Biotechnology & Biotechnological Equipment

ISSN: 1310-2818 (Print) 1314-3530 (Online) Journal homepage: https://www.tandfonline.com/loi/tbeq20

Plant Sources of Galanthamine: Phytochemical and Biotechnological Aspects

S. Berkov, L. Georgieva, V. Kondakova, A. Atanassov, F. Viladomat, J. Bastida & C. Codina

To cite this article: S. Berkov, L. Georgieva, V. Kondakova, A. Atanassov, F. Viladomat, J. Bastida & C. Codina (2009) Plant Sources of Galanthamine: Phytochemical and Biotechnological Aspects, Biotechnology & Biotechnological Equipment, 23:2, 1170-1176, DOI: 10.1080/13102818.2009.10817633

To link to this article: https://doi.org/10.1080/13102818.2009.10817633

PLANT SOURCES OF GALANTHAMINE: PHYTOCHEMICAL AND BIOTECHNOLOGICAL ASPECTS

S. Berkov[1], L. Georgieva[2], V. Kondakova[2], A. Atanassov[2], F. Viladomat[1], J. Bastida[1], C. Codina[1]
University of Barcelona, Faculty of Pharmacy, Department of Natural Products, Plant Biology and Soil Science, Barcelona, Spain[1]
AgroBioInstitute, Sofia, Bulgaria[2]
Correspondence to: Violeta Kondakova
E-mail: violeta.kondakova@gmail.com

ABSTRACT

Galanthamine, an Amaryllidaceae type alkaloid, is an AChE inhibitor marketed as a hydrobromide salt for the treatment of Alzheimer's disease, poliomyelitis and other neurological diseases. Although the chemical synthesis of galanthamine has been successfully performed, plants are the main source of its production. The phytochemical and biotechnological aspects of plants currently used for galanthamine production, namely Leucojum aestivum, Narcissus ssp. Ungernia victoris and Lycoris radiata, are summarized in the present paper.

Keywords: Galanthamine, *in vitro* biosynthesis, plant sources, extraction

Introduction

Four acetylcholinesterase inhibitors have received approval for clinical use in early- to mid-stage Alzheimer's disease: tacrine, donepezil, rivastigmine and galanthamine. Tacrine was the first to gain FDA approval in 1993 but has been largely withdrawn due to adverse side effects. The three remaining drugs, donepezil, rivastigmine and galanthamine, have fewer adverse side-effects and appear relatively safe for general use (50).

Galanthamine, an Amaryllidaceae type alkaloid, is a long acting, selective, reversible and competitive AChE inhibitor (35), which is marketed as a hydrobromide salt under the name of Razadine® (formerly Reminyl®) and Nivalin® for the treatment of Alzheimer's disease, poliomyelitis and other neurological diseases (25). After its discovery in 1952 by Proskurina and Yakovleva in *Galanthus woronowii* (39, 40), the pharmacological properties of galanthamine soon attracted the attention of the pharmaceutical industry. It was first produced by Sopharma (Bulgaria) under the name of Nivalin®. *G. nivalis* plants were used in the early 1960s for industrial extraction but due to their small size and variability of galanthamine content, this species was soon replaced by the considerably bigger *L. aestivum* (49). Galanthamine has been found in many plants from the genera *Amaryllis*, *Hippeastrum*, *Lycoris*, *Ungernia*, *Leucojum*, *Narcissus*, *Galanthus*, *Zephyranthes*, *Hymenocallis*, and *Haemanthus* (15). It is currently being extracted from daffodils (*Narcissus* cultivars) in central and west Europe, snowflake (*Leucojum aestivum)* in East Europe, 'red-tubed lily' (*Lycoris radiata*) in China, and *Ungernia victoria* in Uzbekistan and Kazakhstan.

Although the chemical synthesis of galanthamine has been successfully achieved (19), plants remain the main source of this important natural product. Due to the increased demand for galanthamine and the limited availability of plant sources, *in vitro* culture of galanthamine-producing species has attracted the attention of researchers as an alternative approach for its sustainable production (17, 38).

During the past 30 years considerable efforts have been made to establish *in vitro* production of secondary plant metabolites, mainly using undifferentiated callus or suspension cell cultures that allow scale-up in bioreactors. However, commercial success has been very limited mainly because most secondary metabolites are produced only in very low amounts in undifferentiated cells: moreover, the production capacity of cell lines often declines over time or during scale-up (12).

The production cost of galanthamine is determined by the quality of plant row materials, namely their galanthamine content and its percentage in the alkaloid mixtures. These phytochemical characteristics of the plant sources influence the efficiency of extraction technology and therefore the production costs. Other galanthamine type alkaloids such as narwedine or *N*-demethylgalanthamine are of interest since they could be used as precursors for galanthamine synthesis.

This article aims to provide a brief overview of the phytochemical and biotechnological studies of the current plant sources of galanthamine as well as some future prospects for its production from natural sources.

Leucojum aestivum

Leucojum aestivum L. (snowflake) is a plant species distributed in the Mediterranean region and eastern Europe. The leaves are 30–60 cm long and the bulb 2–4 cm in diameter. It is currently used for the extraction of galanthamine, mainly under licence in Bulgaria. It is gathered from natural habitats, which is causing problems with depletion of wild populations. A plantation of *L. aestivum* has been created in Bulgaria to supply the industry with leaf biomass, which is used for extraction. The galanthamine content in the leaves, referred to DW, was

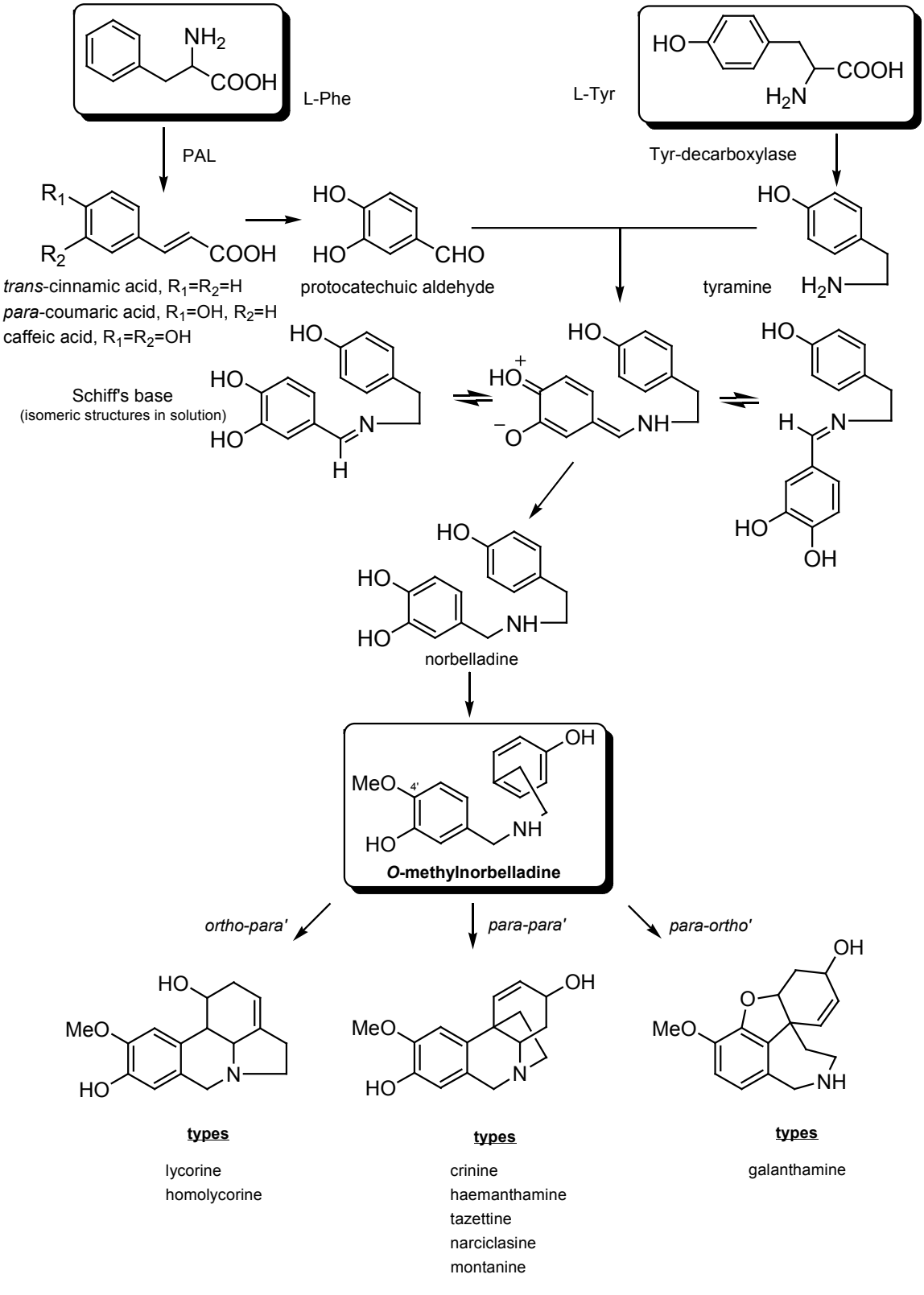

Fig. 1. Phenolic oxidative coupling of *O*-methylnorbelladine in the biosynthesis of Amaryllidaceae alkaloids.

found to vary from trace amounts to 0.5% (generally 0.1-0.3%) depending on the geographical location of the populations (46). The alkaloid synthesis of *L. aestivum* populations can be dominated by different skeleton types (galanthamine, lycorine, homolycorine and haemanthamine) in relation to their origin (22, 46), reflecting the quality of the plant material. South-east Bulgarian populations of snowflake were found to be the richest in galanthamine, dominated by galanthamine type synthesis (46). In contrast, plants collected in Romania were dominated by lycorine type compounds, yielding 0.26% of lycorine and 0.19% of galanthamine (referred to DW) (23). Phytochemical studies have revealed the occurrence of about 36 alkaloids in the alkaloid mixtures of *L. aestivum* (5, 9, 10, 13, 22, 29, 30, 41, 42, 46; **Table 1**), which also show geographical variations in their distribution. As an example, leucotamine and methylleucotamine (29), two galanthamine type alkaloids, were isolated from plants grown in Japan, but they were not detected in plants collected in Bulgaria (9, 22, 46). Apart from galanthamine, three other alkaloids with a higher AChE inhibitory activity than galanthamine, namely sanguinine, *N*-allylnorgalanthamine and *N*-(14-methylallyl) norgalanthamine, have been isolated from snowflake (10, 13, 22). The percentage of galanthamine in the alkaloid mixture of leaves was reported for one population (12%) (9). Galanthamine was found to vary from 4 to 99 % of all compounds in the alkaloid mixtures of dormant bulbs from eighteen Bulgarian populations (22).

***Narcissus* ssp.**

Bulbs of *Narcissus* plants are used as material for extraction of galanthamine. More than one hundred cultivars and different species of *Narcissus* have been screened for this alkaloid, 33 of them having showed more than 0.1% (referred to DW) of galanthamine in their bulbs (16). The cultivar 'Favourite' was found to accumulate 0.15% of galanthamine. Another promising cultivar, 'Ice Follies', was reported to yield 70 mg of galanthamine from 100g of DW (36). The main commercial source of galanthamine, however, is *N. pseudonarcissus* cv. 'Carlton' due to its bulb size (4-5 cm in diameter) and high galanthamine content, which is about 0.10-0.13 % (32). It is commercially cultivated in the UK and Netherlands by various bulb growers.

Despite intensive phytochemical investigations, data on the proportion of galanthamine (and the other alkaloids) in the crude alkaloid mixtures of *Narcissus* cultivars are scarce. Kreh et al. (33) reported 58 % of galanthamine in a bulb alkaloid mixture from *N. pseudonarcissus* cv. 'Carlton' based on a GC-MS study. These authors also reported 23 alkaloids of galanthamine, lycorine, homolycorine and haemanthamine types in the bulbs of this cultivar (**Table 1**). Overall, more than one hundred alkaloids have been found in plants of the genus *Narcissus* (7).

One of the most promising species for cultivation is *N. confusus* - an endemic plant found only in Spain, which has been found to contain around 0.6 % (referred to DW) of galanthamine (6). In a preliminary assay with plants transferred to a greenhouse, the alkaloid pattern of *N. confusus* was studied throughout its phenological cycle (34). What is most interesting in this plant species is the high level of galanthamine accumulated in the total aerial parts of the plant (leaves, stems and flowers) at the end of the ontogenic cycle, which can reach up to 2.5 % referred to DW.

Ungernia victoris

Ungernia victoris Vved. is an endemic plant that is widespread only on the Gissar Ridge and its southern spurs (Tadzhikistan and Uzbekistan). It is a perennial species with leaves up to 20-25 cm long and bulbs 4-7 cm in diameter (47). This plant has been cultivated since 1970 in locations near its natural habitats. The leaves and bulbs have been used as a plant raw material for galanthamine extraction since 1960 (52). The total alkaloids in the leaves and bulbs were found to be 0.27-0.71% and 1.18-1.65 % of DW, respectively. The proportion of galanthamine in the alkaloid mixtures was around 56-57% and 47-48% in the foliage and bulbs, respectively (43). Its accumulation, depending on growing conditions (5), reached up to 0.52% of DW in cultivated plants, which yielded 20-25% more leaf mass compared to wild populations (27). About 10 alkaloids have been reported in this plant species (1, 2, 3, 43, 51, 53; **Table 1**).

Lycoris radiata

Lycoris radiata Grey is a plant species distributed in China, Japan and Korea. The bulbs are 1-3 cm in diameter, while the leaves are up to 50 cm long. Two varieties of *L. radiata* have been described: var. *pumila* which has a diploid genome, and var. *radiata*, with a triploid genome (24). Despite the high amount of work, found by SciFinder Scholar™ 2007 (American Chemical Society; accessed November 2008), mainly in Chinese journals, it was not possible to obtain information on the galanthamine content of this plant species. Twenty-two alkaloids have been reported to occur in *L. radiata* (11, 14, 26, 28, 31, 37, 48; **Table 1**). Since 2002, this plant has been cultivated in China for galanthamine extraction (http://www.galanthamine.cn/plantation.php).

***In vitro* production of galanthamine**

Only two plant species have been studied for their *in vitro* galanthamine production, *N. confusus* and *L. aestivum*. Callus induction is a key point both for micro-propagation and obtaining different methods for alkaloid production. However, the degree of cell and tissue differentiation, strongly influences the galanthamine content in these two species (38, 44), and the lowest levels of galanthamine have been found in calli: 12 ug/g for *L aestivum* and 0.03 ug/g for *N. confusus* both referred to dry weight. As shoot-clump cultures have a higher ability to synthesise this compound, they have been used as a model system for studies on *in vitro* galanthamine production (8, 9, 38, 44, 45). The galanthamine content in shoot-clumps was found to vary when the cultures were cultivated on nutrient media with different combinations of phytohormones (20). Content

TABLE 1

Alkaloids found in the galanthamine plant sources

Compounds	*Leucojum aestivum*	*N. pseudonarcissus cv. Carlton*	*Lycoris radiata*	*Ungernia victoris*
Galanthamine type				
Galanthamine (**1**)	+	+	+	+
Galanhamine-*N*-oxide (**2**)			+	
Epigalanthamine (**3**)	+	+		
N-Demethylgalanthamine (**4**)	+	+		
Sanguinine (**5**)	+			
Leucotamine (**6**)	+			
3-*O*-Methyleucotamine (**7**)	+			
Narwedine (**8**)	+	+		+
Lycoramine (**9**)	+	+	+	
Lycoramine isomer (**10**)	+	+		
Lycoramine-*N*-oxide (**11**)			+	
Epinorlycoramine (**12**)		+		
O-Demethyllycoramine (**13**)			+	
N-Formylnorgalanthamine (**14**)	+			
N-Formylnorgalanthamine isomer (**15**)	+			
N-Allylnorgalanthamine (**16**)	+			
N-(14-Methylallyl)norgalantamine (**17**)	+			
Lycorine type				
Lycorine (**18**)	+		+	+
Ungiminorine (**19**)	+			
3-*O*-Acetylungiminorine (**20**)	+			
Pluvine (**21**)	+		+	
O-Acethylpluvine (**22**)	+			
10-*O*-Demethylpluvine (**23**)		+		
1-*O*-Acetyl-10-*O*-demethylpluvine (**24**)		+		
Dihydrolycorine (**25**)	+		+	
Pseudolycorine (**26**)			+	
Anhydrolycorine (**27**)	+			
O-Acetylcaranine (**28**)	+			
Homolycorine type				
Ungerine (**29**)	+			+
Ungeridine (**30**)				+
Homolycorine (**31**)	+	+	+	
8-*O*-Demethylhomolycorine (**32**)	+		+	

Homolycorine-*N*-oxide (**33**)			+	
Lycorenine (**34**)	+			
O-Methyllycorenine (**35**)		+	+	
O-Methyllycorenine isomer(**36**)		+		
O-Methyllycorenine-*N*-oxide (**37**)			+	
O-Ethyllycorenine (**38**)		+		
Hippeastrine (**39**)	+		+	+
Hippeatrine-*N*-oxide (**40**)			+	
Oduline (**41**)		+		
O-Methyloduline (**42**)		+		
Masonine (**43**)		+		
N-Demethylmasonine (**44**)		+		
Haemanthamine type				
Elwesine (**45**)	+			
Vittatine (**46**)	+	+	+	
11-Hydroxyvittatine (**47**)	+			
Haemanthamine (**48**)	+	+		
11-Oxohaemanthamine (**49**)		+		
8-*O*-Demethylmaritidine (**50**)	+	+		
Haemanthidine (**51**)			+	+
Tazettine type				
Pretazettine (**52**)	+		+	
Tazettine (**53**)	+		+	+
Nortazettine (**54**)				+
Macronine (**55**)	+			
Narciclasine type				
Trisphaeridine (**56**)	+			
Tyramine type				
Hordenine (**57**)	+			+
Methyltyramine (**58**)	+			

of galanthamine also varied in shoot-clumps grown on a sole nutrient medium but induced from different wild populations (22) or calli (38). It was found that the light increases the galanthamine synthesis in a shoot-clump of *L. aestivum* cultivated in a liquid medium (38). Galanthamine content in *L. aestivum* shoot-clumps ranges from traces to 0.5 mg/g of DW (22, 38) and up to 2.5 mg per culture of *N. confusus*, of which 1.97 mg is released into the medium (8). The galanthamine proportion in alkaloid mixtures of *L. aestivum* shoot-clumps varies greatly among different lines, sometimes consisting almost 100 %. Another major alkaloid in *L. aestivum* shoot-clumps, lycorine, can reach up to 77% of total alkaloids (9, 22). Recently initiated bioreactor experiments have shown a galanthamine yield of 2.5 mg/L (38). Increase of sucrose concentration (44) and addition of biotic elicitors (18) resulted in a *ca.* 300% increase of the galanthamine content in *N. confusus* shoot-clumps. Transgenic cultures of *L. aestivum* have been successfully obtained, but galanthamine was not found in them (20).

Conclusions

The introduction of new cultivars with a high galanthamine content and the development of biotechnologies both for *in vitro* production and crop improvement are highly desirable for

pharmaceutical companies to increase their competitiveness in the market. Although European and Asian amaryllidaceous plants are relatively well studied, the highest level of biodiversity in the Amaryllidaceae family is found in South America and South Africa, where the plants have scarcely been studied. The phytochemical investigations of plants used for galanthamine extraction have revealed complex and variable alkaloid patterns, which may be used to control the plant source of galanthamine substances. The introduction of crops with a higher proportion of galanthamine in the alkaloid mixtures is also of interest because it will facilitate the isolation and purification of this compound and could reduce the extraction costs. In this respect, genetic manipulation of the biosynthetic pathway leading to products coming from *para-orto'* oxidative coupling of *O*-methylnorbelladine (**Fig. 1**) in both crops and *in vitro* cultures is still an unexplored field. The *in vitro* studies of galanthamine synthesis are in their initial stages. The introduction of other galanthamine-producing species and selection of galanthamine-rich genotypes should be performed together with optimization of conditions for *in vitro* bioreactor cultivation.

REFERENCES

1. **Abdusamatov A., Abduzaimov K., Yunusov S.** (1962) Uzbekskii Khimicheskii Zhurnal, **6**, 45-55.
2. **Abdusamatov A., Abduzaimov K., Yunusov S.** (1962) Doklady Academii Nauk UzSSR, **19**, 45-47.
3. **Abdusamatov A., Khamidkhodzhaev S., Yunusov S.** (1971) Khim. Prirod. Soed., **7**, 60-64.
4. **Astadzhov N., Dimitrov I., Zachev S., Deneva T., Dgurmanski G., Slatev S., Todorov M., Paskalev G.** (1980) In: The Perspective of Medical Plants. Jusautor, Sofia, 56-74.
5. **Babashkin V.** (1983) Nauchnye Trudy Vsesoyuznyi Nauchno-Issledovatelskii Institut Farmatsii, **20**, 68-75.
6. **Bastida J., Viladomat F., Llabrés J.M., Codina C., Feliz M., Rubiralta M.** (1987) Phytochemistry, **26**, 1519-1524.
7. **Bastida J., Lavilla R., Viladomat F.** (2000) In: The Alkaloids (Cordell G., Ed.), Elsevier Scientific Publishing, Amsterdam, Vol. **63**, 87-179.
8. **Bergoñón S., Codina C., Basida J., Viladomat F., Melé E.** (1996) Plant Cell, Tissue and Organ Culture, **45**, 191-199.
9. **Berkov S., Pavlov A., Ilieva M., Burrus M., Popov S., Stanilova M.** (2005) Phytochem. Anal., **16**, 98-103.
10. **Berkov S., Codina C., Viladomat F., Bastida J.** (2008) Bioorg. Med. Chem. Lett., **18**, 2263-2266.
11. **Boit H., Ehmke H., Uyeo S., Yajima H.** (1957) Chemische Berichte, **90**, 363-368.
12. **Bourgaud F., Gravot A., Milesi S., Gontier E.** (2001) Plant Sci., **161**, 839-851.
13. **Capo M., Saa J.** (1989) Quimica Organica y Bioquimica, **5**, 119-121.
14. **Chen B., Du Z., Zeng F., Hu C., Ma G., Hong S.** (1993) Zhongguo Yaoli Xuebao, **14**, 45-49.
15. **Cherkasov O.** (1977) Khim-Farm. Zhur., **11**, 84-87.
16. **Cherkasov O. and Tokachev O.** (2002) In: Medicinal and Aromatic Plants – Industrial Profiles: *Narcissus* and Daffodil, The Genus *Narcissus*, (Hanks G., Ed.). Taylor and Francis, London and New York, 242-255.
17. **Codina C.** (2002) In: Medicinal and Aromatic Plants – Industrial Profiles: *Narcissus* and Daffodil, The Genus *Narcissus*, (Hanks G., Ed.). Taylor and Francis, London and New York, 215-241.
18. **Colque R., Viladomat F., Bastida J., Codina C.** (2004) Planta Med., **70**, 1180-1188.
19. **Czollner L., Frantsits W., Kuenburg B., Hedenig V., Jordis U., Froehlich J.** (1998) Tetraheron Letters, **39**, 2087-2088.
20. **Diop M.F., Ptak A., Chretien F., Henry M., Chapleur Y., Laurain-Mattar D.** (2006) Nat. Prod. Commun., **1**, 475-479.
21. **Diop M.F., Hehn A., Ptak A, Chretien F., Doerper S., Gontier E., Bourgaud F., Henry M., Chapleur Y., Laurain-Mattar D.** (2007) Phytochem. Rev., **6**, 137-141.
22. **Georgieva L., Berkov S., Kondakova V., Bastida J., Viladomat F., Atanassov A., Codina C.** (2007) Z. Naturforsch., **62c**, 627-635.
23. **Gheorghiu A., Ionescu-Maiu E.** (1962) Ann. Pharm. Franc., **20**, 531-538.
24. **Hayashi A., Saito T., Mukai Y., Kurita S., Hori T.** (2005) Genes Genetic Systems, **80**, 199-212.
25. **Heinrich M., Teoh H.** (2004) J. Ethnopharmacol., **92**, 147-162.
26. **Hung S-H., Ma K-E.** (1964) Yaoxue Xuebao, **11**, 1-14.
27. **Khamidkhodzhaev S.,** (1980) Uzbeksii Biologicheskii Zhurnal, 42-44.
28. **Kihara M., Konishi K., Xu L., Kobayashi S.** (1991) Chem. Pharm. Bull., **39**, 1849-1853.
29. **Kintsurashvili L., Chkhikvadze G., Vachnadze V.** (2000) Izvestiya Akademii Nauk Gruzii, **26**, 194-195.
30. **Kobayashi S., Kihara M., Yyasa K., Imakura Y., Shingu T., Kato A., Hashimoto T.** (1985) Chem. Pharm. Bull., **33**, 5258-5263.
31. **Kobayashi S., Yuasa K., Imakura Y., Kihara M., Shingu T.** (1980) Chem. Pharm. Bull., **28**, 3433-3436.
32. **Kreh M.** (2002) In: Medicinal and Aromatic Plants – Industrial Profiles: *Narcissus* and Daffodil, The Genus *Narcissus*, (Hanks G., Ed.). Taylor and Francis, London and New York, 256-271.
33. **Kreh M., Matush R., Witte L.** (1995) Phytochemistry, **38**, 773-776.

34. **López S., Bastida J., Viladomat F., Codina C.** (2003) Planta Med., **69**, 1166-1168.

35. **Maelicke A., Samochocki M., Jostok R., Feherbacher A., Ludwig J., Albuquerque E.X., Zerlin M.** (2001) Biol. Psychiatry, **26**, 279-288.

36. **Moraes R.** (2002) In: Medicinal and Aromatic Plants – Industrial Profiles: *Narcissus* and Daffodil, The Genus *Narcissus*), (Hanks G., Ed.). Taylor and Francis, London and New York, 273-285.

37. **Numata A., Takemura T., Ohbayashi H., Katsuno T., Yamamoto K., Sato K., Kobayashi S.** (1983) Chem. Pharm. Bull., **31**, 2146-2149.

38. **Pavlov A., Berkov S., Courot E., Gocheva T., Tuneva D., Pandova B., Georgiev V., Yanev S., Burrus M., Ilieva M.** (2007) Process Biochem., **42**, 734-739.

39. **Proskurina N.F., Yakovleva A.P.** (1952) Zurnal Obshchei Khimii, **22**, 1899-1902.

40. **Proskurina N.F., Yakovleva A.P.** (1955) Zurnal Obshchei Khimii, **25**, 1035-1039.

41. **Proskurina N.F.** (1957) Zhurnal Obshchei Khimii, **27**, 3365-3367.

42. **Proskurina N.F.** (1963) Zhurnal Obshchei Khimii, **33**, 1689-1690.

43. **Sadykov Y., Khodzimatov M.,** (1988) Rastitelnie Resursy, **24**, 410-414.

44. **Sellés M., Bergoñón S., Viladomat F., Bastida J., Codina C.** (1997) Plant Cell Tissue and Organ Cultures, **49**, 129-136.

45. **Sellés M., Viladomat F., Bastida J., Codina C.** (1999) Plant Cell Rep., **18**, 646-651.

46. **Stefanov J.** (1990) Ecological, biological and phytochemical studies on natural populations and introduced origins of snow flake (*Leucojum aestivum* L.) in Bulgaria. D.Sc. Thesis, Sofia.

47. **The red data book of republic Uzbekistan.** Volume I – Plants, (http://econews.uz/econews/rus/lib/RedBook/HTMLs_ANG/197.htm).

48. **Uyeo S., Kotera K., Okada T., Takagi S., Tsuda Y.** (1966) Chem. Pharm. Bull., **14**, 793-794.

49. **Valkova A.** (1961) Farmacia, **11**, 17-22.

50. **Viegas C., Bolzani V., Barriero E., Fraga C.** (2005) Mini-Reviews in Medicinal Chemistry, **5**, 915-926.

51. **Volodina A., Dobronravova E., Shakirov T.** (1970) Khimya Prirodnykh Soedinenii, **6**, 450-453.

52. **Yunusov S., Abduazimov K.** (1960) Galanthamine hydrobromide. Patent SU 128111.

53. **Zakirov U., Nasirov S., Kamilov I.** (1966) Meditsinskii Zhurnal Uzbekistana, **12**, 18-19.