

Saponins in *Tribulus terrestris* – chemistry and bioactivity[†]

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Abstract

Tribulus terrestris is a valuable herb known for its application in the folk medicine in many parts of the world. Furostanol and spirostanol saponins of tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin, diosgenin, chlorogenin, ruscogenin and sarsasapogenin type are frequently found in this plant. Four sulphated saponins of tigogenin and diosgenin type are also isolated. Extracts and steroidal saponins have been found to possess various pharmacological activities. Preparations based on the saponin fraction of *T. terrestris* are used for treatment of infertility and libido disorders in men and women, as well as for treatment of cardiac diseases. Food supplements containing *T. terrestris* extracts are on sale in USA and Europe with claim of a general stimulating action.

Abbreviations: Au – Australia; Az – Azerbeidjan; Bg – Bulgaria; Ch – China; In – India; M – Moldova
R – Romania; SA – South Africa; T – Turkey

Introduction

Tribulus terrestris L. (Zygophyllaceae) is an annual plant native of Mediterranean region, but now widely distributed in warm regions of Europe, Asia, America, Africa and Australia (Frohne, 1999). It is known with several common names: puncturevine, caltrop, goat head, bull's head, ground burr nut, devil's thorn.

Description (from Flora Europaea): “Pubescent, procumbent annual 10–60 cm. Stems simple or freely branched. Leaves opposite, often unequal, paripinnate; pinnae 5–8 pairs, elliptical or oblong-lanceolate. Flowers 4–5 mm; petals yellow. Fruit of 5 stellately arranged, hard, rugose

carpels which are keeled and tuberculate on the back, and with 2 or more stout spines on the sides. Dry open habitats, often as a weed. S. Europe, extending locally northwards to N. W. France, S. E. Czechoslovakia and E. C. Russia.

Varies from green and rather sparsely appressed-pubescent to almost silvery-tomentose (Figure 1).

Two subspecies, based on the degree of hairiness and development of spines on the fruit, are sometimes recognized. These do not appear to have any discrete patterns of geographical distribution and are therefore best regarded as varieties” (Tutin, 1968).

The morphology of *T. terrestris* is very variable. Variations in populations based on burr morphology, chromosome numbers and isozyme analysis have been established (Scott and Morrison, 1996; Morrison and Scott, 1996a, b). The burr samples from 30 Australian and 34 overseas (Mediterranean region, South Africa,

[†] In this review the steroidal saponins found in *T. terrestris* are presented, covering the literature up to 2004. The data reveal clear difference in the saponin composition of samples from this plant species collected from different geographical regions. A comprehensive account on the biological activity of extracts and individual saponins is also included.

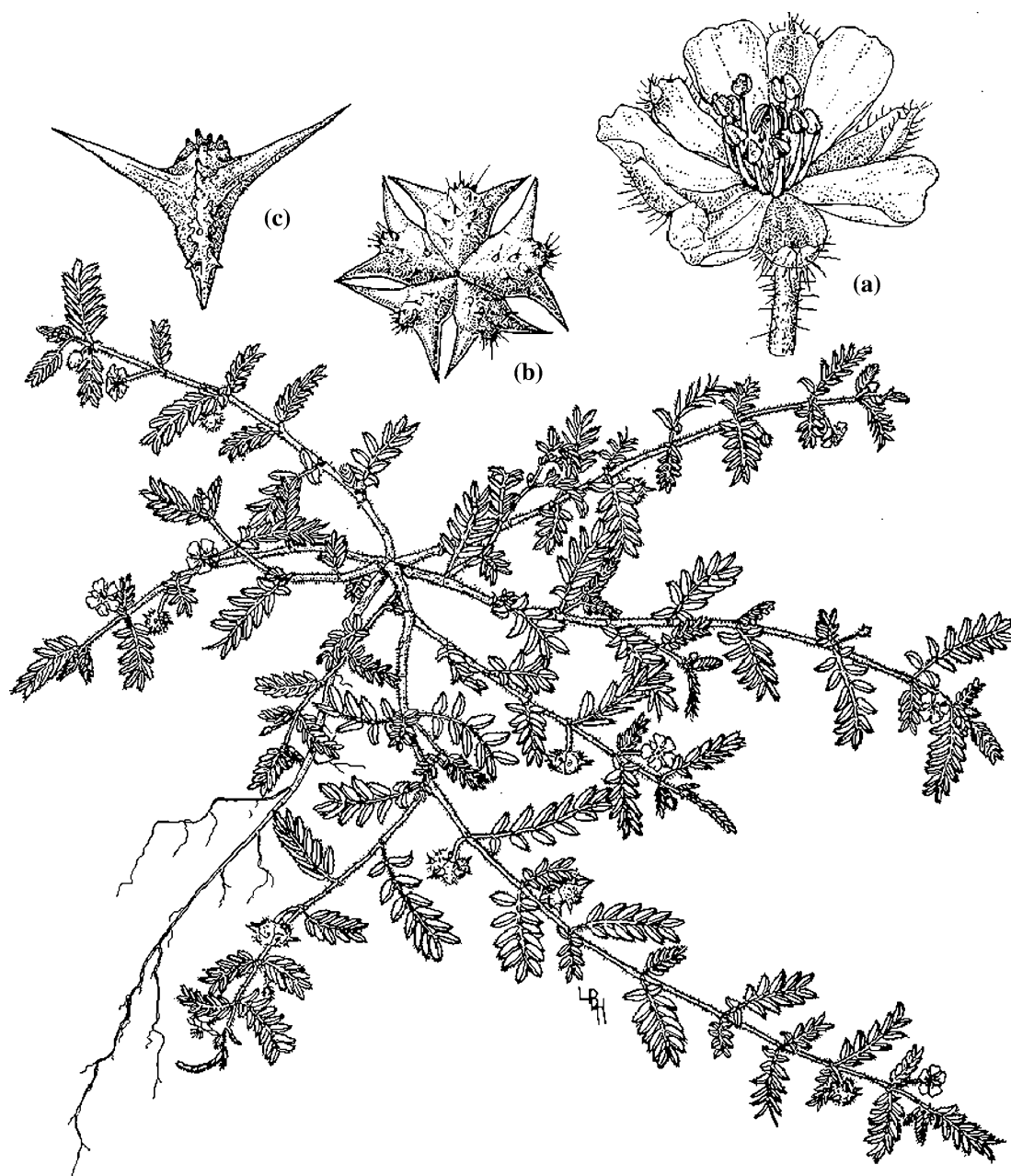


Figure 1. Puncture vine (*Tribulus terrestris*); (a) flower; (b) fruit or seedpod, a cluster of five bony burrs or nutlets; (c) single burr or nutlet containing 2–5 seeds..

India, Israel, USA, Iran, etc.) collection sites have been examined by these authors. They identified four groups of burrs.

Adaikan et al. (2001) studied the morphology of fruits obtained from India. A photograph included in their paper clearly shows the presence of characteristic spines and different morphological types of burrs.

The occurrence of saponins, flavonoids, alkaloids, lignanamides and cinammic acid amides has been reported in *T. terrestris* (Saleh et al., 1982; Bourke et al., 1992; Ren et al., 1994; Li et al., 1998). The presence of spirostanol and furostanol saponins is a characteristic feature of this plant, the latter being considered to be biogenetic precursors of their spiro analogs (Mahato et al.,

1982). Various derivatives of tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin, diosgenin, ruscogenin, chlorogenin and sarsasapogenin are found. Four sulphated furo and spiro saponins have been also isolated.

Tribulus terrestris is an important herb commonly used in the folk medicine of many countries for different purposes. There is a substantial amount of research on its effectiveness in male and female libido disorders, impotence, infertility and sperm motility. Some studies center on its cardiovascular, cytotoxic and antimicrobial activities. Many pharmaceutical preparations and food supplements based on the saponin fraction of this plant are on sale worldwide.

The present review deals with the isolation, structure elucidation and the biological activity of the steroidal saponins in *T. terrestris*, including those up to 2004. The saponin composition has been correlated with the place of origin. This approach revealed interesting differences not only in the type of saponins, but also in the kind and the number of sugars of the saponins in plant samples collected from different geographical regions. The content of protodioscin also depends on the geographical region of collection. This is in line with the considerable product-to-product variations observed in the protodioscin content of the saponin fractions of *T. terrestris* available as market products and requires a proper standardization.

Isolation of saponins

The first indication for the presence of steroidal saponins in plant extracts and/or crude saponin fractions as well as for the type of the isolated individual saponins could be received with the help of spray reagents. Both the spirostanol and furostanol saponins give yellow spots on thin layer chromatography (TLC) with anisaldehyde reagent, but only the furostanol saponins furnish a red colour with Ehrlich's reagent (Cai et al., 2001).

The separation of a saponin mixture into individual components is a difficult task which requires the combined application of various chromatographic techniques. TLC on normal and reversed-phases (HPTLC) in one- or two-dimensional modes (1D and 2D-TLC) provides excellent qualitative information and can be used

for routine analysis (Mangle and Jolly, 1998). High performance liquid chromatography (HPLC) on reversed-phase columns is the most powerful and most frequently used technique for separation of these highly polar compounds. However, the lack of chromophores allowing ultraviolet (UV) detection, limits the choice of gradient and detection methods. Application of a gradient completely excludes detection with a refractive index and this type of detection has been rarely used. The development of the recent new technique for detection with evaporative light scattering detector (ELSD) provides a valuable means for separation and isolation of saponins.

The isolation of eight steroidal saponins including penta- and hexaglycosylated ones has been achieved by repeated silica gel column chromatography (CC) on normal and reversed phase materials (Huang et al., 2003a, b).

Repeated CC on silica gel and HPLC are frequently used for separation of saponins in crude saponin fractions (Cai et al., 2001; Xu et al., 2001).

A combination of liquid vacuum chromatography (LVC) on silica gel, medium pressure liquid chromatography (MPLC, RP-18) with gradient elution, CC on silica gel and semi preparative HPLC on CN phase with UV detection has been successfully applied (Conrad et al., 2004).

The use of CC over RP-18 followed by another CC purification on RP-18 and RPHPLC-ELSD for final purification has been reported (De Combarieu et al., 2003).

In the procedures described by Wang et al. (1996, 1997) the crude saponin fraction is first separated over silica gel CC with a gradient elution $\text{CH}_2\text{Cl}_2\text{-MeOH-H}_2\text{O}$. Some fractions are further chromatographed over RP-18 CC using a gradient elution with 25–50% aq. MeCN. From selected subfractions by a combination of HPLC on RP-18 (MeOH–H₂O) and polyamine (MeCN–H₂O) using refractive index detector the pure saponins were isolated.

In another procedure (Wu et al., 1996) the crude saponin fraction is subjected to CC over silica gel with a gradient $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$. Some fractions are then chromatographed over CC on gel CHP20_p and MPLC (pre-packed column RP-18) with MeOH–H₂O to yield the pure saponins.

A chromatography of the saponin fraction over microporous resin column (eluting with water, 50,

70 and 90% EtOH) and subsequent silica gel CC (CHCl₃-MeOH-H₂O), MPLC (RP-18, H₂O-MeOH gradient) and gel filtration on Sephadex G-25 with H₂O is also described (Xu et al., 2000).

Bedir and Khan (2000) subjected the total EtOH extract to a LVC using a reversed-phase material (C-18) and employing H₂O-MeOH gradient. Some fractions were chromatographed on silica gel CC elution with CHCl₃-MeOH and CHCl₃-MeOH-H₂O to obtain the pure compounds.

Structure elucidation of individual saponins

In general the sugar moieties of the steroidal saponins in *T. terrestris* are oligosaccharides, which consist of 2–4 different kinds of sugar units e.g. D-glucose, D-galactose, D-xylose and L-rhamnose. D-xylose and L-rhamnose usually occur at the terminal positions. Except one (Conrad et al., 2004), the furostanol bisglycosides found in *T. terrestris* contain a glucose unit attached to the C-26 hydroxyl.

The conventional method for structure elucidation starts with acid hydrolysis to yield the aglycone and the monosaccharides, which are separately investigated. In the earlier investigations (Perpelitsa and Kintya, 1975; Mahato et al., 1981; Matschenko et al., 1990) the structures of the sugar moieties of the saponins are determined by identification of the monosaccharides obtained on acid hydrolysis by paper chromatography and gas liquid chromatography, or partial hydrolysis followed by isolation and identification of the prosapogenins. The point of attachment of different sugar units are revealed by permethylation of the saponins followed by hydrolysis or methanolysis and identification of the methylated sugars by paper chromatography or gas liquid chromatography. The mode of sugar linkage in saponins is determined by enzymatic hydrolysis with α - and β -glycosidases, by application of Klyne's rule on molecular rotation difference or on the base of NMR evidence.

At present, the structure elucidation of the steroidal saponins is achieved by a combination of infrared (IR) and mass spectral (MS) methods, extensive 1D and 2D nuclear magnetic resonance (NMR) experiments and chemical transformations.

IR spectroscopy

A confirmatory evidence for the nature of the pure saponins and the absolute stereochemistry at C-25 is obtained by examination of their IR spectra.

All steroidal saponins exhibit a set of four characteristic bands in the region 1000–800 cm⁻¹ – near 980, 920, 900 and 860 (920 > 900 for 25S) cm⁻¹ for the spiro (Wall et al., 1952; Rothman et al., 1952; Eddy et al., 1953) and approximately at 910, 890 sh, 840 and 810 (910 < 840 for 25R) cm⁻¹ for the furosaponins (Kawasaki et al., 1974; Mimaki et al., 1997; Da Silva et al., 1999; De Combarieu et al., 2003).

Mass spectrometry

Mass spectrometry has played an important role in structural analysis of saponins. In early studies derivatization was required for using electron impact mass spectra (Tomova et al., 1974). Later, field desorption and fast atom bombardment spectra were employed to analyze derivatized and underivatized saponins, providing evidence about molecular mass and sugar sequence by cleavage of glycosidic bonds (Perpelitsa and Kintya, 1975; Mahato et al., 1981; Matschenko et al., 1990; Wang et al., 1996, 1997; Wu et al., 1996; Xu et al., 2000). More recently, electrospray ionization (ESI) in the positive and negative ionization mode has become one of the most effective analytical tools for structural characterization of native saponins (Xu et al., 2000; Cai et al., 2001). Multi-stage tandem mass spectroscopy (ESI-MSⁿ) of the molecular ions is used for detailed structural analysis of underivatized saponins (Fang et al., 1998, 1999; Liu et al., 2004). The fragment ions from glycoside cleavage provide information about the mass of aglycone, the type of the monosaccharides, and the sequence and branching of the oligosaccharides. The fragment ions from cross-ring cleavages of sugar residues give information about the linkages between units.

NMR spectroscopy

The application of the contemporary 1D (¹H, ¹³C, TOCSY, selective TOCSY) and 2D (DQF-COSY, HH-LR-COSY, GHSQC, GHSQC-TOCSY, HMBC, NOESY and ROESY) NMR techniques allows a reliable structure elucidation

of the saponins and the complete assignment of all carbons and protons in their molecules.

Usually, the ^1H NMR spectra clearly show the presence of the methyl groups in the steroidal nucleus and the anomeric protons. Large coupling constants values ($J > 7.0$ Hz) observed for the anomeric protons of D-glucose, D-galactose and D-xylose are diagnostic of β -configuration, while $J = 1\text{--}1.5$ Hz for L-rhamnose suggests α -configuration. The anomeric configuration of L-rhamnose could be also confirmed from the chemical shifts of C-3 and C-5. For β -L-rhamnopyranoside these carbons are reported to appear at 75.4 and 73.5 ppm, while for α -L-rhamnopyranoside – at 72.5 and 69.4 ppm, respectively (Agrawal et al., 1985; Sang et al., 1999). The configuration of the anomeric carbons is additionally assured by comparison of the observed ^{13}C shifts with those reported for the corresponding methyl α - and β -glycopyranosides (Cai et al., 2001; De Combarieu et al., 2003). Direct evidence for the sugar sequence and the linkage sites is derived from HMBC experiments.

The ^{13}C chemical shifts of various saponin and saponins having different sugar moieties available in the literature are of great help in structure elucidation.

The *cis* or *trans* A/B-rings juncture is evident from the chemical shifts of C-5, C-9 and C-19 (Agrawal et al., 1985). The ^{13}C NMR data of the numerous saponin and their respective furostanol and spirostanol saponins of tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin, chlorogenin and sarsasapogenin type isolated from *T. terrestris* reveal that for the 5α compounds the chemical shifts of C-5, C-9 and C-19 appear at $\sim 43\text{--}46$ ppm, $\sim 54\text{--}56$ ppm and $\sim 11\text{--}14$ ppm (References in Tables 1–3, 7, 10–13). For the 5β compounds the chemical shifts of these carbons are observed at $\sim 35\text{--}36.5$ ppm, ~ 40 ppm and ~ 24 ppm, respectively (Bedir and Khan, 2000). A cross peak in the NOESY spectrum between H-5 and Me-19 confirms the 5β configuration. NOE correlations from H-5 to H-3 α , H-1 α , H-2 α and H-4 α support the 5α orientation.

The spirostanol saponins could be easily distinguished from the furostanol analogs by the chemical shift of C-22: for the spiro at δ 109–110 and for the furo at δ \sim 112. The 25R spiro-saponins exhibit characteristic ^{13}C chemical shifts of C-20, C-21 and F-ring carbons, different from those of

the corresponding 25S isomers (Wu et al., 1996; Xu et al., 1998).

The C-25 configuration in the furostanol saponins could be deduced on the base of the difference in the chemical shifts ($\Delta_{a,b} = \delta_a - \delta_b$) of the geminal protons Ha-26 and Hb-26. For the 25S compounds this difference is usually more than 0.57 ppm, while for the 25R compounds it is less than 0.48 ppm (Agrawal, 2004).

Chemical transformations

The structure of the sugar moieties of the saponins are determined by TLC comparison of the monosaccharides obtained by acid hydrolysis with authentic samples (Bedir and Khan, 2000; Huang et al., 2003a, b). Acid hydrolysis on HPTLC and G-CMCNa plates is also described (Xu et al., 2000; Sun et al., 2002).

Enzymatic hydrolysis of the furostanol saponins to the corresponding spiro compounds (Xu et al., 2000) and partial hydrolysis under mild acidic conditions to less polar derivatives (Wu et al., 1996) are applied in search for additional structural confirmation.

Identification of steroidal saponins in mixtures

The underivatized saponins from *T. terrestris* extracts have been investigated by ESI multi-stage tandem mass spectrometry (ESI-MSⁿ). The method allows the rapid determination of saponins in extract mixtures without derivatization and prior separation (Fang et al., 1999; Liu et al., 2004).

A separation of some furostanol and spirostanol saponins was achieved by HPLC (C-18) using ELSD and water/acetonitrile gradient as a mobile phase. The method is applied for the quantitative determination of protodioscin in extracts of different origin and market products (Ganzera et al., 2001).

A RP HPLC-ELSD-ESI-MS method has been developed for separation and structural analysis of furostanol saponins in extracts using a gradient of 0.1% formic acid (v/v) in water (A) and acetonitrile (B) (De Combarieu et al., 2003). In an independent study Mulinacci et al. (2003) also proposed the use of HPLC-ESI-MS method for qualitative analysis of saponin mixtures.

Steroidal saponins with a trans A/B-rings juncture**Spirostanol saponins**

Derivatives of 25*R*-5 α -spirostan-3 β -ol (tigogenin, **1**) and 25*S*-5 α -spirostan-3 β -ol (neotigogenin, **2**)

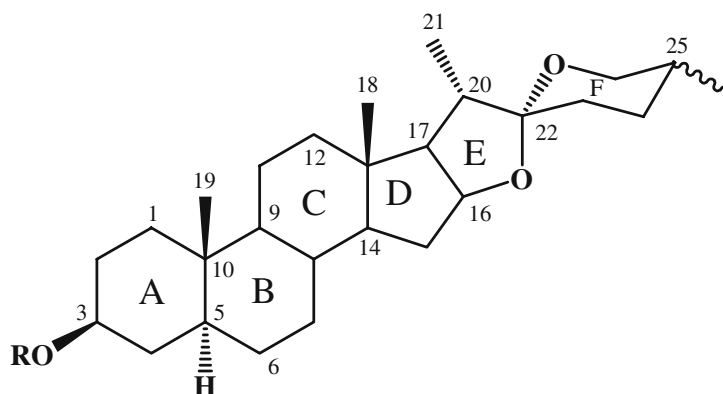


Table 1. Spirosaponins of tigogenin/neotigogenin type.

Compound	R	C-25	Origin
Tigogenin (1) ^{a,b}	H	R	Ch, Au
Neotigogenin (2) ^{c,d}	H	S	In
Tigogenin-3- <i>O</i> - β -glucopyranosyl-(1 \rightarrow 4)- β -galactopyranoside (3) ^e	$-\beta$ -Gal ⁴ - β -Glc	R	Ch
Terrestrosid F (4) ^f	Glc:Rha/2:1	R	Bg
Terrestrosin A (5) ^g	$-\beta$ -D-Gal ⁴ - β -D-Glc ² - β -D-Gal	R,S	Ch
Terrestrosin B (6) ^g	$-\beta$ -D-Gal ⁴ - β -D-Glc α -L-Rha	R,S	Ch
Desgalactotigogenin (7) ^{g,e}	$-\beta$ -D-Gal ⁴ - β -D-Glc ² - β -D-Glc β -D-Xyl	R	Ch
Gitonin (8) ^g	$-\beta$ -D-Gal ⁴ - β -D-Glc ² - β -D-Gal β -D-Xyl	R	Ch
Tigogenin-3- <i>O</i> - β -D-xylopyranosyl-(1 \rightarrow 2)-[β -D-xylopyranosyl-(1 \rightarrow 4)]-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-galactopyranoside (9) ^{e,g,h}	$-\beta$ -D-Gal ⁴ - β -D-Glc ³ - β -D-Xyl α -L-Rha β -D-Xyl	R	Ch
Tribulosin (10) ^{c,i}	$-\beta$ -D-Gal ⁴ - β -D-Glc ³ - β -D-Xyl α -L-Rha β -D-Xyl	S	In

^aXu et al. (1998); ^bMiles et al. (1993); ^cMahato et al. (1981); ^dZafar et al. (1989); ^eXu et al. (2000); ^fTomova et al. (1974); ^gWang et al. (1996); ^hHuang et al. (2003a); ⁱDeepak et al. (2002).

Derivatives of 25R-5 α -spirostan-2 α ,3 β -diol (gitogenin, **11**) and 25S-5 α -spirostan-2 α ,3 β -diol (neogitogenin, **12**)

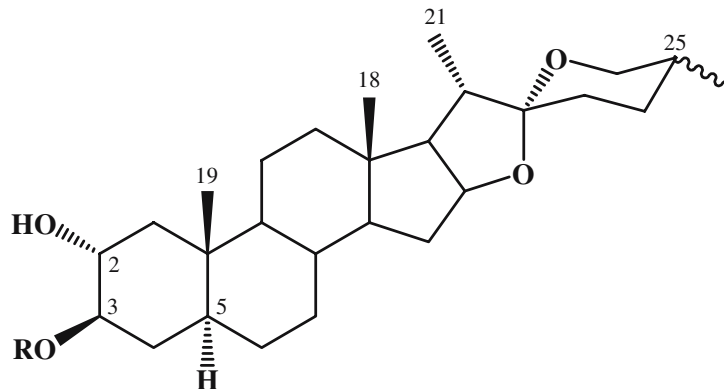


Table 2. Spirosaponins of gitogenin/neogitogenin type.

Compound	R	C-25	Origin
Gitogenin (11) ^{a,b,c,d}	H	R	Ch,SA,R,In
Neogitogenin (12) ^d	H	S	In
Gitogenin-3-O- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside (13) ^e	$-\beta$ -D-Gal ⁴ - β -D-Glc	R	Ch
25R,S-5 α -spirostan-2 α ,3 β -diol-3-O- β -glucopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside (14) ^e	$-\beta$ -D-Gal ⁴ - β -D-Glc	R,S	Ch
Terrestrosin E (15) ^{e,f}	$-\beta$ -D-Gal ⁴ - β -D-Glc ² - β -D-Gal	R,S	Ch
F-Gitonin (16) ^f	$-\beta$ -D-Gal ⁴ - β -D-Glc ² - β -D-Glc β -D-Xyl	R	Ch
Desglucolanatigonin (17) ^f	$-\beta$ -D-Gal ⁴ - β -D-Glc ² - β -D-Gal ³ β -D-Xyl	R	Ch

^aXu et al. (1998); ^bDe Kock and Enslin (1958); ^cGheorghiu and Ionescu-Matiu (1968a); ^dSharma and Narula (1977); ^eWang et al. (1997); ^fWang et al. (1996).

Derivatives of 25*R*-5 α -spirostan-3 β -ol,12-one (hecogenin, **18**) and 25*S*-5 α -spirostan-3 β -ol,12-one (neohecogenin, **19**)

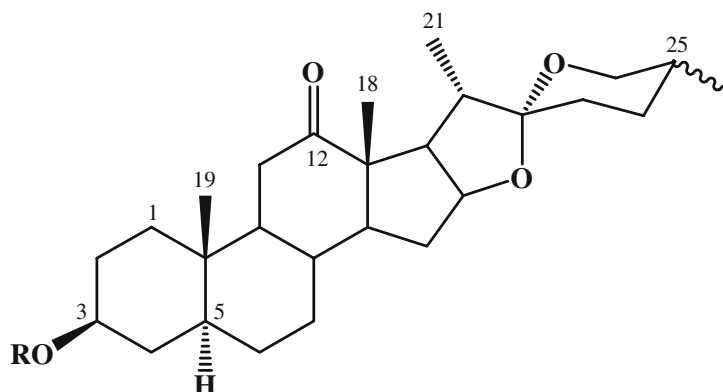


Table 3. Spirosaponins of hecogenin/neohecogenin type.

Compound	R	C-25	Origin
Hecogenin (18) ^{a,b,c,d,e}	H	R	Ch,In,Bg
Neohecogenin (19)	H	S	
Agovoside A (20) ^f	- β -Gal	R	Ch
Neohecogenin-3- <i>O</i> - β -D-glucopyranoside (21) ^g	- β -D-Glc	S	In
Hecogenin-3- <i>O</i> - β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside (22) ^{b, h,i}	- β -Gal ⁴ - β -Glc	R	Ch,In
Terreside B (23) ^j	- β -Gal ⁴ - β -Glc	S	Ch
Hecogenin-3- <i>O</i> - β -glucopyranosyl-(1 \rightarrow 2)- β -glucopyranosyl-(1 \rightarrow 4)- β -galactopyranoside (24) ^h	- β -Gal ⁴ - β -Glc ² - β -Glc	R	Ch
Terreside A (25) ^j	- β -Gal ⁴ - β -Glc ² - β -Glc	S	Ch
Hecogenin-3- <i>O</i> - β -xylopyranosyl-(1 \rightarrow 3)- β -glucopyranosyl-(1 \rightarrow 4)- β -galactopyranoside (26) ^h	- β -Gal ⁴ - β -Glc ³ - β -Xyl	R	Ch
Terrestrosin C (27) ^{k,l}	- β -D-Gal ⁴ - β -D-Glc ² - β -D-Gal - β -D-Gal ⁴ - β -D-Glc ³ - β -D-Xyl	R,S	Ch
Hecogenin-3- <i>O</i> - β -D-glucopyranosyl-(1 \rightarrow 2)-[β -D-xylopyranosyl-(1 \rightarrow 3)]- <i>O</i> - β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside (28) ^{f,h,m,n}	β -D-Glc	R	Ch,In
Terrestrosin D (29) ^{f,k}	- β -D-Gal ⁴ - β -D-Glc ² - β -D-Gal β -D-Xyl	R	Ch
Hecogenin-3- <i>O</i> - β -D-xylopyranosyl-(1 \rightarrow 2)-[β -D-xylopyranosyl-(1 \rightarrow 3)]- β -D-glucopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-galactopyranoside (30) ^{f,m}	- β -D-Gal ⁴ - β -D-Glc ³ - β -D-Xyl α -L-Rha β -D-Xyl	R	Ch
25 <i>R</i> , 5 α -Spirostan-12-one-3- <i>O</i> - β -xylopyranosyl-(1 \rightarrow 2)-[β -xylopyranosyl-(1 \rightarrow 3)]- β -glucopyranosyl-(1 \rightarrow 4)-[α -rhamnopyranosyl-(1 \rightarrow 2)]- β -galactopyranoside (31) ^o	- β -Gal ⁴ - β -Glc ³ - β -Xyl α -Rha β -Xyl	R,S	Ch

^aXu et al. (1998); ^bZafar et al. (1989); ^cZafar and Aeri (1992); ^dTomova et al. (1977); ^eHuang et al. (2002); ^fHuang et al. (2003a); ^gMahato et al. (1981); ^hXu et al. (2000); ⁱWu et al. (1996); ^jXu et al. (2001); ^kWang et al. (1996); ^lWang et al. (1997); ^mBedir et al. (2002); ⁿGanzera et al. (2001); ^oCai et al. (2001).

Derivatives of 25*R*-spirost-5-ene,3 β -ol (diosgenin, **32**) and 25*S*-spirost-5-ene, 3 β -ol (yamogenin, **33**)

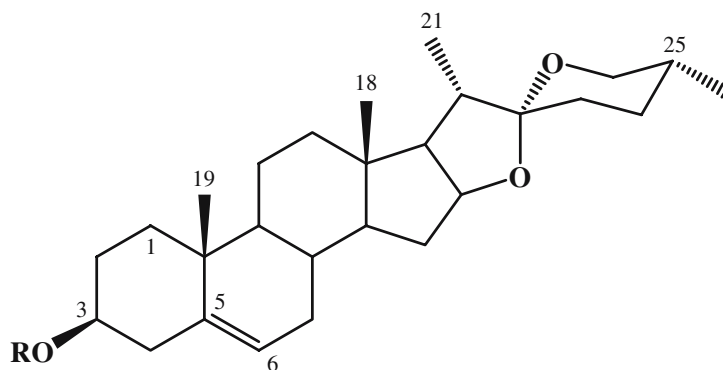


Table 4. Spirosaponins of diosgenin/yamogenin type.

Compound	R	C-25	Origin
Diosgenin (32) ^{a-j}	H	R	Bg, T, M, R, SA, In, Az, Au
Yamogenin (33)	H	S	
Trillin (34) ^{k,l}	- β -D-Glc	R	Bg, M
Trillaridin (35) ^{k,l}	- β -D-Glc- β -D-Glc	R	Bg, M
Dioscin (36) ^{h,k,m,n}	- β -D-Glc ⁴ - α -L-Rha ² α -L-Rha	R	In, Bg, M
Gracillin (37) ^{k,l}	- β -D-Glc ³ - β -D-Glc ² α -L-Rha	R	Bg, M
Prosapogenin A of dioscin (38) ^l	- β -D-Glc ² - α -L-Rha	R	Bg
Tribestin (39) ^{l,n}	- β -D-Glc ² - α -L-Rha ⁴ SO ₃ Na	R	Bg

^aTomova et al. (1974), ^bDe Kock and Enslin (1958), ^cGheorghiu and Ionescu-Matiu (1968a), ^dZafar and Aeri (1992), ^eKintya et al. (1972); ^fTomova and Panova (1965), ^gTosun et al. (1991), ^hIskenderov (1970), ⁱSharma and Narula (1977), ^jMiles et al. (1993), ^kPerepelitsa and Kintya (1975), ^lMatschenko et al. (1990), ^mMahato et al. (1981), ⁿConrad et al. (2004).

Yamogenin (**33**) and its derivatives with 25S configuration are not reported to occur in *T. terrestris*.

Derivatives of 25R-spirost-5-ene-1 β , 3 β -diol (ruscogenin, **40**)

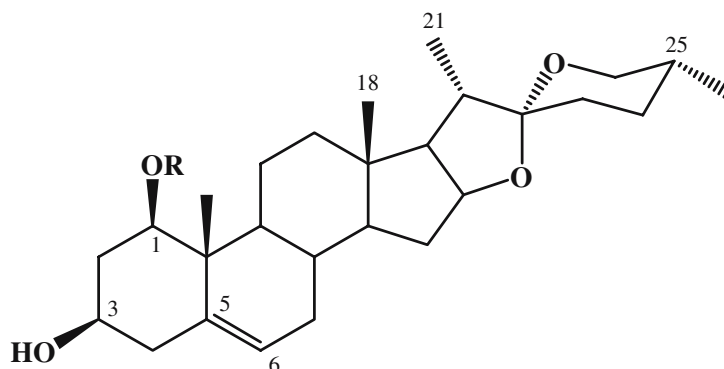


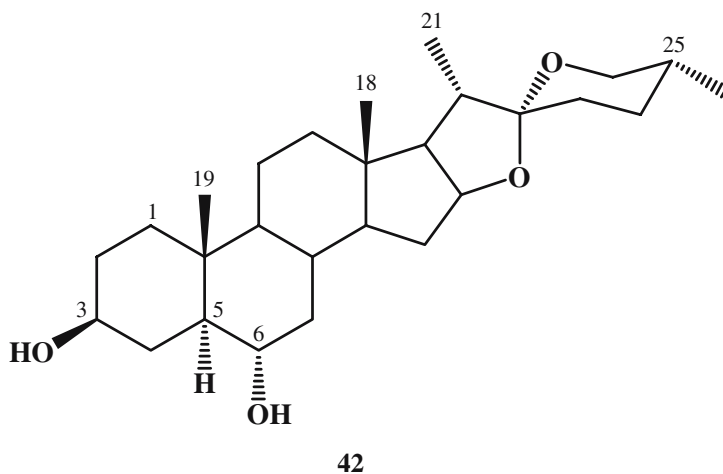
Table 5. Spirosaponins of ruscogenin type.

Compound	R	Origin
Ruscogenin (40) ^{a,b,c,d}	H	In,SA,Az
Saponin C (ruscogenin-1-O- α - L-rhamnopyranosyl- (1 \rightarrow 2)- β -D-6-acetylglucopyranoside, 41) ^e	- β -D-Glc ² - α -L-Rha 6 OAc	SA

^aDe Kock and Enslin (1958), ^bZafar and Aeri (1992), ^cBrown and De Kock (1959), ^dIskenderov (1970), ^eWilkins et al. (1996).

The sapogenins **42–50** are found only free. Of them chlorogenin (**42**) is a representative of the 6-OH-spiro compounds (Gheorghiu and Ionescu-Matiu, 1968a, b).

The 25R-spirost-3,5-dienes **43** and **44** occur in *T. terrestris* growing in India and China (Zafar and Aeri, 1992; Huang et al., 2002). Compound **43** has been also isolated from the sapogenin mixture ob-



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tained after hydrolysis of the saponin fraction of *T. terrestris* from Bulgaria (Tomova and Panova, 1965).

25R-5 α -Spirost-3,12-dione (hecogenone, **45**), 25R-5 α -spirost-3,6,12-trione (**46**) and their

respective 4-ene derivatives **47** and **48** as well as the 25R-spirost-4-ene-12-one sapogenins **49** and **50** have been isolated from *T. terrestris* of Chinese origin (Xu et al., 1998; Wu et al., 1999).

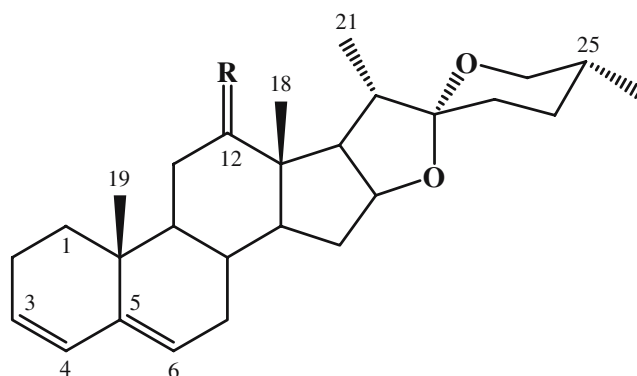


Table 6. Derivatives of 25R-spirost-3, 5-diene.

Compound	R	Origin
25R-Spirost-3,5-diene (43) ^{a,b,c}	H ₂	In,Bg,SA
25R-Spirost-3,5-diene-12-one (44) ^d	O	Ch

^aZafar and Aeri (1992), ^bTomova and Panova (1965), ^cDe Kock and Enslin (1958), ^dHuang et al. (2002).

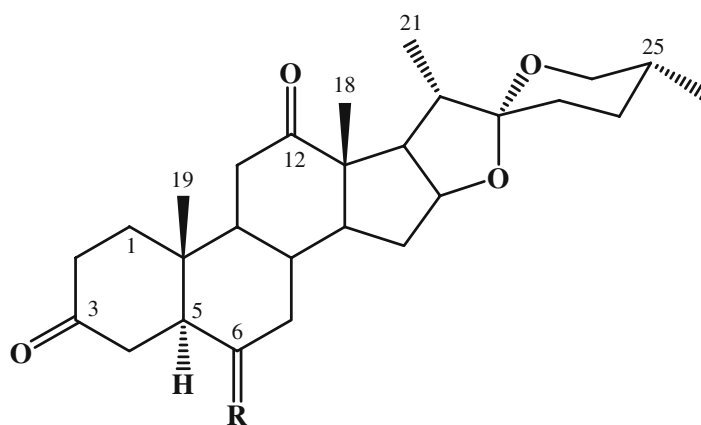


Table 7. Derivatives of hecogenone.

Compound	R	Origin
Hecogenone (45) ^a	H ₂	Ch
25R-5 α -Spirost-3,6,12-trione (46) ^a	O	Ch

^aXu et al. (1998).

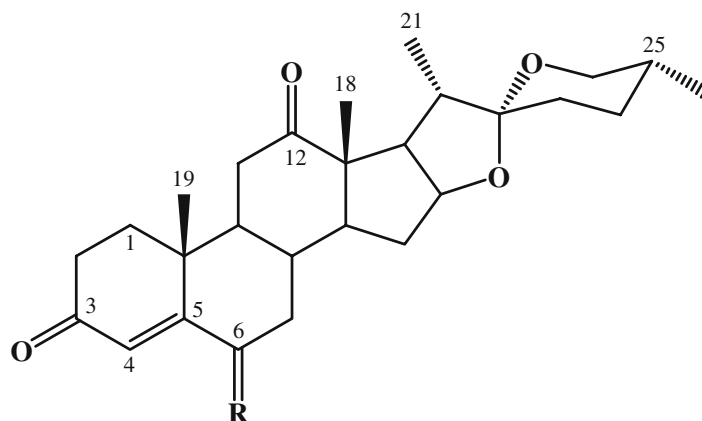


Table 8. Derivatives of 25R-spirost-4-ene-3,12-dione.

Compound	R	Origin
25R-Spirost-4-ene-3,12-dione (47) ^{a,b,c,d}	H ₂	Ch
25R-Spirost-4-ene-3,6,12-trione (48) ^a	O	Ch

^aXu et al. (1998), ^bWu et al. (1999), ^cHuang et al. (2002); Huang et al. (2003b).

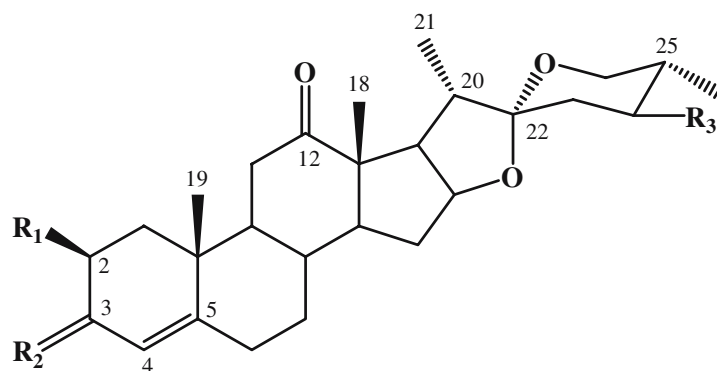


Table 9. Derivatives of 25R-spirost-4-ene-12-one.

Compound	R ₁	R ₂	R ₃	Origin
25R-Spirost-24 β -ol-4-ene-3,12-dione (49) ^a	H	O	β OH	Ch
25R-Spirost-2 β ,3 α -diol-4-ene-12-one (50) ^a	β OH	α OH, H	H	Ch

^aHuang et al. (2002).

Furostanol saponins

Precursors of tigogenin (1) and neotigogenin (2) spirosaponins

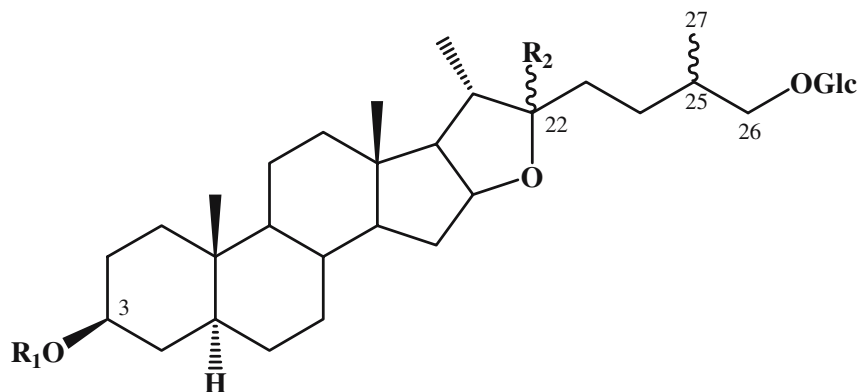


Table 10. Derivatives of tigogenin/neotigogenin type.

Compound	R ₁	R ₂	C-25	Origin
25R-5 α -Furostan-22-methoxy-3 β ,26-diol-26-O- β -D-glucopyranosyl-3-O- β -D-xylopyranosyl-(1 \rightarrow 2)-[β -D-xylopyranosyl-(1 \rightarrow 3)]-O- β -D-glucopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-galactopyranoside (51) ^{a,b,c}	- β -D-Gal ⁴ - β -D-Glc ³ - β -D-Xyl ² α -L-Rha β -D-Xyl	α OCH ₃	R	Ch,In
Terrestroneoside A (52) ^d	- β -D-Gal ⁴ - β -D-Glc ³ - β -D-Xyl ² α -L-Rha β -D-Xyl	OCH ₃	N.D.	Ch
Terrestrosin H (53) ^e	- β -D-Gal ⁴ - β -D-Glc ² - β -D-Gal	α OH	R,S	Ch
Neoprotodioscin (5,6-dihydroprotodioscin, 54) ^f	- β -D-Glc ⁴ - α -L-Rha ² α -L-Rha	α OH	R	Bg
Neoprototribestin (5,6-dihydroprototribestin, 55) ^f	- β -D-Glc ² - α -L-Rha ⁴ SO ₃ Na	α OH	R	Bg
Terrestrinin B (56) ^b	- β -D-Gal ⁴ - β -D-Glc ³ - β -D-Xyl ² ² α -L-Rha β -D-Xyl	α OH	S	Ch

^aXu et al. (2000), ^bHuang et al. (2003a), ^cBedir et al. (2002), ^dSun et al. (2002), ^eWang et al. (1997), ^fCombarieu et al. (2003).

According to Sun et al. (2002) terrestroneoside A (**52**) is the predominant component of *T. terrestris* (aerial parts) from China.

Precursors of gitogenin (11) and neogitogenin (12) spirosaponins

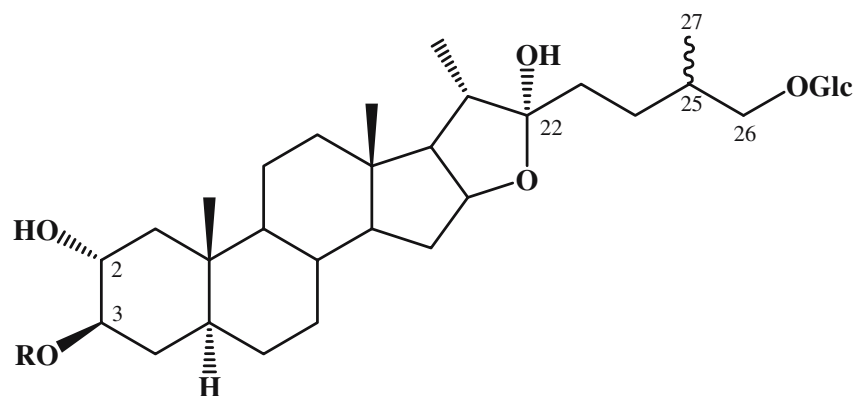


Table 11. Furostanol derivatives of gitogenin/neogitogenin type.

Compound	R	C-25	Origin
Terrestrosin F (57) ^a	-β-D-Gal ⁴ -β-D-Glc	R	Ch
26-O-β-[D-glucopyranosyl-(25R,S)-5α-furostan-2α,3β,22α,26-tetrol-3-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (58) ^a	-β-D-Gal ⁴ -β-D-Glc	R,S	Ch
Terrestrosin G (59) ^a	-β-D-Gal ⁴ -β-D-Glc ² -β-D-Gal	R,S	Ch

^aWang et al. (1997).

Precursors of hecogenin (18) and neohecogenin (19) spirosaponins

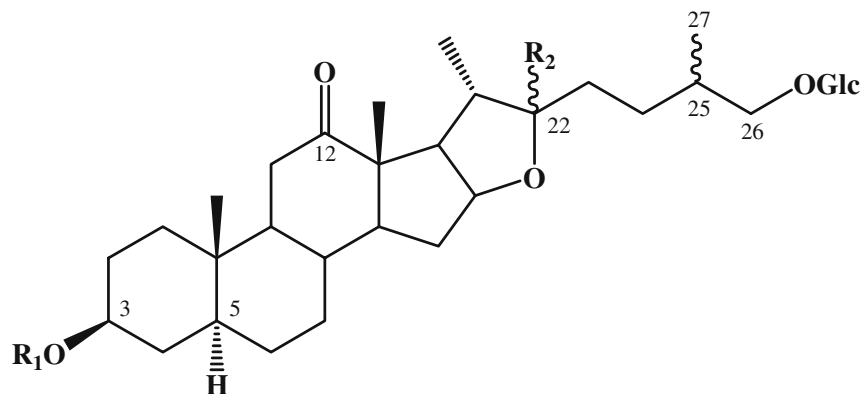


Table 12. Furostanol saponins of hecogenin/neohecogenin type.

Compound	R ₁	R ₂	C-25	Origin
26-O-β-Glucopyranosyl-(25S)-5α-furostan-12-one-3β,22α,26-triol-3-O-β-glucopyranosyl-(1 → 2)-β-galactopyranoside (60) ^a	-β-Gal ² -β-Glc	αOH	S	Ch
26-O-β-Glucopyranosyl-(25R)-5α-furostan-12-one-3β,22α,26-triol-3-O-β-glucopyranosyl-(1 → 2)-β-galactopyranoside (61) ^b	-β-D-Gal ² -β-D-Glc	αOH	R	Ch
26-O-β-Glucopyranosyl-(25S)-5α-furostan-12-one-3β,22α,26-triol-3-O-β-glucopyranosyl-(1 → 4)-[α-rhamnopyranosyl-(1 → 2)]-β-galactopyranoside (62) ^a	-β-Gal ⁴ -β-Glc α-Rha	αOH	S	Ch
26-O-β-D-Glucopyranosyl-5α-furostan-12-one-3β,22,26-triol-3-O-[[β-D-xylopyranosyl-(1 → 3)]-β-D-galactopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside (63) ^c	-β-D-Gal ⁴ -β-D-Glc ³ -β-D-Xyl β-D-Gal	OH	N.D.	Ch
Terrestrosin I (64) ^d	-β-D-Gal ⁴ -β-D-Glc ² -β-D-Gal	αOH	R,S	Ch

^aCai et al. (2001), ^bCai et al. (1999), ^cWu et al. (1996), ^dWang et al. (1997).

Precursors of diosgenin (32) and yamogenin (33) spirosaponins

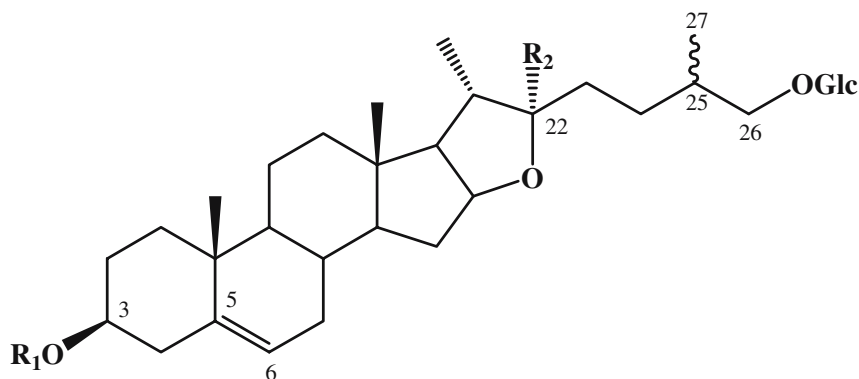


Table 13. Furostanol derivatives of diosgenin/yamogenin type.

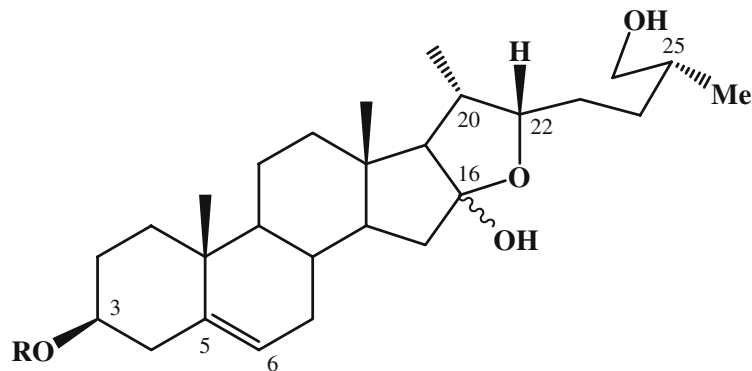
Compound	R ₁	R ₂	C-25	Origin
Protodioscin (65) ^{a-i}	-β-D-Glc ⁴ -α-L-Rha α-L-Rha	OH	R	Bg,Ch,In,M
Methylprotodioscin (66) ^c	-β-D-Glc ⁴ -α-L-Rha α-L-Rha	OCH ₃	R	Bg
Terrestrosin J (67) ^j	-β-D-Gal ⁴ -β-D-Glc ² -β-D-Gal	OH	R,S	Ch
Prototribestin (68) ^{d,e}	-β-Glc ² -α-Rha SO ₃ Na	OH	R	Bg
Methylprototribestin (69) ^e	-β-Glc ² -α-Rha SO ₃ Na	OCH ₃	R	Bg
Protogracillin (70) ^{a,c,g,i,k}	-β-D-Glc ³ -β-D-Glc α-L-Rha	OH	R	Bg,M

^aTomova (1980), ^bBedir et al. (2002), ^cMatschenko et al. (1990), ^dCombarieu et al. (2003), ^eKostova et al. (2002), ^fTomova and Gjulemetova (1978a), ^gGjulemetova et al. (1982), ^hGanzera et al. (2001), ⁱPerepelitsa and Kintya (1975), ^jWang et al. (1997), ^kTomova et al. (1981).

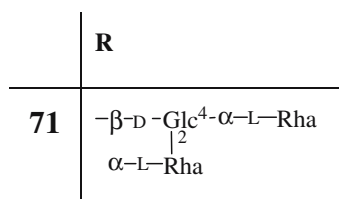
According to several studies protodioscin (65) and protogracillin (70) are the main components of the aerial parts of *T. terrestris* from Bulgaria (Tomova et al., 1981; Tomova, 1980; Obreshkova et al., 1998; Gyulemetova et al., 1982). The clear predominance of 65 over 70 is

reported by the same authors. However, recent investigations of Kostova et al. (2002) and De Combarieu et al. (2003) on samples (aerial parts) from Bulgaria unambiguously showed the main components as protodioscin (65) and prototribestin (68) (65 > 68).

Saponins of furostanol type with a hydroxyl group at C-16



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The unique furostanol saponin tribol (**71**) with a hydroxyl group at C-16 instead at C-22 has been isolated from the aerial parts of

T. terrestris of Bulgarian origin (Conrad et al., 2004).

Pseudofurostanol precursors of hecogenin (18) and neohecogenin (19) spirosaponins

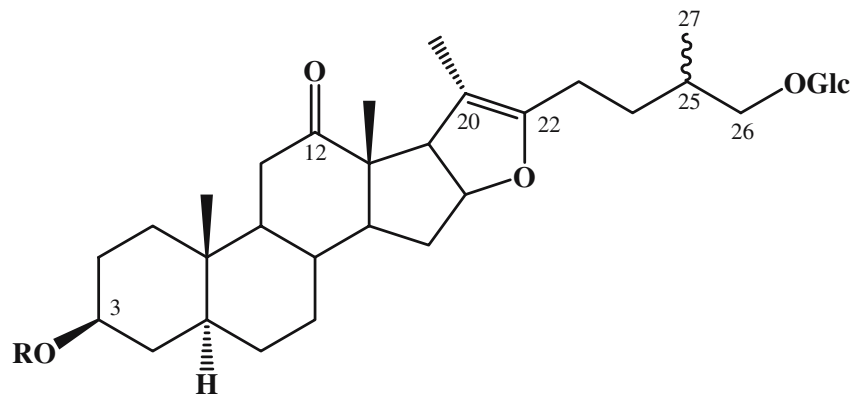
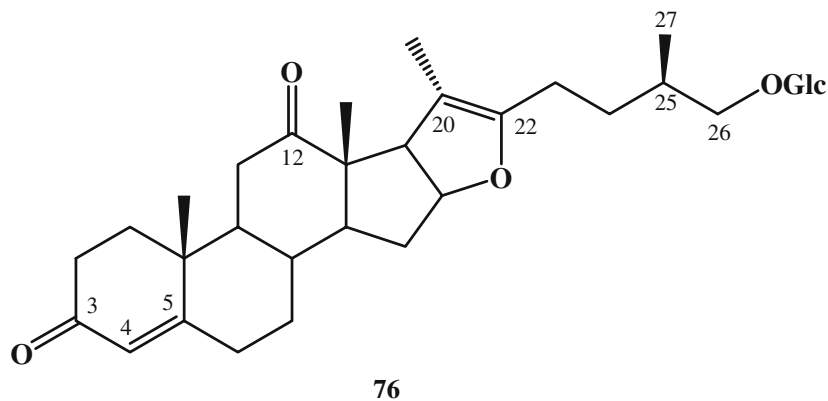


Table 14. Pseudofurostanol saponins of hecogenin/neohecogenin type.

Compound	R	C-25	Origin
26-O-β-D-Glucopyranosyl-(25R,S)-5α-furostan-12-one-20(22)-ene-3β,26-diol-3-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside(72) ^a	-β-D-Gal ⁴ -β-D-Glc	R,S	Ch
26-O-β-D-Glucopyranosyl-5α-furostan-12-one-20(22)-ene-3β,26-diol-3-O-β-D-xylopyranosyl(1 → 3)-[β-D-galactopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside (73) ^b	-β-D-Glc ⁴ -β-D-Glc ³ -β-D-Xyl β-D-Gal	N.D.	Ch
Terrestrosin K (74) ^c	-β-D-Gal ⁴ -β-D-Glc ² -β-D-Gal	R	Ch
26-O-β-D-Glucopyranosyl-(25R,S)-5α-furostan-12-one-20(22)-ene-3β,26-diol-3-O-β-D-galactopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (75) ^c	-β-D-Gal ⁴ -β-D-Glc ² -β-D-Gal	R,S	Ch

^aCai et al. (1999), ^bWu et al. (1996), ^cWang et al. (1997).



Terrestrosin A (76) was found in *T. terrestris* from China (Huang et al., 2003a, b).

Steroidal saponins with a cis A/B-rings juncture

Three saponins of this type have been isolated so far. The three of them are found in *T. terrestris* of Chinese origin and have 25S configuration (Bedir and Khan, 2000; Bedir et al., 2002).

Spirostanol saponins

Derivatives of 25S-5 β -spirostan-3 β -ol (sarsasapogenin, 77)

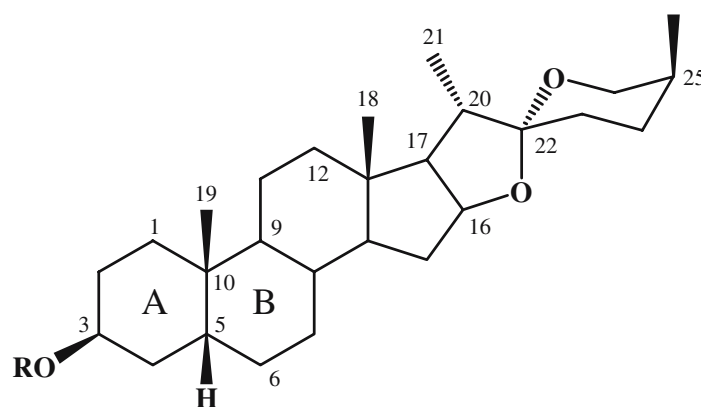


Table 15. Spiro saponins of sarsasapogenin type.

Compound	R	Origin
Sarsasapogenin (77)	H	
Isoterrestrosin B (78) ^{a,b}	- β -D-Gal ⁴ - β -D-Glc α -L-Rha	Ch

^aBedir et al. (2002), ^bBedir and Khan (2000).

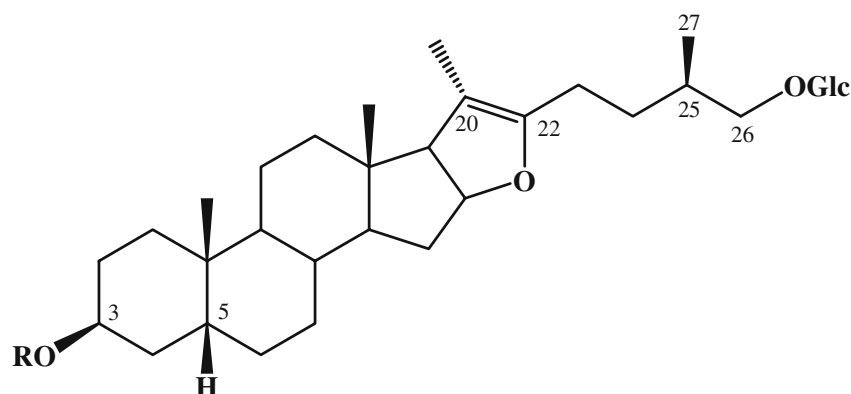


Table 16. Pseudofurostanol saponins of sarsasapogenin type.

Compound	R	Origin
Tribulosaponin A (79) ^{a,b}	-β-D-Glc ⁴ -α-L-Rha ²	Ch
Tribulosaponin B (80) ^{a,b}	-β-D-Gal ⁴ -β-D-Glc ² α-L-Rha	Ch

^aBedir et al. (2002), ^bBedir and Khan (2000).

Saponin composition and saponin content of *T. terrestris* from different geographic regions

Most of the phytochemical investigations described in the literature refer to *T. terrestris* of Chinese, Indian and Bulgarian origin. *T. terrestris* of Chinese origin is the best studied. There are limited studies on the saponins in the same plant species from Turkey, Moldova, South Africa, Australia, Azerbeidjan and Romania.

A careful examination of the literature data presented above reveals some differences in the saponin content and the saponin composition of *T. terrestris* growing in different geographic regions of the world:

- Saponins with a *cis* A/B-rings juncture (Tables 15 and 16) are found only in *T. terrestris* of Chinese origin.
- Saponins of gitogenin/neogitogenin type (Tables 2 and 11) are not present in *T. terrestris* of Indian and Bulgarian origin.
- The saponins of tigogenin, gitogenin and hecogenin type (Tables 1–3 and 10–12) predominate in *T. terrestris* from China.
- The diosgenin type spiro saponins (Table 4) are reported to occur in *T. terrestris* of Bulgarian, Moldovian, Romanian, Turkish and South African origin. They are not found in *T. terrestris* from China. Dioscin (36) is the only compound of this type reported for samples from India.
- The high content of furostanol saponins of diosgenin type (Table 13) is a characteristic feature of *T. terrestris* from Bulgaria.
- So far, the furostanol saponin protodioscin (65) is isolated only from *T. terrestris* of Bulgarian and Moldovian origin (Table 13). According to Tomova and co-workers (Tomova et al., 1981) it is the main component of samples from Bulgaria. Different extracts of *T. terrestris* from Bulgaria, India and China were compared by HPLC-ELSD (Ganzera et al., 2001). The results showed large variations not only in the content but also in the saponin composition depending on the geographical region. All the samples from Bulgaria were found to contain a rather high percentage (0.245–1.337%) of protodioscin. The samples from China contained either no

or only small amounts (0.063 and 0.089%) of this compound. Protodioscin was a minor component (0.024%) in the sample from India. This sample showed a totally different profile of the saponin content.

Three *T. terrestris* market products were also analyzed in the same investigation. The product manufactured in Bulgaria and containing a standardized plant extract again showed the highest protodioscin content (6.49%), whereas the other two of unspecified origin were significantly lower (0.85 and 0.17%). Based on these results the authors suggested the presence of different chemotypes of *T. terrestris*. This study is the first report for the occurrence of protodioscin in Chinese and Indian *T. terrestris*.

- Sulphated spirostanol and furostanol saponins (Tables 4 and 13) are isolated only from *T. terrestris* of Bulgarian origin.
- The saponins of *T. terrestris* from Bulgaria contain maximum 4 sugar units. Penta- or hexaglycosides are found in samples from India and China.
- The saponins of samples from India and China contain galactose, glucose, rhamnose and xylose, while those from Bulgaria – only glucose and rhamnose.

For now it is difficult to explain the above described differences. The publications on the isolation and structure elucidation of the chemical components (including saponins and flavonoids) of *T. terrestris* do not offer any morphological description of the studied plant samples. This makes impossible any correlation between the morphology and the chemical composition, as well as any taxonomic conclusion. However, it is interesting to note that preliminary HPLC investigations of two pairs of hairy and glabrous fruits collected from Turkey and Bulgaria revealed clear quantitative differences in the content of protodioscin, prototribestin and rutin (Dinchev and Kostova, 2005).

Ethnobotany

Tribulus terrestris is a famous herb traditionally used by different cultures for a number of conditions.

In India the fruits have been long used as a tonic and against calculus infections, urinary discharges and impotence; in the form of infusion it is recommended as a diuretic and against kidney diseases and gravel (Chopra et al., 1956). In Ayurveda the herb is known for its anti-urolithiatic, diuretic and aphrodisiac properties (Deepak et al., 2002).

The fruits have been applied in the traditional Chinese medicine for treatment of eye trouble, edema, abdominal distention, emission, morbid leucorrhea, sexual dysfunction and veiling (Wu et al., 1996; Xu et al., 2000; Cai et al., 2001). In the Shern-Nong Pharmacopoeia (the oldest known pharmacological work in China) *T. terrestris* is described as a highly valuable drug to restore the depressed liver, for treatment of fullness in the chest and mastitis and also used to dispel the wind and clear the eyes, for treatment of acute conjunctivitis, headache and vitiligo (Wu et al., 1999).

In South Africa it is used medicinally as a tonic for diarrhea and diseases of the throat and the eyes (Drewes et al., 2003).

In the Bulgarian folk medicine *T. terrestris* is recommended for purification of blood and haemorrhoids (Stoyanov, 1973)

Biological activity of extracts and individual saponins

Treatment of impotence and sexual deficiency

One of the most well known properties of *T. terrestris* is as an aphrodisiac and as a traditional medicine for treating male infertility (Gauthaman et al., 2002). Based on this information a preparation from the saponin fraction of the aerial parts of *T. terrestris* for veterinary application has been developed by Tomova and co-workers and introduced in production in Bulgaria (Tomova et al., 1966; Tomova and Gjulemetova, 1978b). During the clinical trials this preparation has shown a general stimulating action with emphasis upon sexual system (spermatogenesis, *libido sexualis*). The biological activity of different subfractions and pure components of the preparation has been checked quantitatively on “Wistar” rats, counting up the number of sertolium cells, spermatogons, spermatocides and spermatides in stage VII according to Lebland and Clermont (Tomova et al., 1981).

As a result of these investigations and clinical trials on humans (Protich et al., 1981; Viktorov et al., 1982) the original Bulgarian preparation Tribestan has been created and standardized (Tomova et al., 1978). It is recommended and widely used for treatment of infertility and libido disorders in men and women. According to Tomova et al. (1981) the furostanol saponins and in particular the two main components of the saponin fraction protodioscin (65) and protogracillin (70) (65 > 70) are responsible for the observed biological activity. Later, the structure of the second main component was revised from 70 to 68, which proved to be a new structure and the name prototribestin given to this saponin.

Libilov is another preparation based on the saponin fraction of *T. terrestris* and claimed to possess similar activities. Research carried out by different scientific teams from Indonesia and Singapore on *T. terrestris* and pure protodioscin (65) indicates that Libilov increases men's sex drive. In a multi-center, placebo-controlled, randomized, double-blinded clinical trial, protodioscin proved to be effective in treatment of male infertility (Adimoelja and Adaikan, 1997).

By clinical trials protodioscin is proved to increase the level of dehydroepiandrosterone (DHEA) in infertile men. The authors concluded that protodioscin in *T. terrestris* could be the precursor of DHEA in patients with low serum level of this hormone. It is suggested that this hormone participates in improvement of cell membrane integrity and function at cellular level, in improvement of circulation, health and sense of well being. This indirectly results in improved sex drive (Adimoelja and Adaikan, 1997; Adimoelja, 2000).

Investigations of Milanov et al. (1981) reveal that Tribestan elevates the level of the luteinizing hormone and testosterone in the orally treated healthy males, not affecting follicle-stimulating hormones. In the females, the concentrations of follicle-stimulating hormone and estradiol were increased, where the testosterone concentration was not significantly changed.

It is suggested that protodioscin works by increasing the conversion of testosterone into the potent dehydrotestosterone, which not only stimulates increase in the sex drive, but as well as the production of red cells, and the muscle developments, thus contributing to improvement of blood circulation and the oxygen transport systems, and

therefore contributing to the optimal health (Arsyard, 1996).

The proerectile activity of *T. terrestris* extracts has been reported by Adaikan et al. (2000).

Recently, two pharmaceutical formulations useful for treatment of male and female impotence and containing *T. terrestris* extract have been patented (Bombardelli et al., 2003a, b).

Preparations containing *T. terrestris* extracts are currently on sale in USA and Europe as food supplements with claim of a general stimulating action on motor activity, muscle tone and restorative tonic for vigor (Ganzera et al., 2001; De Combarieu et al., 2003; Mulinacci et al., 2003). It was found that chronic ingestions of the nutritional supplement AND-NB containing androstenediol, saw palmeto, γ -linolenic acid, indol-3-carbinol, chrysin and *T. terrestris* increase serum androstenedione, free testosterone, dihydrotestosterone and estradiol concentrations in healthy men (Brown et al., 2002).

Treatment of cardiac diseases

The drug named Xinnao Shutong is made of the crude saponin fraction of Chinese *T. terrestris* (leaves and stems). It shows significant effect in treatment of various cardiac diseases including coronary disease, myocardial infraction, cerebral arteriosclerosis and the sequelae of cerebral thrombosis (Cai et al., 1999, 2001; Xu et al., 2000).

A clinical trial shows that a saponin of *T. terrestris* has the action of dilating coronary artery and improving coronary circulation. It is recommended for treatment of angina pectoris (Wang et al., 1990).

Antimicrobial activity

Literature data reveal that the antibacterial activity of *T. terrestris* varies depending on the origin and the part of plant under investigation. The ethanolic extracts of Yemeni *T. terrestris* have demonstrated no detectable antibacterial activity against any of the reference bacteria (Ali et al., 2001). The methanolic extracts of different parts (fruits, roots and stems plus leaves) of the same herb growing in Iran inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*

at concentrations of 2–4 mg/ml. The observed activity is similar to that of *T. terrestris* of Turkish origin (Kianbakht and Jahaniani, 2003).

The ethanolic extracts of the fruits and leaves of the Indian herb exhibited activity against *E. coli* and *S. aureus* (Kianbakht and Jahaniani, 2003). The chloroform extract of air-dried finely ground plant material of Spain showed weak activities against *S. aureus*, *Candida albicans* and *Mycobacterium phlei* (Recio et al., 1989).

In a study on the antimicrobial effects of seven individual saponins isolated from *T. terrestris* the spirostanol saponins **78**, **28** and **30** exhibited remarkable antifungal activity against *C. albicans* and *Cryptococcus neoformans*, while none of the furostanol derivatives **79**, **80**, **65**, **51** showed activity. The minimum inhibitory concentrations of the three spirostanol saponins varied from 1.5–6.2 µg/ml. All the compounds were inactive against *S. aureus*, *Aspergillus fumigatus*, *P. aeruginosa* and *Mycobacterium intracellulare*. The authors suggested some structure–activity correlations (Bedir et al., 2002).

Cytotoxic activity

The inhibitory effect of saponin mixture from *T. terrestris* of Chinese origin on Bcap37 breast cancer cell line was determined and results showed a potent inhibitory effect in a concentration-dependent manner (Sun et al., 2003).

A study on the cytotoxic effects of the steroidal saponins **28**, **30**, **51**, **65**, **78**, **79**, **80** isolated from *T. terrestris* of different geographical regions (Bulgaria, China and India) and different parts of the plant (stems and fruits) shows that only the spiro compounds **28**, **30** and **78** exhibit remarkable activity. The most active spirostanol glycoside **28** demonstrates a broad range of anticancer activity against cell lines SK-MEL, KB, BT-549 and SK-OV-3 at IC₅₀ of 6.0, 7.0, 6.0 and 8.2 µg/ml, respectively, while **30** and **78** showed selective cytotoxicity against SK-MEL at 6.7 and 9.1 µg/ml (Bedir and Khan, 2000; Bedir et al., 2002).

Dioscin (**36**) and prosapogenin A of dioscin (**38**) exhibited cytotoxic activity against the cancer cell line K562 *in vitro* (Hu et al., 1996). Methylprotodioscin (**66**) and protodioscin (**65**) were found to inhibit the growth of human leukemia HL-60 cells in culture and macromolecular synthesis in a dose-dependent manner (Shao et al., 1997).

The potential anticancer activity of protodioscin (**65**) and methylprotodioscin (**66**) were tested *in vitro* against 60 human cancer cell lines. Protodioscin was found cytotoxic against most cell lines from leukemia and solid tumors, especially selectively against one leukemia line (MOLT-4), one NSCLC line (A549/ATCC), two colon cancer lines (HCT-116 and SW-620), one CNS cancer line (SNB-75), one melanoma line (LOX IMVI), and one renal cancer line (786-0) with GI₅₀ (Growing Inhibition) ≤2.0 µM (Hu and Yao, 2002). Methylprotodioscin (**66**) showed strong cytotoxicity against most human cell lines from solid tumors with GI₅₀ ≤10.0 µM, especially selectively against one colon cancer line (HCT-15) and one breast cancer line (MDA-MB-435) with GI₅₀ <2.0 µM but moderate cytotoxicity was shown against leukemia cell lines with GI₅₀ = 10–30 µM. Based on an analysis using the compare computer program with methylprotodioscin as a seed compound, the authors suggested a potential novel mechanism of its anticancer action (Hu and Yao, 2003).

Dioscin (**36**), protodioscin (**65**) and methylprotodioscin (**66**) showed a tumor inhibitory effect *in vivo* in C6 glioma cells. In addition, dioscin (**36**) inhibits the DNA synthesis of C6 glioma cells at 10 µg/ml (Chiang et al., 1991).

Anthelmintic activity

The anthelmintic activity of successive extracts of Indian *T. terrestris* (whole plant) prepared using petroleum ether, chloroform, 50% MeOH and water was evaluated using the nematode *Caenorhabditis elegans*. Only the 50% methanol extract showed activity. Further bioassay-guided investigation confirm tribulosin (**10**) and sitosterol glucoside as active components with ED₅₀ of 76.25 and 82.50 µg/ml, respectively (Deepak et al., 2002).

Others

Investigations of Lin et al. (1999) support the traditional use of *T. terrestris* fruits for treatment of vitiligo.

An ethanolic extract of the fruits of the herb showed significant dose dependent protection against urolithiasis in albino rats. Maximum activity was localized in the 10% aqueous methanol fraction (Anand et al., 1994).

The lyophilized saponin mixture of the plant caused a significant decrease on peristaltic movements of isolated sheep ureter and rabbit jejunum preparations in a dose dependent manner. According to these results the saponin mixture may be useful for some smooth muscle spasms or colic pains (Arcasoy et al., 1998).

The preventive and therapeutic effects of saponins from *T. terrestris* on diet induced hyperlipidemia in mice have been studied. The results showed that the saponins could significantly low the levels of serum TC (P , 0.05), LDL-c (P , 0.01) and liver TC (P , 0.05), TG (P , 0.05) and increase the activities of superoxide-dismutase in liver (Chu et al., 2003). Hypoglycemic effect of saponins from *T. terrestris* was investigated. The saponins were found to reduce the level of serum glucose significantly (Li et al., 2002).

Conclusions

Tribulus terrestris is a well known herb used in the folk medicine of many countries for a number of diseases. The high content of steroidal saponins is a characteristic feature of this plant. Derivatives of tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin, diosgenin, ruscogenin, chlorogenin and sarsasapogenin are found. The sugar moieties of the isolated furostanol and spirostanol saponins are oligosaccharides, which contain 2–4 different kind of sugars – glucose, rhamnose, galactose and xylose.

The plant is an industrial source for production of medicinal preparations based on its saponin fraction. Food supplements with a claim of general stimulating action are also currently on sale in Europe and USA.

Tribulus terrestris still remains a plant object of further studies. By now the saponin composition of this plant collected only in China, India, Bulgaria and Moldova has been well studied. According to literature data the saponin composition and the saponin content of *T. terrestris* from different geographical regions is different. To explain the reason of the observed differences investigations on the correlation between the morphology and the saponin composition of the plant will be required. Further studies on the biological activities of extracts and individual compounds are of special

importance. Development of analytical methods for qualitative and quantitative determination of saponins in mixtures is another direction for the future investigations.

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