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MEDICINAL PROPERTIES, PHYTOCHEMISTRY AND PHARMACOLOGY OF *TRIBULUS TERRESTRIS* L. (ZYGOPHYLLACEAE)

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Abstract

Tribulus terrestris (puncture vine) belongs to family Zygophyllaceae and it is a herbaceous, mat forming plant in nature. It extensively grows in warm dry tropics all over the world and ecologically adaptated as a typical C_4 xeromorphic plant. *T. terrestris* is a noxious weed along with its use in many countries as a folk medicine for different purposes from time immemorial. Ancient records describe various medicinal properties of *T. terrestris* as a popular source to cure variety of different disease conditions in China, India, and Greece. The plant is used directly as a herb or as a main component for production of a number of medicines and food supplements such as for physical rejuvenation, therapy for the conditions affecting liver, kidney, cardiovascular system and immune systems. Also it is used as a folk medicine for increased muscle strength, sexual potency and in treatments of urinary infections, heart diseases and cough. It is considered invigorating stimulant, aphrodisiac, and nutritive. This review discusses the most commonly recognized medicinal properties of this herb. The chemistry of *T. terrestris* extracts to establish the relationship between medicinal properties of this important plant will also be reviewed.

Introduction

Despite great progress in medicine, use of herbal medicines is still popular. Herbal medicines have been in use since ancient times for general health and for specific diseases (Shinwari & Qaisar, 2011). Particularly, in developing countries, great number of people depends on the traditional system of medicine for a variety of diseases. There are more than 35,000 plants species being used in various human cultures around the world for medicinal purposes. The World Health Organization reported that 80% of the world's population rely mainly on traditional medicine and a major part of the traditional therapies involve the use of plant extracts or their active constituents (Anon., 1993). What to say of humans, positive effects of regular use of medicinal plants have been observed on birds and animals (Khan, 2009; Javed *et al.*, 2012; Khushdil *et al*., 2012).

Tribulus terrestris L., is commonly known as puncture vine, caltrop, yellow vine, goat head and devil's horn. It is is a member of the Zygophyllaceae family and is widely distributed in both tropical and mild temperate regions (Hegnauer, 1973). The genus *Tribulus* contains 25 species and many are regarded as noxious weeds. Because of the spiny nature of the fruits, they are a hazard to grazing animals. Also, sheep staggers is reported to be the result of beta-carboline alkaloids contained in the fruit (Bourke *et al*., 1992). *T. terrestris* is of medicinal and pharmaceutical interest as it contains a number of steroidal saponins which may account for its use in muscle building, conditioning and treatment of certain ailments (de-Combarieu *et al*., 2003; Cai *et al*., 2001; Xu *et al*., 2000; 2008; Dinchev *et al*., 2008; Kostova & Dinchev, 2005; Conrad *et al*., 2004; Huang *et al*., 2003; Bedir & Khan, 2000). The extract is also used for urinary dysfunction, asthma and opthalmia and different (Sarwat *et al*., 2008; Qureshi *et al*., 2010) and has been shown to have antihypertensive and vasodilatory properties (Phillips *et al*., 2006, Sharifi *et al*., 2003).

It may also protect against oxidative stress and exhibits antitumor, cytotoxic, antifungal and antihelmentic properties (Pandey *et al*., 2007; Hu & Yao 2002; 2003; Perrone *et al*., 2005; 2003; Neychev *et al*., 2006; Zhang *et al*., 2005, Deepak *et al*., 2002). Studies have shown that saponins in the extracts of *Tribulus* species exhibited hypoglycemic and hypolipidemic effects in diabetic rats (El-Tantaway & Hassanin, 2007). These saponins were also shown to increase cardiocyte survival by attenuating induced apoptosis under experimental conditions (Hong-Li & Yang, 2008).

Since many of *T. terestris* uses have not been fully validated on scientific basis, it is natural to have doubts when presented with herb purportedly useful in so many ailments. It is of immense importance to review the use of *T. terrestris* for different uses from the point of view of recent scientific findings.

Habitat: *T. terrestris* is native to warm temperate and tropical regions of southern Europe, southern and western Asia, throughout Africa, and Australia (GRIN; http://www.ars-grin.gov). *T. terrestris* is widely distributed in Africa, Southern Europe, China, Japan, Korea and western parts of Asia (Al Ali *et al*., 2003; Sharifi *et al*., 2003). *T. terrestris* grows well in light textured soils however it grows over a wide range of soil types. Generally, it can be found in cultivated crops, overgrazed pastures, roadsides, lawns and neglected areas.

Botanical description: *T. terrestris* is a small silky, hairy and prostrate herb with stems up to 2m long (Fig. 1). Leaves are pinnate, short (~1.25 cm length), opposite and each consisting of 4-8 pairs of spear shaped leaflets. *T. terrestris* is characterized by small (8-15 mm diameter) yellow petal flowers and thorny fruits. Fruits are woody burr about 1 cm diameter with sharp spines upto 6 mm long. Burr consists of 5 wedge shaped segments. Each segment has 2 unequal pairs of spines. Seeds are enclosed in a woody star-shaped structure (carpels) of around 5-7 mm length and 5-6 mm width. There are up to five seeds in each carpel and each seed is 1.5-3 mm long and yellow in color. Each plant can have upto 2000 seeds. They taste astringent and it is agreeable. The root when fresh is slender, fibrous, cylindrical and of light brown color. The stomata of *T. terrestris* are anomocytic in which the guard cells are not surrounded with any subsidiary cell (Perveen *et al*., 2007).

Fig. 1. *T. terrestris* plant.

Chemistry: The chemistry of *T. terrestris* has been extensively studied and 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). This plant is extremely rich in substances having potential biological significance, including: saponins, flavonoids, alkaloids and other nutrients (Wang *et al*., 1997). The quantities and presence of these important metabolites depend on the various parts of the plant used. The fruit and root of *T. terrestris* contains pharmacologically important metabolites such as phytosteroids, flavonoids, alkaloids and glycosides (Wu *et al*., 1996). A good number of various saponins and their different derivatives have also been identified in *T. terrestris,* of which diosgenin, gitogenin and chlorogenin are in the leaf tissue. 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. In general saponins, flavonoids, alkaloids, lignanamides and cinnamic acid amides are of therapeutic values, of which spirostanol and furostanol saponins are main ingredient (Kostova & Dinchev, 2005). Many pharmaceutical preparations / food supplements are on sale in market based on saponins content. The saponin composition has been correlated with the place of origin. This approach revealed interesting differences not only in the type of sapogenins, 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. The freshly expressed juice of the aqueous extract of the whole plant contains inorganic nitrates, mostly potassium nitrate. The diuretic property of the plant is due to the nitrates. The fruit contains alkaloid, resin, fat, ascorbic acid, minerals (14%) and essential oils. The amount of ascorbic acid increases from roots to fruits in *T. terrestris*. *T. terrestris* is also rich in iron. See Fig. 2 for the chemical structures of Spirostanol and Fig. 3 for Furostanol saponins.

Variation in saponin composition and content with geographical regions: Survey of the literature data reveals some differences in the saponin content and the saponin composition of *T. terrestris* growing in different geographic regions of the world (Kostova & Dinchev, 2005). Phytochemically, the *T. terrestris* of Chinese, Indian and Bulgarian origin have been investigated and there are limited phytochemical analysis reports of *T. terrestris* from Turkey, Moldova, South Africa, Australia, Azerbeidjan & Romania. For example, saponins with a cis A/B-rings juncture (gitogenin/neogitogenin type) are found only in *T. terrestris* of Chinese origin (Xu *et al*., 1998; Kostova & Dinchev, 2005). The diosgenin type spiro saponins are reported to occur in *T. terrestris* of Bulgarian, Moldovian, Romanian, Turkish and South African origin (Tomova *et al*., 1974; Miles *et al*., 1993).

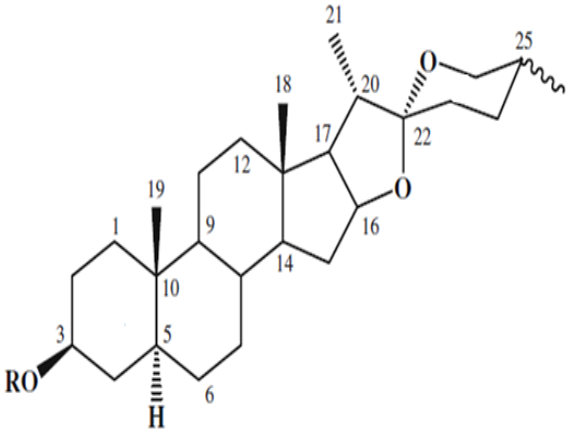

Fig. 2. Chemical structure of a typical spirostanol saponin.

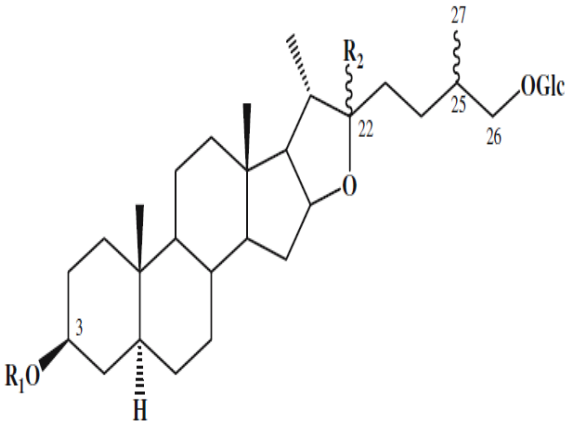

Fig. 3. Chemical structure of furastanol saponin.

Sulphated spirostanol and furostanol saponins have been isolated only from *T. terrestris* of Bulgarian origin (Conrad *et al*., 2004). Interestingly, the saponins analyzed from samples of India and China contained sugar units such as galactose, glucose, rhamnose and xylose, while those from Bulgaria had only glucose and rhamnose (Ganzera *et al*., 2001). At the moment it is difficult to explain the above described differences among *T. terrestris* of different origin. However, the variations in the saponins contents and saponin compositions of *T. terrestris* from different origin do support the different bioactivities and different medicinal uses of the herb in different regions of the world.

Biological activities of extracts and saponins of *T. terrestris*: *T. terrestris* has been in use for medicinal purposes since time immemorial. In India and China, the medicinal use of this herb is traced 5,000 year back (Balch, 1990; Bartram, 1995). In Ayurveda this herb is known for its diuretic, aphrodisiac and anti-urolithiatic properties (Deepak *et al*., 2002). In the traditional Chinese medicine, its fruits have been used for treatment of eye trouble, abdominal distention, emission, edema, morbid leucorrhea, sexual dysfunction and veiling (Xu *et al*., 2000; Cai *et al*., 2001). In South Africa it is recommended in the form of a herbal tonic for diarrhea and diseases of the throat and the eyes (Drewes *et al*., 2003). In the Bulgarian folk medicine, *T. terrestris* is used for blood purification and in haemorrhoids (Stoyanov, 1973).

Sexual disorders: Since long *T. terrestris* is known for its aphrodisiac properties and as a traditional medicine for treating male infertility (Gauthaman *et al*., 2002). A formulation of the saponins from *T. terrestris* was developed for veterinary application and production in Bulgaria (Tomova *et al*., 1966; Tomova & Gjulemetova, 1978b). This formulation was effective in stimulating the sexual system (spermatogenesis, *libido sexualis*). Using *T. terrestris* extracts, an increase in sexual function in rats was demonstrated and attributed to an increase in testosterone, dihydrotestosterone, and dehydroepiandrosterone (Gauthaman *et al*., 2002; Gauthaman & Ganesan. 2008). A Bulgarian formulation with the name of Tribestan was optimized and widely used for treatment of infertility and libido disorders in men and women (Tomova *et al*., 1978a; Protich *et al*., 1981; Viktorov *et al*., 1982). The furastanol saponins (protodioscin, protogracilin) from *T .terrestris* were reported to have stimulating effect on spermatogenesis via Luteinizing Hormone (LH) stimulating the secretion of male hormone Testosterone. Testosterone in turn significantly improved the quality and quantity of sperms (Tomova *et al*., 1981; Brown *et al*., 2002). Libilov is another formulation of the saponin fraction of *T. terrestris* and is reported for similar activities. Protodioscin, main component of Libilov, proved to be effective in treatment of male infertility and it increased the level of dehydroepiandrosterone (DHEA) in infertile men. Protodioscin in *T. terrestris* was suggested to be DHEA precursor in patients with low serum level of this hormone (Adimoelja & Adaikan, 1997; Adimoelja, 2000). Different formulations containing *T. terrestris* extracts are marketed in USA and Europe as food supplements for stimulation, vigor (De Combarieu *et al*., 2003; Mulinacci *et al*., 2003) and for other multiple ailments.

Cardiac diseases: Research studies revealed that saponins of *T. terrestris* can play a role in dilating coronary artery and improving coronary circulation. The results of a clinical trial on 406 patients, when treated with saponin of *T. terrestris* showed that the total efficacious rate of remission angina pectoris was 82.3% and the total effective rate of ECG improvement was 52.7% higher (Wang *et al*., 1990). Therefore, *T. terrestris* was recommended for treatment of angina pectoris (Wang *et al*., 1990). Many research studies revealed that aqueous extract of *T. terrestris* fruits have some antihypertensive effect (Yang *et al*., 1991, Chui *et al*., 1992 and Lu *et al*., 1994). These beneficial effects have partly been attributed to its ability to increase nitric oxide (NO) release from the endothelium and nitrergic nerve endings (Adaikan *et al*., 2000). Antihypertensive mechanism of *T. terrestris* was studied in 2K1C hypertensive rats by measurement of circulatory and local ACE activity in aorta, heart, kidney and lung. The systolic blood pressure and ACE of *T. terrestris* fed hypertensive rats was significantly decreased compared to hypertensive rats indicating a negative correlation between

consumption of *T. terrestris* and ACE activity in serum and different tissues in 2K1C rats (Sharifi *et al*., 2003). An another research study indicated that dietary intake of *T. terrestris* can significantly lower serum lipid profiles, decrease endothelial cellular surface damage and rupture and may partially repair the endothelial dysfunction resulting from hyperlipidemia in New Zealand rabbits fed a cholesterol-rich diet (Altug Tuncer *et al*., 2009).

Antimicrobial activity: Antimicrobial activities of *T. terrestris* are reported to vary with the origin of the plant and the part of the plant used. The ethanolic extracts of Yemeni *T. terrestris* did not show antibacterial activity against bacteria tested whereas the methanolic/ethananolic extracts of different parts (fruits, roots and stems with leaves) of Iranian, Indian or Turkish *T. terrestris* inhibited the growth of different microorganisms tested (Bedir & Khan, 2000; Ali *et al*., 2001; Kianbakht & Jahaniani, 2003). Among the seven different saponins tested from *T. terrestris*, only the spirostanol saponins showed antifungal activity against *C. albicans* and *Cryptococcus neoformans*, while none of the furostanol derivatives exhibited inhibitory activity. Further, these compounds were not effective against *S. aureus*, *Aspergillus fumigatus*, *P. aeruginosa* and *Mycobacterium intracellulare* (Bedir *et al*., 2002). Antimicrobial activity of organic and aqueous extracts from fruits, leaves and roots of *T. terrestris* from Iraq was examined against 11 species of pathogenic and non-pathogenic microorganisms. All the extracts from the different parts of the plant showed antimicrobial activity against most tested microorganisms. The most active extract was ethanol extract from the fruits with a minimal inhibitory concentration of 0.15 mg/ml against different bacteria and fungi (Al-Bayati & Al-Mola, 2008).

Anti-cancerous activity: Among the different saponins analyzed from different parts (stem & fruit) of *T. terrestris* collected from different regions (Bulgaria, China & India), only spiro compounds exhibited remarkable activity (Kostova & Dinchev, 2005). The most active spirostanol glycoside (Hecogenin) exhibited a broad range of anticancer activity against cell lines SK-MEL, KB, BT-549 and SKOV- 3 (Bedir & Khan, 2000; Bedir *et al*., 2002). Dioscin & also prosapogenin A of dioscin showed anti-cancerous activity against the cancer cell line K562 *in vitro* (Hu *et al*., 1996). Protodioscin proved to be cytotoxic against cell lines from leukemia and solid tumors and particularly 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 (Hu & Yao, 2002). Based on computer analysis of methylprotodioscin as a seed compound, a potential novel mechanism was suggested for its anti-cancer action (Hu & Yao, 2003). The saponin mixture from Chinese origin on Bcap37 breast cancer cells had potent inhibitory effects in a concentration dependent manner (Sun *et al*., 2003). In addition, it has been reported that the extract of *T. macropterus*, which is in the same family as *T. terrestris*, has cytotoxic activity against a human liver cancer cell line. Since long, *T. terrestris* extracts have been used as anticancer therapy in oriental medicine, however the mechanisms of these effects have not been well elucidated.

Anthelmintic activity: Anthelmintic activity was observed only in the 50% methanol extracts of Indian *T. terrestris* (whole plant) using the nematode *Caenorhabditis elegans*. Further investigation revealed tribulosin and sitosterol glucoside as active components responsible for Anthelmintic activity (Deepak *et al*., 2002).

Miscellaneous uses: Lin *et al*., (1999) investigated the use of *T. terrestris* fruits for treatment of vitiligo with positive results. The lyophilized saponin mixture of the plant caused a significant decrease on peristaltic movements of isolated sheep ureter and rabbit jejunum preparations and it was proposed that 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. It was showed that the saponins could lower the levels of serum TC, LDLc and liver TC, TG and increase the activities of superoxide-dismutase in liver (Chu *et al*., 2003). Hypoglicemic effect of saponins from *T. terrestris* was investigated and the saponins were found to reduce the level of serum glucose significantly (Li *et al*., 2002).

Conclusion

The herb *T. terrestris* is a well known and 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. Different derivatives of tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin, diosgenin, ruscogenin, chlorogenin and sarsasapogenin are found in the different parts of this herb originated from different regions. 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, depending upon on the origin of the plant. 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. *T. 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. The biodiversity of this plant on molecular basis have not been studied in detail and detail studies should be carried out. Little information is available on the molecular markers. Similarly, there is little genomics work done on this important plant and EST data base is not available for *T. terrestris*. Development of analytical methods for determination of different saponins in mixtures and molecular exploitation of *T. terresstris* are areas for future research.

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