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Studies on species of the *Solanaceae*, with an enphasis on *Withania somnifera*

Abstract

Nicoletti, M.:Studies on species of the *Solanaceae*, with an enphasis on *Withania somnifera*. – Bocconea 13: 251-255. 2001. – ISSN 1120-4060.

The family of *Solanaceae* includes about 90 genera and over 2000 species. Among them a great number of important plants has agronomic and pharmaceutical uses. They contain alkaloids and triterpenoids as main active constituents. Among the last compounds, it is important to consider a group of compounds specific to *Solanaceae*, the withanolides, present in particular in *Withania somnifera*. *W. somnifera* is reported to have several properties: analgesic, antipyretic, anti-inflammatory, abortificient, but in particular it is well known and used in the Indian traditional medicine as a tonic. Our research on *W. somnifera* was prompted by the possibility of studying the Italian plants of *W. somnifera* and verifying their chemotype on the basis of withanolide composition. These plants were repropagated by *in vitro* culture, but only traces of withanolides. with prevalence of withanolide L, were detected by HPLC in the regenerated tissues.

Introduction

The family of *Solanaceae* includes about 90 genera and over 2000 species. Among them a great number of important plants has agronomic and pharmaceutical uses, such as potato, tobacco, pepper, deadly nightshade, poisonous jimsonweed. Many species are well known for their contents of alkaloids of many different types which generally have chemotaxonomic bearings. A wide distribution is registered for tropane alkaloids, that comprise the well known group of esters of monohydroxytropane, like hyoscine and hyosciamine/atropine, as well as the more recent calystegines, polyhydroxy-nor-tropanes, mainly present in *Duboisia* and *Solanum* and at the present under study for their antifeedant and insecticide activities (Guntli 1999).

Another important class of natural constituents of Solanaceae is constituted by triterpenoids, including steroid alkaloids, glycoalkaloids and several steroidal saponines. Among the last compounds, it is important to consider a group of compounds specific to the Solanaceae, the withanolides, ergostane-type steroidal 26-22 lactones, present in particular in the genera *Physalis* and *Withania*, from which in fact they derive their name. The distribution of withanolides, based on the data of Borin & Gottlieb (1993), divides the *Solanaceae* between the primitive and the most advanced genera (Table 1).

Table 1. Distribution of triterpenoids (TR), in particular steroids (ST) and withanolides (WI) in *Solanaceae* (data from Borin 1993, the numbers refer to the number of isolated compounds).

Tribes	Genera	TR	ST	WI
Cestroideae		59	51	-
Cestraea	*Cestrum*	5	-	-
	Petunia	34	34	-
	Vestia	3	-	-
Nicotianeae	*Nicotiana*	17	17	-
	Solanoideae	375	75	189
Solaneae-Solaninae		139	30	2
	Lycopersicon	10	5	-
	Solanum	118	24	-
	Capsicum	9	1	-
	Tubocapsicum	2	-	2
Solaneae-Physalinae		161	6	153
	Physalis	77	4	71
	Withania	82	2	80
	Witheringia	2	-	2
Jaboroseae	*Actinistus*	24	21	2
Datureae	*Datura*	27	8	19
Nicandreae	*Nicandra*	14	2	11
Lycieae	*Lycium*	10	8	2

W. somnifera (L.) Dunal (WS) is the most studied species in *Solanaceae* for its withanolide content. This species is an evergreen perennial shrub occurring in several regions of Europe, Asia and Africa. Three main centres of distribution can be considered: the Mediterranean basin, the Indian subcontinent and the South Africa. This geographical distribution is well evidenced by the different chemical composition of the plants of each region. *W. somnifera* contains several classes of natural products, including flavonoid glycosides, flavonols, phytosterols and alkaloids, but the scientific interest has mainly been concentrated on withanolide constituents, either for their biological properties and for their use as chemical markers. Since the first classical work of Abraham & al. (1968) on chemotypes of Israel, based on the discriminating presence of withanolides, the chemical variation of this species has been well documented and proved as one of the best examples of chemotypology. Although so far this variation is considered only at the chemical level, some morphological differences have also been observed at least in the case of Indian plants. More than 90 withanolides have been reported from *Withania somnifera* (Glotter 1991), isolated from different parts of the plant, and this figure must be considered uncompleted since also recently the isolation of five new withanolides has been reported from stem bark of plants collected in the southern region of New Delhi (Ali & al. 1997).

W. somnifera is reported to have several properties: analgesic, antipyretic, anti-inflammatory, abortificient, but in particular it is well known and used in the Indian traditional medicine (Ayurvedic and Unani medicines), with the name of aschwangandha, as a curative of many ills, and as a general powerful tonic. For this reason and in consideration of

the adaptogenic activity, the roots of the plant have been commercialised also in western countries and considered as a sort of Indian Ginseng. As a matter of fact, *W. somnifera* pertains to a series of vegetal drugs, which do not share taxonomic relationship or chemical composition with the true ginsengs of the *Panax*, but due to their adaptogenic properties they are commonly referred to as "pretend ginsengs" (Table 2). The anti-stress activity of *W. somnifera* has been an object of pharmacological investigation and is regarded comparable to that of ginseng (Grandhi 1994).

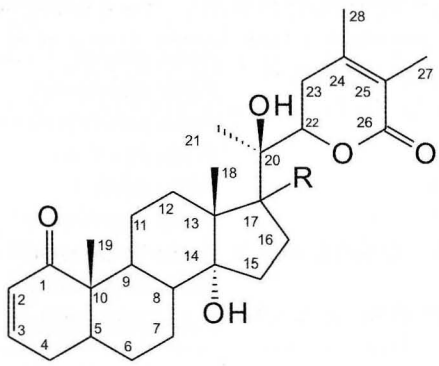
Table 2. List of *Panax* species of Asian or American origin considered as true vs. pretend ginseng.

TRUE GINSENGS		
Asian, Chinese and Korean Ginseng		*Panax ginseng*
		Panax notoginseng
		Panax pseudo-ginseng
		Panax japonicus
		Panax trifolius
		Panax zingeberinsis
		Panax stipuleanatus
		Panax vietnamensis
American Ginseng		*Panax quinquefolius*
PRETEND GINSENGS		
OTHER SPECIES RELATED TO GINSENG FOR THEIR SIMILAR ACTIVITY		
Chemotaxomically related to true Ginseng		
	Species	Family
Siberian Ginseng	*Eleutherococcus senticosus*	*Araliaceae*
Alaskan Ginseng	*Echinopanax horridum*	*Araliaceae*
Wild Ginseng	*Aralia nudicans*	*Araliaceae*
Not chemotaxomically related to true Ginseng		
Brazilian or South American Ginseng	*Pfaffia paniculata*	*Amaranthaceae*
Wild Red American Ginseng	*Rumex hymenosepalus*	*Polygonaceae*
Ginseng of the Andes, maca	*Lepidium meyenii*	*Brassicaceae*
North-western China native Ginseng	*Pedicularis* spp.	*Scrophulariaceae*
Indian or Ayurvedic Ginseng, aschwangandha	*Withania somnifera*	*Solanaceae*

Results and discussion

Our study was prompted by the possibility of studying the Italian plants of *W. somnifera* and verifying their chemotype on the basis of withanolide composition (Vitali & al. 1996). Presence of *W. somnifera* in the Mediterranean region is reported for several countries, i.e. apart the aforementioned Israel, also in Egypt and other countries of North Africa. In Italy the species occurs only in the main islands, Sicily and Sardinia, where however it is more and more difficult to find and for this reason it must be considered in a serious danger of extinction.

Separation by CC of the methanolic extract of the leaves of plants cultivated from seeds collected in Sicily led to the isolation of five withanolides (Table 3): withanolide L 1, withanolide E, 2, 17-isowithanolide E, 3, 5-ethylwithanolide S, 4, and withanolide S, 5 (Fig. 1). The identification of the compounds was obtained by NMR spectroscopic methods. All the compounds isolated present the chemical characters reported as typical of chemotype III of Israelian plants, (viz. absence of substitution at C-4, configurations 20S and 14α-OH, prevalence of 17α-OH compounds), with the difference from that chemotype due to the



Compound	R	others
1	17α-OH	Δ^4
2	17β-OH	5β,6β-epoxy
3	17α-OH	5β,6β-epoxy
4	17β-OH	5α-OH,6β-OH
5	17β-OH	5α-OH,6β-OEt
6	17α-H	Δ^4

Fig. 1. Withanolides isolated from Italian plants of *Withania somnifera*.

prevalence of withanolide J instead of withanolide E (Fig. 1). Analogous results were obtained from plants collected in Sardinia with isolation of withanolides l, 2, 3, 5 and withanolide G, 6 (Table 3). Considering that chemotype III is concentrated in the coastal regions of Israel and that the isolation of similar withanolides has been achieved in Egyptian plants (El-Olemy & al. 1986), it appears that this chemotype should be typical of the Mediterranean basin populations, thus differing from chemotypes of other centres of distribution.

Table 3. Withanolides isolated from Italian plants (values in % w/w of fresh leaves).

	Sicily	Sardinia
Withanolide J	0.12	0.17
Withanolide E	0.04	0.05
17-Iso-withanolide E	0.02	0.03
5-Ethylwithanolide S	0.01	-
Withanolide S	0.05	0.06
Withanolide G	—	0.06

Surprisingly no withanolides were found in the roots of plants from Sicily and Sardinia, where only phytosterols were evidenced. This result appears in contrast with other reports,

but it can be considered a further evidence of the chemical variation of this species.
In order to maximise the withanolide content and its production, *W. somnifera* has been the object of several studies for the *in vitro* culture (Stoller & al. 1974, Roja & al. 1991, Yu & al. 1974). In our experiments with Italian plants the best results in growth increase were obtained in the hormone-free MS medium, whereas addition of BAP or 2,4-D resulted in a lower production. Only traces of withanolides, with prevalence of withanolide J, were decteted by HPLC in the regenerated tissues. Also in the *in vitro* experiments performed by other researchers on plants of different origins the production of withanolides resulted decreased in comparison with wild plants.
Inoculation of *Agrobacterium rhizogenes* induced the formation of hairy roots, which in results were deprived of withanolides. This result is in contrast with the experiments of Ali & al. (1997), which recently reported the production of withanolides in hairy roots of *W. somnifera*, and points out the maintenance of the original chemical characters in the *in vitro* products.

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