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An Overview On Chemical, Pharmacological and Botanical Aspects of *Tribulus pentandrus* Forssk

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In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract
Tribulus pentandrus Forssk. belongs to the genus Tribulus L., a genus that was previously considered one of the main genera of Zygophyllaceae R. Br. but later, it was transferred to the newly established family Tribulaceae Hadidi. Several databases were searched to collect data for this review within the scope of phytochemistry, bioactivity, and plant taxonomy such as Web of Science, Scopus, PubMed, Google Scholar, Reaxys, and Jstor from 1890 until now. Several chemical classes were previously reported from T. pentandrus extract, with flavonoids and steroidal saponins being the most commonly reported classes of compounds. Other reported chemical classes included amides, fatty acids, alkaloids, derivatives of cinnamic acid, and phytosterols. The plant's medicinal value was also reviewed with declaring the conflicts that occur while identifying this plant and the possible consequent biological application inefficiency. This is the first integrated review to cover the chemical, biological, molecular, and botanical previously reported literature.
Keywords: Tribulus pentandrus; Zygophyllaceae; Medicinal applications; Phytochemical constituents; Taxonomic treatment; Molecular data

1. Introduction

Zygophyllaceae R.Br. taxa are distributed in the Old and New World's tropical and arid regions. Many species of the family are economically important and they have been used for timber as in Guiacum spp., or for waxes and medicinal resins (Bulnesia spp.). Moreover, some were used for their edible fruits (*Balanites* spp.) and dyes, as well as incorporating them in different medicines (*Peganum harmala*) [1].

Tribulus L. is a polymorphic genus belonging to family Zygophyllaceae R.Br. and possesses about 32 accepted species [2] that prefer to grow under arid and semi-arid ecological conditions along Mediterranean (southern), Western Asia (Arabia to Pakistan), Himalayas, India, Mauretania to Sudan, and Somalia, while being introduced in Ecuador and South America [3]. Many of its species have significant biological applications, and some of them exist in markets as health and nutritional supplements [4].

According to El Hadidi [5], *Tribulus* species are highly polymorphic and show morphological variations under different ecological conditions and growth stages that may cause conflicts when identifying them. So, taxonomic studies were necessary for solving such conflicts to ensure accurate identification of medicinal plants and, in turn, ensure their made-drug efficiency [6]. Several taxonomic works dealt with *Tribulus pentandrus* Forssk. for solving the problem of the plant misidentification due to its similarity to some other *Tribulus* species as *T. longipetalus* Viv., *T. alatus* Del., *T. megistopteris* Kralik, *T. bimucronatus* Viv., *T. parvispinus* C. Presl, *T. ochroleucus* (Maire) Ozenda and Quézel, and *T. mollis* Ehrenb. ex Schweinf. [5, 7-12].

T. pentandrus Forssk. was reported to possess a rich content of flavonoids, steroidal saponins, and alkaloids, as well as cinnamic acid derivatives, amides, fatty acids, and phytosterols that provided it with biological effectiveness against various diseases [13-15].

This review aims to provide the available literature about *T. pentandrus* from chemical, botanical, molecular, and biological perspectives, as well as the previously reported taxonomic works that dealt with declaring the aspects of similarity among *T. pentandrus* and other *Tribulus* species as a step for

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solving any possible authentication conflicts that negatively could affect their biological applications.

2. Botanical aspects

2.1. Distribution

T. pentandrus is an annual or perennial species. It prefers to grow on waste grounds and sandy plains. It is native to North Africa, (doubtfully recorded in Algeria), Sahara desert to India and South Africa, introduced in to Ecuador (Fig. 1) [3].

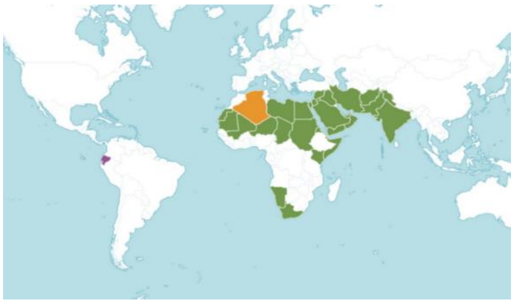

Fig. 1: World distribution of *T. pentandrus* native localities (adopted from: https://www.kew.org).

2.2. Morphological characterization

Tribulus species are generally annual or biennial, prostrate or erect. Leaves compound paripinnate with 5-10 unequal leaflets with oblique bases. Flowers solitary, floral parts inserted on 10-lobed annular disc; stamens 5-10. Fruit schizocarp (Fig. 2, A).

The mature carpels provide the most reliable characters for distinguishing *Tribulus* species, as they show considerable variations in the shape of spines and wings during the development and ripening of the fruit. This may explain the confusion of false interpretations that are commonly seen [5]. According to Hosni [16], the most reliable characters for *Tribulus* species, which are of significant systematic value, are those of mature carpels and the size of flowers. Whereas, variable characters such as habit of the plant and hairiness degree are of minor systematic value.

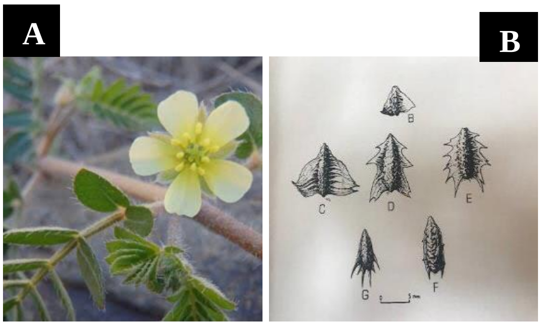

Fig. 2. A: *Tribulus pentandrus* (Vegetative part), **B:** Different forms of *T. pentandrus* fruits: (B: var. *micropterus*; C-F: var. *pentandrus* G: var. *acanthopterus*) [16].

2.3. Plant classification

There is no satisfying classification of the genus *Tribulus*. Engler [17] distinguished three groups of species on the basis of mericarps appendage characters that varied from being spiny (with 2-4 spines per carpel), to others with two lateral wings or being spineless. Engler [18] subdivided Zygophyllaceae into seven subfamilies, concluding many tribes, of which *Tribulus* was grouped to tribe Tribuleae within the subfamily Zygophylloideae. However, El Hadidi [19, 20] proposed a new family of Tribulaceae based on Engler's tribe, Tribuleae.

El Hadidi [5] proposed a new classification of *Tribulus* based on the characteristics of mature carpels. It was divided into three sections: *Terrestris* Hadidi (includes *T. terrestris*) with spiny carpels; *Alata* Hadidi (includes *T. pentandrus*) characterized by winged carpels; and *Inermis* Hadidi with unarmed carpels.

Tribulus pentandrus Forssk. is one of genus *Tribulus* species placed in Section Alata Hadidi due to possessing detectable winged mericarps. It is believed to be the first reported *Tribulus* with winged mericarps [10]. Confusingly, this plant appeared very similar to *T. longipetalus* Viv. and *T. alatus* Del.. So, El Hadidi [5] examined their type specimens and realized that *T. pentandrus* and *T. longipetalus* possessed the same characters and reported them as conspecific species, but he ignored any detailed descriptions about *T. alatus* Del.. Later on, El Hadidi [21] reported *T. alatus* and *T. longipetalus* as synonyms of *T. pentandrus* Forssk. in the Flora of Tropical East Africa.

A high level of similarity also existed between *T. pentandrus* and *T. megistopteris* Kralik (Syn. *T. pterocarpus*), but Mandaville [9] proved that they were different and related this confusion to species polymorphism [8].

Moreover, *T. pentandrus* and *T. bimucronatus* were considered conspecific species due to defects in the identification process, and some specimens of *T. pentandrus* possessed small flowers and unarmed mericarps with reduced basal spinules that misled to identifying them as *T. bimucronatus* [5]. Another reported mistake was the wrong identification of *T. parvispinus*, which is characterized by possessing spiny fruits, as being *T. pentandrus*, a failure that resulted from the difficulty in detecting those spines in immature fruit specimens. Also, confusion between *T. ochroleucus*, which has basal small spines, and *T. pentandrus* was also reported. *T. mollis* was described as having unarmed fruits, but its isotype that existed in Kew showed fruits with dentate wings, a character that is characteristic of *T.*

pentandrus, and this caused confusion that was solved by examining the plant isotype present in Stockholm that showed unarmed fruits as stated in *T. mollis* original description [5, 8].

2.4. Comparative anatomical investigations

A comparative anatomical investigation was performed for a number of Egyptian *Tribulus* species, which revealed the existence of anatomical distinctive characters that could help in distinguishing *T. pentandrus* from its close and similar species [22]. The leaflet-blade of *T. pentandrus* possessed one basal vascular bundle, which distinguished it from *T. terrestris*, *T. mollis*, and *T. megistopterus*, whose leaflet-blades had two basal vascular bundles. This noticeable difference could help in solving the frequent confusion that was previously reported by many morphological and chemical studies between *T. pentandrus* and *Tribulus* species like *T. terrestris*, *T. mollis*, and *T. megistopterus* [8, 11, 12]. Moreover, this anatomical distinction was in accordance with Saleh *et al.* [7] results that realized the difference between *T. pentandrus* and *T. terrestris*.

Another comparative anatomical study that involved *T. pentandrus* Forssk., *T. longipetalous* Viv., and *T. terrestris* L., reported their highly similar anatomical structures. Little differences were recorded among them, including variations in stem and leaflet hairness degrees as well as the existence of sclrenchymatous fibers embedded in pith of *T. pentandrus* and *T. longipetalous* and their absence from *T. terrestris* [23].

2.5. Palynological characters

Pollen grains of Tribulaceae are polypantoporate, spheroidal, sometimes slightly elliptical, and reticulate, in which each lumen contains a pore [24].

The palynological description of both *T. pentandrus* and *T. terrestris* didn't show any significant differences, as both of them possessed pantoporate, spheroidal, reticulate pollen grains of nearly equal sizes [25].

3. Chemical aspects

3.1. Chemical constituents of T. pentandrus

Many authors dealt with the isolation and identification of different chemical classes from *T. pentandrus*, such as flavonoids and saponins; all the identified compounds are reported in **Table 1**. While Hilmi *et al*. [15] reported the existence of many other biologically effective chemical classes including, alkaloids, amides, lignans, cinnamic acid derivatives, phytosterols, and fatty acid compounds. Moreover, they detected the compound's percents of many of those classes from different parts of the plant using GC-MS technique.

3.2. Chemotaxonomic characters of T. pentandrus

*C*hemical investigation was performed by El Nur *et al*. [12], where the phenolic constituents of three *Tribulus* species (*T. terrestris*, *T. pentandrus*, *T. bimucronatus*) were detected using TLC. The paired affinities among the chemical profiles of the studied taxa were estimated, and an average paired affinity of 68.4% was reported among the three *Tribulus* species, despite the stem and fruit chemical constituents of *T. terrestris* and *T. pentandrus* were more similar and different from those of *T. bimucronatus.* Those chemical results were inconsistent with the morphological variations in the studied plants, where *T. pentandrus* shows much more similarity with *T. bimucronatus* than with *T. terrestris*.

This difference between *T. pentandrus* and *T. terrestris* was supported by Saleh *et al*. [7], who proved the existence of difference in glycosylation patterns of flavonoids between the two species based on kaempferol, quercetin, isorhamnetin and tricin skeleton. It was found that both species possessed quercetin 3-*O*-gentiobioside as their first major flavonoid glycoside, whereas they differed in their second major glycoside, quercetin 3-*O*-rutinoside for *T. terrestris*, but in *T. pentandrus*, kaempferol 3-*O*-gentiobioside, kaempferol 3-*O*-gentiobioside-7-*O*-glucoside, and quercetin 3-*O*-gentiobioside-7-*O*-glucoside were its second major ones. Moreover, *T. pentandrus* was characterized by a larger variation of kaempferol glycosides, unlike *T. terrestris*, which possessed a wider variation of isorhamnetin glycosides (Fig. 3).

Another chemical profiling process was performed using HPLC-ESI-MS for *T. pentandrus*, *T. megistopterus* subsp. *pterocarpus*, and *T. parvispinus* which helped in detecting their contents of steroidal saponins that varied from cholestane-, spirostane- to furostane- aglycones and their relative glycosides. Comparing their chemical profiles, *T. pentandrus* showed a high similarity to *T. megistopterus* subsp. *pterocarpus*, whereas *T. parvispinus* was noticeably different from them [11].

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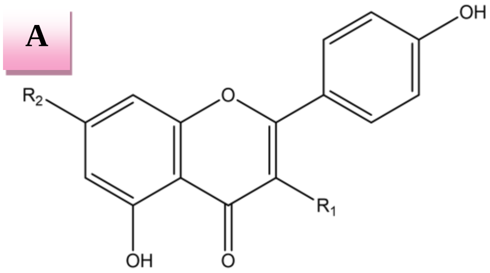
Table (1): Chemical compounds isolated and identified from *T. pentandrus*.

No.	Compound	Plant part	Reference
	Flavonoids		
1	Kaempferol -3-*O*-glucoside	Whole plant/ Aerial parts	[7, 26]
2	Kaempferol-7-*O*-glucoside	Whole plant	[7]
3	Kaempferol -3-*O*-gentiobioside	Whole plant	[7]
4	Kaempferol 3-*O*-(3″-*O*-*E*-*p*-coumaroyl)-α-D-glucopyranoside	Whole plant	[7, 26]
5	Tribuloside; Kaempferol 3-*O*-(6″-*O*- *E* -*p*-coumaroyl)-α-D-glucopyranoside	Aerial parts	[26]
6	Kaempferol 3-*O*-(3″,6″-di-*O*-*E*-*p*-coumaroyl)- *β*-D-glucopyranoside	Aerial parts	[26]
7	Kaempferol -3,7-*O*-diglucoside	Whole plant	[7]
8	Kaempferol -3-*O*-gentiobioside-7-*O*-glucoside	Whole plant	[7]
9	Quercetin -3-*O*-glucoside	Whole plant/ Aerial parts	[7, 26]
10	Quercetin -7-*O*-glucoside	Whole plant	[7]
11	Quercetin -3-*O*-gentiobioside	Whole plant	[7]
12	Quercetin -3-*O*-rutinoside	Whole plant	[7]
13	Quercetin -3-*O*-gentiotrioside	Whole plant	[7]
14	Quercetin -3,7-*O*-diglucoside	Whole plant	[7]
15	Quercetin -3-*O*-gentiobioside-7-*O*-glucoside	Whole plant	[7]
16	Quercetin -3-*O*-rutinoside-7-*O*-glucoside	Whole plant	[7]
17	Isorhamnetin -3-*O*-gentiobioside	Whole plant	[7]
18	Isorhamnetin -3-*O*-coumaroylglucoside	Whole plant	[7, 26]
19	Isorhamnetin -3,7-*O*-diglucoside	Whole plant	[7]
20	Isorhamnetin -3-*O*-gentiobioside-7- *O*-glucoside	Whole plant	[7]
21	Tricin -7-*O*-diglucoside	Whole plant	[7]
	Saponins		
22	Pentandroside A	Aerial parts	[27, 13, 11, 26]
23	Pentandroside B	Aerial parts	
24	Pentandroside C	Aerial parts	
25	Pentandroside D	Aerial parts	
26	Pentandroside E	Aerial parts	
27	Pentandroside F	Aerial parts	
28	Pentandroside G	Aerial parts	
29	(22S,25S)-11α,16*β*,22,26- tetrahydroxycholest-4-en-3-one 16-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranoside	Aerial parts	[11]
30	(25S)-α-spirostan-3*β*-ol-3-O-β -D-glucopyranosyl-(1→2)-O- [β-D-glucopyranosyl-(1→3)]-O-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside	Aerial parts	[26]
31	(25S)-26-*O*-*β*-D-glucopyranosyl-5α-furostan-3*β*,22α,26-triol-3-*O*-*β*-D-galactopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside	Aerial parts	[26]
32	(25S)- 26-*O*- *β*-D-glucopyranosyl-5α -furostan-3-*β*,22 α,26-triol-3-*O*-*β* -D-glucopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*O*- *β* -D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside	Aerial parts	[26]
33	(22S,25S)-16-*O*- *β*-D-xylopyranosyl-5α -cholestan-3-*β*,16- *β*,22, 26-tetraol-3-*O*- *β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*O*- *β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside.	Aerial parts	[26]
34	(25S)-5α-spirostan-2α, 3 *β*-diol-3-*O*- *β*-D-galactopyranosyl-(1→2)-O-[*β*-D-glucopyranosyl-(1→3)]-O- *β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside	Aerial parts	[26]
35	(25S)-5 α-spirostan-3-*β*-ol-3-*O*- *β*-D-galactopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]- *O*- *β*-D-glucopyranosyl-(1→4)- *β*-D-galactopyranoside.	Aerial parts	[26]
36	(22S,25S)-16 *β*,22,26-trihydroxycholest-4-en-3-one-16-*O*- *β*-D-xylopyranoside.	Aerial parts	[26]

A



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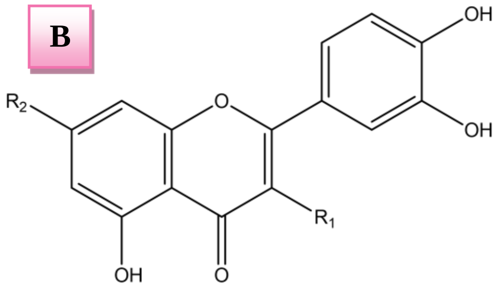


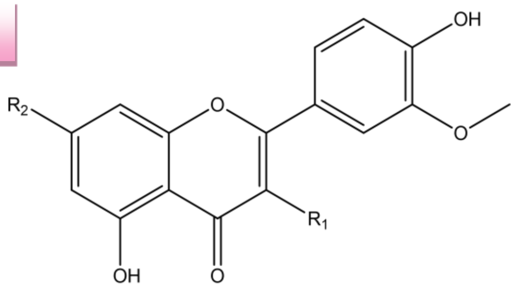
Kaempferol derivatives

(1) R1 = Glucose, R2= OH.
(2) R1= OH, R2 = Glucose.
(3) R1 = Gentiobiose, R2= OH.
(4) R1 = 3′′-*O*-E-*p*-coumaroyl -glucose, R2= OH.
(5) R1 = 6′′-*O*- E -*p*-coumaroyl -glucose, R2 = OH.
(6) R1= Dicoumaroyl-glucose, R2= OH.
(7) R1= Glucose, R2 = Glucose.
(8) R1 = gentiobiose, R2 = Glucose.

Quercetin derivatives

(9) R_1 = Glucose, R_2= OH.
(10) R_1 = OH, R_2 = Glucose.
(11) R_1 = Gentiobiose, R_2= OH.
(12) R_1 = Rutinose, R_2= OH.
(13) R_1 = Gentiotriose, R_2= OH.
(14) R_1 = Glucose, R_2 = Glucose.
(15) R_1 = Gentiobiose, R_2 = Glucose.
(16) R_1 = Rutinose, R_2 = Glucose.

C



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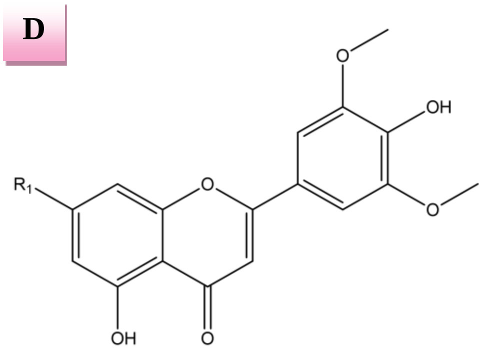

Isorhamnetin derivatives

(17) R_1 = Gentiobiose, R_2= OH.
(18) R_1 = *p*-coumaroylglucose, R_2= OH.
(19) R_1 = Glucose, R_2 = Glucose.
(20) R_1 = Gentiobiose, R_2 = Glucose.

Tricin derivative

(21) R_1= OH, R_2 = Diglucose.

Fig. 3. Structures of flavonoids identified from *T. pentandrus*.

3.3. Pharmacological aspects

In India, the stem, leaves, root, fruits, and seeds of *T. pentandrus* are traditionally used in folk medicine as anti-inflammatory agents, aphrodisiacs, astringents, cooling effects on the body, diuretics, general tonics for rejuvenation, kidney disease, menstruation, urinary bladder disease, and urination disorder [28]

The phytochemical analysis of *T. pentandrus* revealed a noticeable high total flavonoid content. Moreover, many studies reported its high contents from steroidal saponins that exceeded its value as a medicinal plant due to its diverse chemical composition [11, 14]. Ahmed *et al.* [14] reported the high antidiabetic activity of its seeds. Moreover, the wide antibacterial activity of *T. pentandrus* was reported by Mahalel [13], who related its activity to the production of steroidal saponins. Pentandroside A is one of its isolated saponins. It is a cholestane-type saponin that proved to have high effectiveness against different gram-positive and gram-negative bacterial strains (*S. aureus*, *E. coli*, *S. marcescens*, *P. putida*, and *B. cereus*) and was found to be more effective if compared with ampicillin, cephaloridine, and pencillin G [13]. On the other side, Olas *et al.* [29] evaluated the effect of *T. pentandrus* crude extract on blood platelet adhesion as a step for evaluating its antiplatelet activity, which revealed its low significance for this purpose. El Tantawy *et al*. [30] investigated the effect of *Tribulus pentandrus* (= *T. alatus*) on free serum testosterone levels in male rats, and they revealed that the plant possessed an aphrodisiac activity as a result of its androgen elevating effect. Goswami *et al.* [31] studied the effect of the steroidal extract of *Tribulus alatus* on semen parameters in adult male mice. The result showed a significant enhancement in sperm concentration and motility. The increase in testosterone levels by *T. pentandrus* promoted protein synthesis activity.

3.4. Molecular aspects

Traditional taxonomy depends on classifying plants based on their morphological characteristics, and it has to take into consideration maturity stages of the specimens. Therefore, modern molecular techniques like DNA fingerprinting and DNA barcoding are found to be more efficient as taxonomic tools. DNA barcoding is based on utilizing variable short DNA regions called DNA barcodes like ITS, rbcL, trnH-psbA and matK which are universal DNA barcodes that are ideal for identifying plants down to the species level [32]. Thus, several works depended on DNA barcoding for authenticating *T. pentandrus* together with its morphologically close species, *T. terrestris* using the universal DNA barcodes: ITS, rbcL, and matK [33, 34]

4. Conclusions

Despite, *T. pentandrus* is a promising medicinal plant due to its chemical constituent variability, it faces obstacles that limit its medical uses. This plant identification process is not so easy as it shows close morphological similarity to many other *Tribulus* species. Those identification conflicts negatively may lead to suspected plant-made drug efficiency. This review helped in overviewing all the previous trials for solving such conflicts with dependence on chemical, molecular, and different botanical investigations in a step for ensuring the plant authentication and it's made-drugs efficiency.

5. Conflicts of interest

There are no conflicts to declare.

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