

## ON THE PRESENCE OF PHYTOSTEROLS IN SOME SPECIES OF *EUPHORBIA* L.

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**Keywords:** Chemotaxonomy, *Euphorbia*, Friuli- Venezia Giulia, Phytosterols.

**Abstract:** In five species of *Euphorbia* of the Friuli-Venezia Giulia region (NE Italy) (*E. amygdaloides*, *E. cyparissias*, *E. helioscopia*, *E. fragifera* and *E. flavicomma* subsp. *verrucosa*) ten phytosterols were isolated and identified, by means of HPLC and spectroscopic techniques, as  $\beta$ -sitosterol, stigmasterol, campesterol, colestan-3, 5, 6-triol, cycloartenol, squalene, stigmastanol, stigmasterol acetate, cholesterol and lanosterol. The ratio  $\beta$ -sitosterol/stigmasterol can be used to distinguish some of the species.

### Introduction

The interest in plant sterols (phytosterols) has been growing over the last few years because of their biological importance. Numerous studies show that phytosterols are widespread, from the simplest organisms such as bacteria, algae and fungi (Chardon-Loriaux *et al.* 1976), to vascular plants, where they were isolated both in the vegetative and reproductive systems (Ingram *et al.* 1968). Recent studies have also identified them in the genus *Euphorbia* (Atallah & Nicholas 1972, Nielsen *et al.* 1979, Sekula & Nes 1980), together with various other secondary metabolites such as triterpenes, flavonoids, resins and essential oils. The highest concentrations were found in chloroplasts, microsomes and mitochondria, and in the plasma-lemma (Nes & Heftmann 1981), where they probably are structural elements of the membranes, regulating their stabilisation and the permeability (Grunwald 1968 and 1971). Studies were also carried out on the biosynthesis and the functions of phytosterols (Sharma 1970, Heftmann 1971) and of some derivatives, in particular steryl esters (Waller *et al.* 1981) and steryl glycosides (Smith 1969). Their concentration varies during the life cycle, as proved by the studies of Davis (1972) and Grunwald (1975) on tobacco, and of Ingram *et al.* (1968) on some *Cruciferae*. Germination (Bush & Grunwald 1972), growth (Geuns 1975), anthesis (Biswas *et al.* 1967) and senescence (Grunwald 1975) are significant stages in the biosynthesis of phytosterols, whose concentration is also influenced by environmental factors such as water availability,

temperature (Davis & Finkner 1972) and light (Bush *et al.* 1971). Moreover, the concentration of sterols particularly increases during the germination of the seeds, which seems to be linked to the biogenesis of the membranes (Duperon 1971; Bush & Grunwald 1972).

According to some authors (Atallah *et al.* 1975), all plant tissues can potentially synthesize sterols, but the degree of biosynthesis depends on the type and on the age of the tissue (Geuns 1975). As regards the pharmacological properties of phytosterols, Gupta *et al.* (1980) demonstrated their anti-inflammatory and antipyretic properties. Experiments on their contraceptive effects, due to stigmast-5ene-3-7 diol and to stigmast-5ene-3, 7 diol isolated in leaves of *Ananas comosus*, were conducted on Guinea pigs by Pakrashi *et al.* (1975). Phytosterols also have antitumoral and cytotoxic (Miles *et al.* 1974), antihyper-cholesteremic (Chandler *et al.* 1979), anticonvulsant (Farnsworth & Cordell 1976), antibacterial, antitubercular and expectorant effects (Benoit *et al.* 1976).

### Examined species

The genus *Euphorbia* comprises about 700 species and has a world-wide distribution (except Antarctica), especially in hot and dry areas. They are herbaceous annuals and perennials, suffrutescent plants and trees, mainly with simple leaves. Thermophytic elements prevail also among the almost thirty species of the flora of the Friuli-

Venezia Giulia region (Pignatti 1982, Poldini 1980, 1991), the highest concentration of the species being in the Karst area around Trieste and Gorizia, with a submediterranean climate (Poldini *et al.* 1991).

Our study is based on five among the most widespread species of the subgenus *Esula* Pers.: *E. amygdaloides* L. and *E. cyparissias* L., of the sect. *Esula*; *E. helioscopia* L., *E. fragifera* Jan and *E. flavicoma* DC. subspecies *verrucosa* (Fiori) Pign. of the sect. *Helioscopia* Dumort.

*E. amygdaloides* is found growing all over Europe (Meusel *et al.* 1978). In the Friuli-Venezia Giulia region it mainly occurs in the mountains, but it also occurs sporadically in the lowlands (Poldini 1991).

The distribution of *E. cyparissias*, originally limited to central-southern and south-eastern Europe has become wider because of anthropization, and it now extends to the United States and New Zealand (Hult n & Fries 1986). In north-eastern Italy, it is very common in open, stony grasslands.

*E. helioscopia*, the only annual species considered, occurs in Europe and western Asia (Meusel *et al.* 1978). It is mainly found growing in synanthropic stands on loose, well-manured soil from sea level to about 1300 m.

The Illyrian endemic *E. fragifera* occurs from Montenegro to the Trieste Karst, where it is an important element of the sage garrigue. Two chemical varieties were distinguished in the Trieste Karst on the basis of essential oils composition. The different terpenoidic composition is probably correlated to different pollinators (Coassini Lokar *et al.* 1986).

Between the two intraspecies varieties of *E. flavicoma*, only subsp. *verrucosa*, with a Mediterranean-Pontic-Illyrian distribution, occurs in the Friuli Venezia-Giulia region (Pignatti 1982, Poldini 1991), usually in dry grasslands on limestone.

## Data and Methods

*Plant material* - The samples (30 plants of each species at full anthesis) were randomly collected in the following areas of the Karst of Trieste (NE Italy): *E. amygdaloides*: Zolla di Monrupino and Mt. Orsario, 330 m; *E. cyparissias*: Opicina, 330 m; *E. fragifera*: Prosecco, 250 m; *E. flavicoma* subsp. *verrucosa*: between Gabrovizza and Sgonico, 260 m; *E. helioscopia*: Trieste, Scorcola, 100 m.

Voucher specimens were deposited in the Herbarium of the Department of Biology, University of Trieste (TSB). Nomenclature follows Pignatti (1982).

*Preparation of crude extracts* - The samples (epigeal and aerial organs) were dried at 50°C until constant weight and pulverized under liquid nitrogen in a stainless steel ball-mill prior to extraction. 5 g of each pulverized sample accurately weighted and exhaustively extracted according to the method proposed by Ghosh *et al.* (1985), were shaken again with a mixture of chloroform: methanol: water (30:60:30) in the ratio 5:120 p/V at room temperature (exhaustion control by preparative TLC). After centrifugation (20,000 rpm for 10 min) the supernatant was separated from the methanolic phase with chloroform, evaporated *in vacuo* at 30°C; the residue was dissolved in 10 ml of n-hexane for HPLC in defined volumes (10 ml) and used directly for analysis. The purity and identity of the peaks in the crude extracts were controlled by UV spectra detection and compared with the standards (SIGMA Chem. Co., St. Louis, USA). The standards and the crude extracts were filtered on Millipore before the injection (7µ 1/10 ml of crude extract).

*HPLC equipment* - We used a Spectra Physic M-8750, Spectra Physic Programmer M 8700, septumless injector Model 8750, with a 10 µl sample loop, Bio Rad BIO-SIL-HP10 column (4×250 mm i.d. with BIORAD HP-10 pre-column, Spectra Physic SP Mod. 8840 wavelength detector, multiple Mega Series (C. Erba, Milano, Italy) Computing integrator.

*Operating conditions* - Flow rate 1,5 ml/min, room temperature, detection UV 220 nm (detector sensitivity 0.01 a.u.f.s.), PSI 150, chart speed 10 mm/min; all the determination were performed at room temperature (22°C) and at isocratic condition of elution. Standard deviation for retention times values 0.4-1.0 %; peak area reproducibility (for 5 analyses of standards) 1.9-3.1 %; mobile phase: n-hexane/ isopropanol/methanol (96:3.5:0.5 v/v).

## Results

Ten of the phytosterols contained in aerial and hypogeal parts of the various samples of the five *Euphorbia* species considered were identified and quantified, as mean percentages (Tab.1): β-sitosterol, stigmasterol, campesterol, colestano-3β, 5α,

Tab.1 - Composition in phytosterols (% dry weight) in epigeal and hypogeal parts of the populations of the five species of *Euphorbia*.

Phytosterols	<i>E. amygdaloides</i>		<i>E. fragifera</i>		<i>E. helioscopia</i>		<i>E. cyparissias</i>		<i>E. verrucosa</i>	
	epig. %	hypog. %	epig. %	hypog. %	epig. %	hypog. %	epig. %	hypog. %	epig. %	hypog. %
$\beta$ -sitosterol	88.95	84.61	93.97	79.53	79.90	71.92	64.28	50.30	83.50	37.95
stigmasterol	6.35	6.62	2.77	2.20	12.34	16.93	27.13	18.28	12.27	15.53
campesterol	2.20	2.53	1.94	3.09	3.09	4.21	5.01	6.78	1.78	6.49
colestan - 3 $\beta$ , 5 $\alpha$ , 6 $\beta$ - triol	1.17	2.14	-	3.65	2.41	1.75	-	20.32	0.28	9.63
cycloartenol	0.11	0.31	0.05	0.32	0.76	0.90	0.71	1.00	1.02	1.51
squalene	-	1.64	-	0.32	-	1.75	0.13	0.03	-	0.82
stigmastanol	-	-	0.05	0.58	0.83	1.27	2.62	2.91	1.07	1.33
stigmasterol acetate	1.22	2.15	1.22	-	-	-	-	-	-	16.07
cholesterol	-	-	-	0.19	-	-	-	-	-	-
lanosterol	-	-	-	-	0.04	-	-	-	-	-
$\Sigma$ others unidentified	-	-	-	7.65	-	0.80	0.12	0.38	0.08	2.81

6 $\beta$ -triol, cycloartenol, squalene, stigmastanol, stigmasterol acetate, cholesterol and lanosterol.  $\beta$ -sitosterol, stigmasterol and campesterol are present both in epigeal and hypogeal organs, with  $\beta$ -sitosterol prevailing in the aerial parts. Stigmasterol is present in lower percentages, and generally prevails in the hypogeal parts with the exception of *E. fragifera* and *E. cyparissias*, which present the greatest percentages. Campesterol and cycloartenol are always most abundant in the hypogeal parts. Squalene, in very low quantities, was only found in the hypogeal parts of all samples. Stigmastanol is absent only in *E. amygdaloides* and tends to prevail in the hypogeal parts. Stigmasterol- acetate is scarcely represented, with the exception of the hypogeal parts of *E. verrucosa*. The hypogeal parts of *E. cyparissias* and *E. verrucosa* contained the highest percentages of colestan- 3 $\beta$ - 5 $\alpha$ - 6 $\beta$ - triol.

Small quantities of lanosterol (in *E. helioscopia*) and of cholesterol (*E. fragifera*) were isolated. These triterpenic alcohols were also isolated by Falsone (1972) and Falsone & Schneider (1985) in *E. biglandulosa* Desf., together with their acetylic derivatives.

The relative position of the five species may be defined by the chemical variables, taken three by three. The analysis proved that they are discriminated only by the quantities of  $\beta$ -sitosterol, stigmasterol,  $\Sigma$  others in the hypogeal parts (Fig. 1A) and by the quantities of  $\beta$ -sitosterol, stigmasterol, campesterol in their epigeal organs (Fig. 1B).

If we examine the sterolic composition of the various plant organs, there is evidence that, as already established by Grunwald (1975), the ratio  $\beta$ -sitosterol/ stigmasterol in the hypogeal and aerial

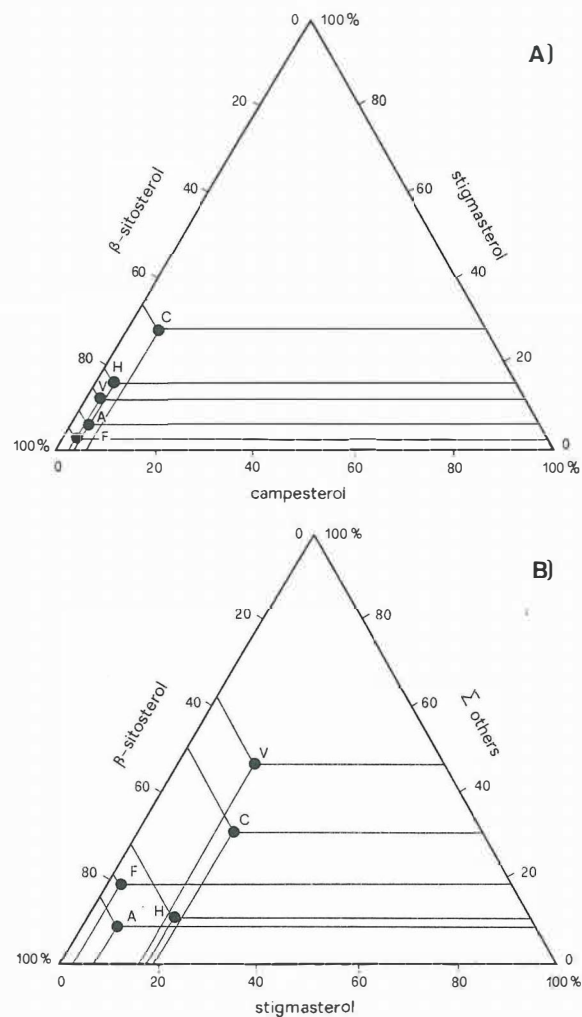


Fig. 1 - Triangular diagram showing the phytosterol discriminant combination of epigeal (A) and of hypogeal (B) organs of the five examined species (A = *E. amygdaloides*; C = *E. cyparissias*; F = *E. fragifera*; H = *E. helioscopia*; V = *E. verrucosa*).



Tab. 2 - Ratio  $\beta$ -sitosterol/stigmasterol in epigeal and hypogeal parts in the populations of the examined species of *Euphorbia*.

Species	$\beta$ -sitosterol/stigmasterol	
	epigeal	hypogeal
<i>E. fragifera</i>	33.92	36.15
<i>E. amygdaloides</i>	14.00	12.76
<i>E. helioscopia</i>	6.47	4.25
<i>E. verrucosa</i>	6.27	2.44
<i>E. cyparissias</i>	2.37	2.75

parts (Tab. 2) is effective to distinguish the examined species. If both hypogeal and aerial organs are considered, *E. amygdaloides*, *E. fragifera* and *E. cyparissias* are clearly distinguished in their epigeal parts, while *E. amygdaloides* and *E. fragifera* are distinguished by the ratio value in the hypogeal parts. *E. helioscopia* and *E. verrucosa* are distinguishable only on the basis of the ratio value in the hypogeal parts.

## Conclusions

This study was focused on the qualitative and quantitative presence of sterols in some populations of five *Euphorbia* species occurring in the Friuli-Venezia Giulia region, analyzed in their anthesis phase. It highlighted the high productivity of these biologically active compounds, and in particular of  $\beta$ -sitosterol, which tends to prevail in the green parts of the plants, especially in the leaves, where, on the other side, the biosynthesis takes place. Most of the identified sterols may play an active role in the florigenic activity, particularly  $\beta$ -sitosterol and lanosterol, whose concentration of 100 p.p.m. is sufficient to lead to flowering in 50% of the plants of *Chrysanthemum* (Biswas *et al.* 1967). The presence of single sterol derivatives was proved to be linked to developmental stages, and a particular value was attributed to the ratio  $\beta$ -sitosterol /stigmasterol (Grunwald 1975). In our study, the value of the ratio remains sufficiently constant in the populations, both in epi- and hypogeal parts, and may be utilized for the discrimination at population level. Lanosterol was only present in *E. helioscopia*, and therefore is a significant discriminant. Lanosterol and its ester derivatives, which are not usually present in plant extracts, are also typical of other *Euphorbiae* with a more southern distribution

(Falsone 1972, Falsone & Schneider 1985); lanosterol and cycloartenol may result from the primary cyclization of squalene and are often found as acetylic derivatives, especially in latex. The 4-demethylsterols ( $\beta$ -sitosterol, campesterol and stigmasterol) prevail in the examined species, while saturated compounds (= stanols) are present in smaller amounts. Among sterol-esters, only stigmasterol-acetate was found.

It would be interesting to continue this analysis on other species of the genus, to define the composition of steroidal derivatives in plants and in latex alone, where ester derivatives and triterpenic alcohols seem to prevail, probably because of their highest solubility in water, which makes them less linked to the membrane processes. This could contribute to understand their role in various parts of the plant, and to establish the chemosystematic value of some of them inside the genus, or among other critical entities, such as *E. characias* and *E. wulfenii*.

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