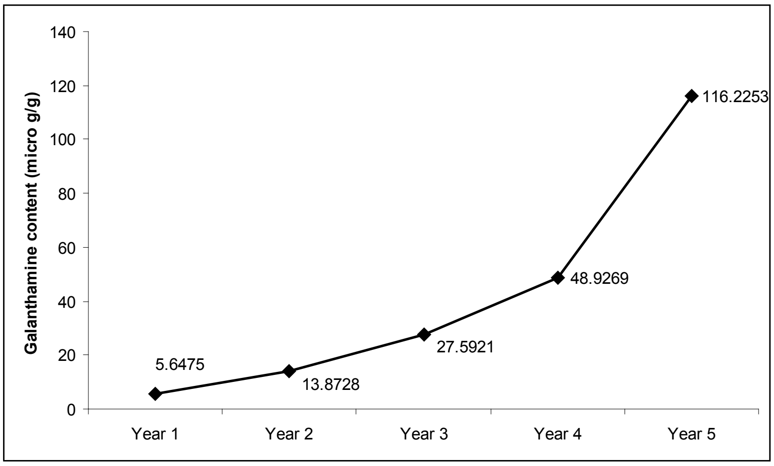

Figure 2. Galanthamine content of *L. radiata* bulb in different year after planting

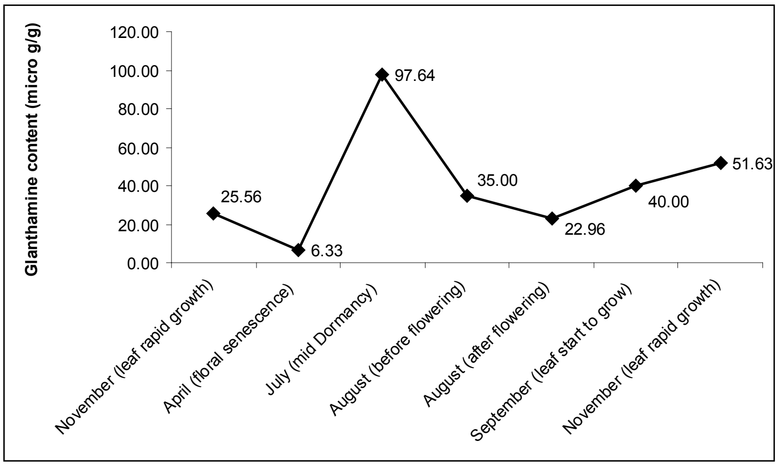

Figure 3. Galanthamine content of *L. radiata* bulb in different growth phases

The major problem for propagation to produce enough raw materials is the low natural multiplication rate which is ca 2.495-2.656 per annum [27]. Although the highest multiplication rate was obtained from *L. chinensis* which was 7.09, the variation was very high between 1 and 19 [5]. Therefore the effectiveness of propagation techniques has been developed in the last two decades particularly in the last ten years [19]. The macro-propagation of Lycoris, such as chipping has been investigated and suggested that cutting 4 or 8 chips per bulb were the best solutions [14,28,29]. Bulbs were cut while immersed in dilute Consan 20 solution. The bulbs were partitioned vertically cut into halves, then into quarters, and into eighths. The chips were then placed in trays or pots of vermiculite and perlite in a warm room at 25°C days and 20°C nights [28].

Whereas macro propagation techniques are simple and require minimal facilities, however they cannot produce the multiplication rates required for the effective bulking of a stock in a reasonable period. Therefore, micro-propagation became more than necessary. Since the first reported tissue culture of *L. radiata* in 1994, several reports have been published [14,19,29,30]. However, most reports focused on *L. aurea* [31] and/or *L. radiata* [5,30,32] and no mature and effective plant regeneration or transformation system has been established [29]. Yang et al. [5] reported that for inducing embryonic callus of *L. aurea*, the best constitution of the medium was: MS' + BA (5 mg/L) + KT (0.7 mg/L) + NAA (3 mg/L) + Peptone (50 mg/L) + PVP (30 mg/L) + VC (50 mg/L) + sugar (5%) + amoxicillin (25 mg/L). For multiplication and differentiation of embryonic callus of *L. aurea*, the best medium was: MS + BA (1.2 mg/L) + KT (0.5 mg/L) + NAA (1.8 mg/L) + PVP (30 mg/L) + VC (50 mg/L) + sugar (3%) + YE (100 mg/L).

CHEMICAL COMPOUNDS AND EXTRACTION TECHNIQUE DEVELOPMENT

There are more than 500 amaryllidaceae alkaloids have been isolated over the last three decades. Apart from galanthamine, there are some other alkaloids, such as, lycorine, narciclasine and haemanthamine have anti-cancer, anti-leukemia or anti-inflammatory properties [33]. In China, since 1960s researchers started to look at natural alkaloids from Lycoris for medicinal uses [19,34,35] **(Table 4)**. It was reported that the level of alkaloids and level of galanthamine are significantly different amongst different species [19,34] **(Table 5)**.

There are several conventional extraction techniques used, such as, impregnation extraction, percolation extraction, boiling extraction, Solvent Reflux Extraction (SRE) and continuous solvent reflux extraction method. Based on solubility, activity (no reaction with interested compounds), economy and safety, commonly used solvents are water; hydrophilic organic solvents such as ethanol, methanol, acetone, etc.; and lipophilic organic solvents such as petroleum spirit (ether), benzene, chloroform, ethyl

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ether, ethyl acetate, dichloroethane etc. Meanwhile, the degree of crushing of raw materials, extraction time, extraction temperature, pH value, equipment conditions and other factors which could affect the extraction efficiency, were also taken into account [24,37].

Table 4. Alkaloids in *Lycoris* plants [19, 34-36]

Compound	Species								
	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ	Ⅵ	Ⅶ	Ⅷ	Ⅸ
Allynorgalanthamine			+						
Crinine	+	+	+						
Demethylgalanthamine	+			+	+		+		
Galanthamine	+	+	+	+	+	+	+	+	+
Haemanthidine	+	+			+		+		+
Hippamine				+					
Hippeastrine	+	+			+	+	+		
Epigalanthamine	+			+	+	+	+		
Homolycorine	+	+		+		+	+		
Lycoramine	+	+	+	+	+	+	+		
Lycorenine	+			+	+	+	+		
Lycoricidine	+			+			+	+	
Lycoricidinol		+					+	+	
Lycoricidinoside									
Lycorine	+	+	+	+	+	+	+	+	+
Narciclasine		+		+					
Norpluviine				+		+	+		
Narwedine			+						
Pluviine	+	+		+		+	+		
Pseudolycorine	+		+	+		+	+	+	
Squamigerine				+		+	+		
Tazettine				+		+	+		
Vittatine						+			
O-demethyllycoramine	+	+					+		
Haemanthamine	+								
O-methyllycorenine	+								
Lycosinine A	+								
Lycosinine B	+								
Pretazettine							+		
Isotazettine							+		
Demethylhomolycorine							+		
Lycorinine							+		
Ethyllycorinine							+		
Norgalanthamine									
Norsanguinine								+	
Norbutsanguinine								+	
Sanguinine				+				+	
Hippadine								+	
Galanthine								+	

Note: Ⅰ. *L aurea;* Ⅱ. *L. chinenesis;* Ⅲ. *L. guangxiensis;* Ⅳ. *L. longituba;* Ⅴ. *L. sprengeri;* Ⅵ. *L. squamigera;* Ⅶ. *L. radiata;* Ⅷ. *L. sanguinea;* Ⅸ. *L. incarnata*

Table 5. Comparison of three key compound contents of some *Lycoris* and Chinese daffodil [19, 34]

Materials	Content of compounds (mg/g, dry based)		
	Lycorine	Lycoramine	Galanthamine
L. albiflora	3. 31 ± 0. 04	0. 67 ± 0. 02	5. 03 ± 0. 13
L. anhuiensis BG	3. 14 ± 0. 18	1. 24 ± 0. 04	2. 56 ± 0. 18
L. anhuiensis LYS	3. 61 ± 0. 32	2. 20 ± 0. 26	3. 46 ± 0. 21
L. aurea	5. 59 ± 0. 29	0. 20 ± 0. 01	2. 24 ± 0. 10
L. chinensis	4. 53 ± 0. 16	0. 70 ± 0. 01	1. 73 ± 0. 03
L. haywardii	2. 65 ± 0. 39	1. 94 ± 0. 09	0. 43 ± 0. 02
L. houdyshelii	2. 78 ± 0. 31	0. 64 ± 0. 04	0. 30 ± 0. 01
L. incarnata	8. 36 ± 0. 35	0. 75 ± 0. 01	2. 39 ± 0. 01
L. longituba BG	1. 84 ± 0. 11	1. 80 ± 0. 14	2. 24 ± 0. 06
L. longituba JN	1. 89 ±0. 25	1. 14 ±0. 14	5. 15 ±0. 28

L. radiata	4. 02 ±0. 16	1. 58 ±0. 07	0. 56 ±0. 04
L. radiata var. pumila	3. 07 ±0. 02	1. 53 ±0. 09	0. 18 ±0. 01
L. rosea	2. 27 ±0. 25	2. 94 ±0. 15	0. 53 ±0. 06
L. sprengeri	3. 57 ±0. 12	2. 62 ±0. 19	0. 86 ±0. 03
L. squamigera	4. 82 ±0. 37	1. 31 ±0. 09	1. 74 ±0. 03
L. straminea	3. 27 ±0. 41	0. 78 ±0. 11	0. 36 ±0. 02
N. tazetta L. var. chinensis	3. 79 ±0. 10	0. 28 ±0. 01	0. 46 ±0. 01

The most popular used method is solvent (continuous) reflux extraction in which ethanol (75-95%) is used as the solvent and solid/liquid ratio is 1/6 to 1/20. The optimum temperature is between 85 and 95°C and pH is 5 [24,36-40]. Microwave-Assisted Extraction method has been developed in recent years [41-44], and the Closed Microwave-Assisted Extraction is being used in most of the research however the container is only enough for small quantity extractions. Therefore, Post-Microwave Irradiated Reflux Extraction (PMIRE) technique has been developed and commonly used for a bigger quantity extraction. It is much quicker and efficient than SRE. In this method before solvent extraction the materials were treated by 300 W microwave (the length of time depends on quantity) [24,45-47]. In the last two decades, quite a few patents about the process of galanthamine production in terms of extraction, crystallization and purification on daffodil, Leucojum and Lycoris have been filed and granted; however they cannot be freely used.

FUTURE WORKS

It has been suggested that *L. longituba* and *L. albiflora* have the highest level of galanthamine, however, due to the natural distribution, most of the research projects focused on *L. aurea* and *L. radiata*. Only a few papers researched into other species, e.g. Zhao et al. [14] investigated plant growth and development of eight species of Lycoris, i.e. *L. sprengeri*, *L. longituba*, *L. anhuiensis*, *L. aurea*, *L. chinensis*, *L. radiata diploid, L. albiflora, L. haywardii* and *L. incarnata*, to generate a guide for further cultivation practices. Therefore, it is important to establish Gene banks (germplasm collections) and conduct genetic diversity studies for some specific purposes, such as breeding.

Another essential task is to establish Lycoris regeneration and genetic transformation system to solve the breeding and propagation problems to establish a sustainable supply chain [5,17,29,48]. With the development of cultivation and propagation practices for establishing a sustainable supply chain, suitable planting and lifting machines should be developed, encouraging and feasible innovation for vast scale creation of plants.

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