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Original

Phytochemical Analysis of Ethanolic Extracts of Three Sudanese *Tribulus species*

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Abstract:

This study aims to explore the phytochemically bioactive compounds of three Sudanese *Tribulus species.* Threewild species of Tribulus; *T. terrestris, T.longipetalous* and *T.pentandrus,*were collected from KhartoumState. Their bioactive compounds were extracted by ethanol, from their roots, aerial parts, and fruits. Gas-Chromatography-Mass Spectroscopy (GC-MS) was used for phytochemical analysis of their bioactive compounds.In *T. terrestris* different compounds (20 from roots), (27 from aerial parts) & (29 from fruits) were identified by GC/MS. Six classes of chemical compounds were found; sugars, fatty acids, terpenes, steroids, alkanes and alcohols. Major components were sucrose (28.72%) in roots and 4-O-methyl-mannose in aerial parts (49.60%) and in fruits (43.66%).

In *T.longipetalous* many compounds were identified; 23 from roots, 20 from aerial parts, and 29 from fruits. These compounds were classified into four groups; Sugars, fatty acids, terpenes alcohols. The most commonly detected compound was 4-O-methyl mannose; 73.11% (in roots), 43.24% (in aerial parts) and 35.75% (in fruits). In *T.pentandrus* more compounds were identified, 31 (roots), 31 (aerial parts) and 42 from fruits. These compounds were grouped into eight classes; sugars, fatty acids, terpenes, steroids, alkanes, alcohols, steroidal sapogenins and alkaloids. The major component also was 4-O-methylmannose 47.86 % (in roots), 44.75 % (in aerial parts) and 55.05 % (in fruits).The most commonly found classes of compounds in the three *Tribulus species* were; sugars, fatty acids, terpenes, steroids and alcohols.

The major compound found in all parts of the three *Tribulus species* was 4-O-methylmannose. In addition other compounds were identified from *T.pentandrus*

Keywords:*Tribulus sp*, phytochemical analysis, ethanolic extracts, sucrose, 4-O-methylmannose, gamolenic acid, alkaloids, saponins.

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Introduction:

Tribulus species are a group of annual herbs belonging to theZygophyllaceae family with 25 genera and 250 species. It is widely distributed in the tropical and subtropical regions. Their leaves, fruits and roots contain pharmacologically important metabolites such as phytosteroids, flavonoids, alkaloids and glycosides. Different *Tribulus species*are widely used in traditional folk medicine from Greek civilization, to India, China, Europe, Asia, Africa and Latin America[1].

Tribulus is of therapeutic, medicinal and pharmaceutical interest as it contains; saponins,flavonoids, alkaloids, glycosides, phytosterol, lignanamides, tannins, terpenoids, amides derivatives (cinammic) and proteins. The most important bioactive metabolites were Steroidal saponins and flavonoids which are used for muscle building, conditioning and treatment of certain ailments [1].

The aqueous fresh extract of the whole plant contains inorganic nitrates, mostly potassium nitrate, that has diuretic effects. The *Tribulus* species extract is also used for urinary dysfunction, asthma, ophthalmic and different diseases [2, 3] and has been shown to have antihypertensive properties and protection against oxidative stress and also has vasodilating properties [4, 5]. It may exhibit antitumor, cytotoxic, antifungal and anthelmintic properties [6].

In *traditional* medicine *T. terrestris* is believed to have aphrodisiac properties for treatment of male's infertility [7]. Saponins formulation from *T. terrestris* were applied for veterinary production in Bulgaria, as it effectively stimulated their spermatogenesis, libido sexuality [8,9]. *T. terrestris* extracts increased sexual function in rats, which attributed to increase in testosterone, dihydrotestosterone, and Dehydroepiandrosterone. Tribestan is a Bulgarian formulation widely used for treatment of infertility and libido disorders in men and women [10,11]. The furastanol saponins (protodioscin, protogracilin) from *T .terrestris* increased male spermatogenesis via Luteinizing Hormone which stimulated secretion of Testosterone that significantly improved sperms quality and quantity [12, 13].

T.pentandrus extracts were used in different pharmacological, and clinical trials including anticancer [14], ant cholinergic, antifilarial [15] anti-malarial [16] and as a CNS depressant and stimulant [17], hypoglycemic effect [18] immunologic effect, smooth muscle relaxant and stimulant activity. [19, 20].

The present study describes the chemical profiles of ethanolic extracts of roots, aerial parts and fruits of three Sudanese *Tribulus species:Tribulus, T. terrestris,*

T.longipetalous and *T.pentandru* growing wild in Khartoum State, Central Sudan.

Materials and Methods:

Plant materials:

The roots, aerial parts (leaves and stem) and fruits from three *Tribulus species*; *T. terrestris, T. longipetalus and T.pentandrus* were collected from the Faculty of Agriculture, University of Khartoum, Shambat, Khartoum North.

Preparation of extracts:

From each plant a sample of 20g were mixed with 120 mL of 80% ethanol in 500 mL conical flask. The conical flask was sealed tightly with aluminum foil for 10 days at room temperature. The filtrate was transferred to Petri dishes; the ethanolic crude extract was collected after evaporation.

Gas Chromatography /Mass Spectrophotometry :

The qualitative and quantitative analysis of the sample was carried out by using GM/MS apparatus (Model; GC/MS-QP2010-Ultra, Japan) with capillary column (Rtx-5ms-30m×0.25 mm×0.25µm). The sample was injected by using split mode, Helium as the carrier gas passed with flow rate 1.61 mL/min, the temperature program was started from 60^{o} C with rate 10c/min to 300^{o} C as final temperature degree with 3 min hold time, the injection port temperature was 300^{o} C, the ion source temperature was 200 300^{o} C and the interface temperature was 250^{o} C. The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 26 min.

The samples contents were analyzed and identified by comparing their retention times and mass fragmentation patterns with already deposited data in the library, of the National Institute of Standards and Technology (NIST).

Results and Discussion:

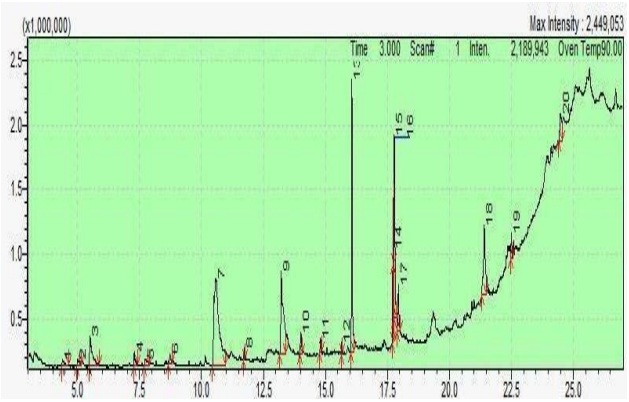
The chemical composition of ethanolic extract of *Tribulus terrestris*:

GC/MS chromatogram of ethanolic extract of roots of *T.terrestris* (Fig.1) clearly showed 20 peaks indicating the presences of 20 compounds. The major compounds were listed in Table.1; sucrose (28.72%), 1-(+)–ascorbic acid, 2,6-dihexadecanoate (13.34%); alpha-d-manno-furanoside, methyl (13.18%) 9,12,15–octa decatrienoic acid (Z,Z,Z) – (8.54%); diosgenin (8.32%) and cyclopentane1-acetyl-1,2 –epoxy (4.99%).

GC/MS analysis of aerial parts extracts of *T.terrestris* identified 27 compounds (Fig.2). The main compounds were; 4-O-methylmannose (49.60%), gamolenic acid (10.73%), 1-(+)–ascorbic acid 2,6-dihexadecanoate (10.64%),

Sucrose (6.33%) and phytol acetate (3.33%) (Table.1).

GC/MS chromatogram of ethanolic extract of fruits of *T.terrestris* (Fig.3) showed the presence of 29 compounds. The major compounds were: 4-O-methylmannose (43.66%), 1-(+) – ascorbic acid 2,6-dihexadecanoate (10.35%), linoleic acid ethyl ester (5.94%), 5-hydroxy methyl furfural (5.37%), gamolenic acid (5.15%) and 4-o-methylmannose (4.06%) (Table.1).



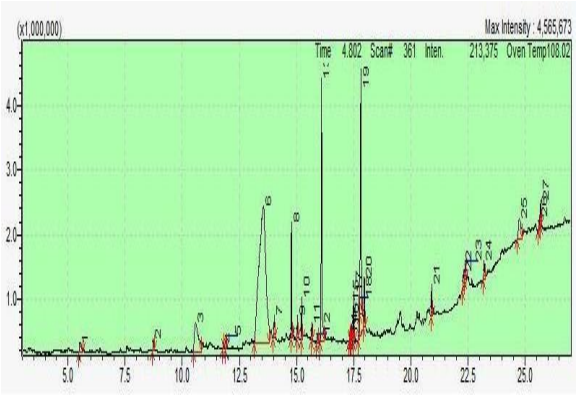


Fig.1 GC/MS chromatogram
Fig.2 GC/MS chromatogram of

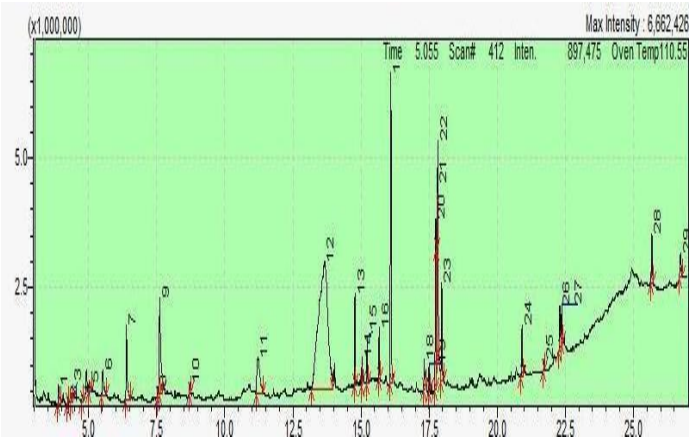


Fig.1

T. terrestris roots
T. terrestris aerial parts

GC/MS chromatogram of *T. terretris* fruits

The chemical compositions of ethanolic extract of *Tribulus longipetalous*:

GC/MS chromatogram of ethanolic extract of roots *T.longipetalous* (Fig.4) 23 compounds was identified. The major compounds were (Table.1); 4-O-methylmannose (73.11%), 1-(+)–ascorbic acid 2,6-dihexadecanoate (12.75%), sucrose (11.92%), 9,12,15 – Octadecatrienoic acid (Z,Z,Z) (5.56%), 9,12–octadecadienoic acid (Z,Z)-, methyl ester (5.30%) and 9 – octadecenoic acid methyl ester (e) (4.99%).

Twenty compounds were detected in the aerial parts of *T. longipetalous* (Fig.5) (Table.1).

The major compounds were; 4-o-methylmannose (43.24%), 1-(+)–ascorbic acid 2,6-dihexadecanoate (14.72%), oleic acid (10.08%), 9,12,15 octadecatrienoic acid (Z,Z,Z) – (8.76%); phytol acetate (4.69%) and octadecenoic acid (4.73%).

Analysis of *T. longipetalous* fruits extract detected 29 compounds (Fig.6) (Table.1). The compounds were; 4-o-methylmannose (35.75%); octadec-9-enoicacid (23.35%), palmitoleic acid (1.86%), stigmastane (5.18%); squalene (4.25%) and octadecenoic acid (4.19%).

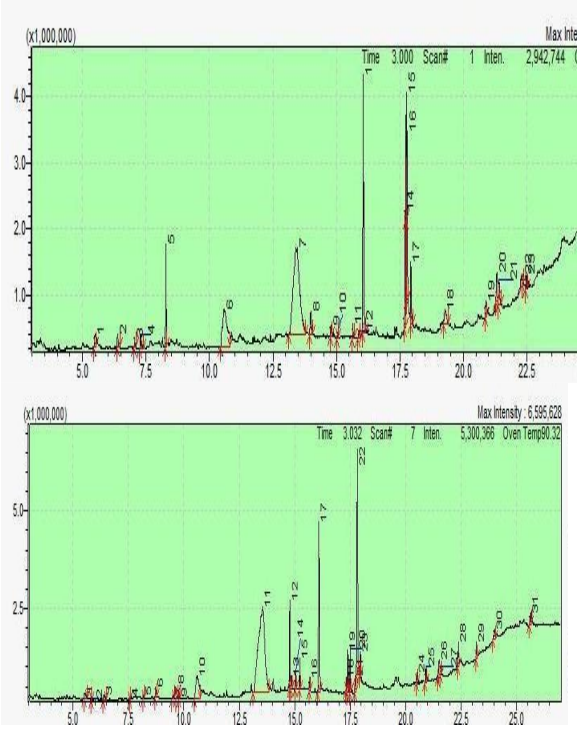


Fig.4 G

C/MS chromatogram of

Fig.5 GC/MS chromatogram of

T. longipetalous roots

T.longipetalous aerial parts

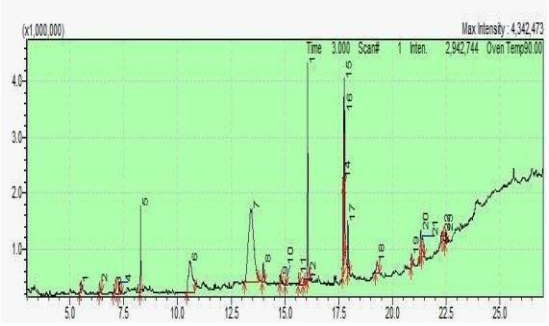


Fig.6 GC/MS chromatogram of *T.longipetalous* fruits

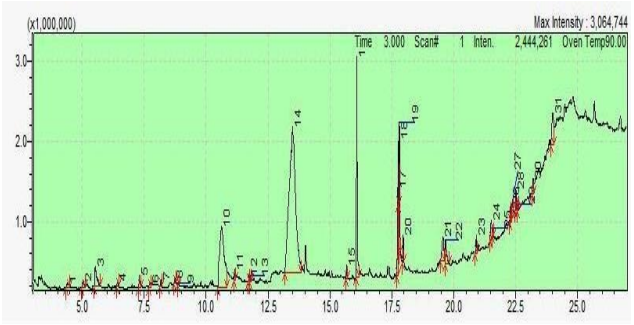
The chemical contents of ethanolic extract of *Tribulus pentandrus*:

Thirty one compounds were identified from extracts of *T.pentandrus* roots (Fig.7, Table.1). The compounds were; 4-o-methylmannose (47.86%), sucrose (14.55%), 1-(+)-ascorbic acid 2,6-dihexadecanoate (8.87%), gamolenic acid (4.23%), 5-alpha-hydroxy-6-beta-methyltigogenin (3%) and diosgenin (1.46%).

Thirty one compounds were detected in aerial parts of *T.pentandrus* (Fig.8, Table.1). The compounds were; 4-o-methyl mannose (44.75%); gamolenic acid (15.215%), 1-(+)–ascorbic acid 2,6-dihexadecanoate (10.14%), sucrose (6.16%) and diosgenin (2.08%).

Forty two compounds were identified in fruits of *T.pentandrus* (Fig.9, Table.1). The compounds were; 4-O-methylmannose (55.05%), 1-(+)–ascorbic acid 2,6-dihexadecanoate (10.97%); Linoleic acid ethyl ester (3.96%); Octadecenoic acid (3.30%); Sucrose (3.27%) and Diosgenin (1.55%) as the major phytochemical constituents.

Chemical compounds	***T. terrestris***			***T.longipetalous***			***T.pentandrus***		
	Roots	**Aerial Parts**	**Fruits**	**Roots**	**Aerial Parts**	**Fruits**	**Roots**	**Aerial Parts**	**Fruits**
Sucrose	28.72	6.33	-	11.92	-	-	14.55	6.16	3.27
1-(+)–Ascorbic acid.2,6-dihexadecanoate	13.34	10.64	10.35	12.75	14.72	-	8.87	10.14	10.97
alpha-d-manno-furanoside,methyl	13.18	-	-	-	-	-	-	-	-
Linoleic acid	8.54	-	-	5.56	8.76	-	-	-	-
Diosgenin	8.32	-	-	-	-	-	1.46	2.08	1.55
cyclopentane,1-acetyl-1,2 –epoxy	4.99	-	-	-	-	-	-	-	-
4-O-Methyl mannose	-	49.60	43.66	73.11	43.24	35.75	47.86	44.75	55.05
Gamolenic acid	-	10.73	5.15	-	-	-	4.23	15.21	-
Phytol acetate	-	3.33	-	-	4.69	-	-	-	-
Linoleic acid ethyl ester	--	-	5.94	-	-	-	-	-	3.96
5-hydroxy methyl furfural	-	-	5.37	-	-	-	-	-	-
9,12–octadecadienoic Acid (z,z)-,methyl ester	-	-	-	5.30	-	-	-	-	-
9 – Octadecenoic acid methyl ester	-	-	-	4.99	-	-	-	-	-
Oleic acid	-	-	-	-	10.08	-	-	-	-
Stearic acid	-	-	-	-	4.37	4.19	-	-	3.30
Octadec – 9 - enoic acid	-	-	-	-	-	23.35	-	-	-
Palmitoleic acid	-	-	-	-	-	1.86	-	-	-
Stigmastane	-	-	-	-	-	5.18	-	-	-
Squalene	-	-	-	-	-	4.25	-	-	-
5.alpha.-hydroxy-6-beta-methyl tigogenin	-	-	-	-	-	3.26	-	-	-



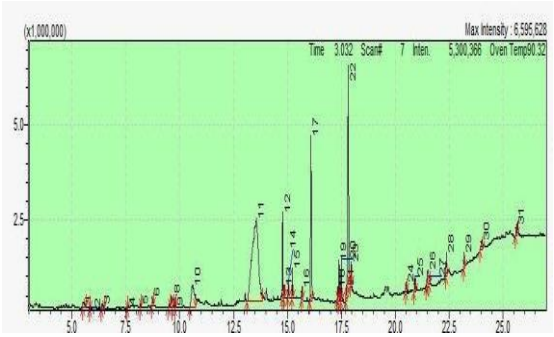


Fig.7 GC/MS chromatogram of *Tribulus pentandrus* roots

Fig.8 GC/MS chromatogram of *T.pentandrus* aerial parts

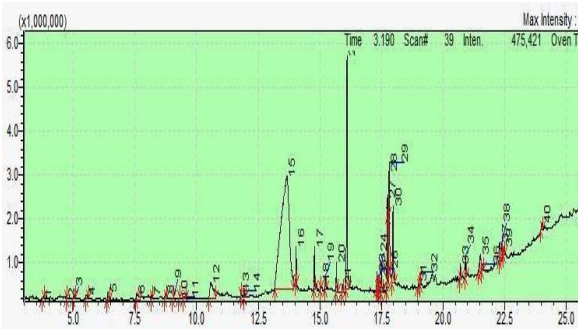


Fig.9 GC/MS chromatogram of *T.pentandrus* fruit

Table.1 Chemical compositions of three *Tribulus* species: *T. terrestris, T.longipetalous and T.pentandrus*

Chemical classes identified in studied samples:

All of the studied samples (aerial parts, fruits and roots) of *T. terrestris, T.pentandrus* and *T. longipetalus* contain **sugar** (range between 62.4% in *T.pentandrus* roots and 28.7% in *T.terrestris* roots) (Table.2). All samples (except *T. terrestris* roots) contain **terpenes** (range between 6.9% in *T. longipetalus* aerial parts and 0.3% in *T.pentandrus* roots). *Tribulus pentandrus* roots and *T. terrestris* aerial parts contain **steroids** 4.2%, 3.3% respectively (Table.2).

Alkanes were identified in *T. longipetalus* fruits (12%) and in *T.terrestris* roots (4%).

Alcohols were detected in only *T.terrestris* (4%).

Steroidal sapogenins were identified in roots of *T.terrestris, T.longipetalous and T.pentandrus* (9.5%, 5%, and 4.7%, respectively). Also **alkaloids** were found in roots of *T.pentandrus* (1%) *T. terrestris* (0.6%).

Fatty acids were detected in all species (range between 42.6% in *T. longipetalus* fruits and 20.8% in *T.pentandrus* roots). Nine fatty acid were identified T.terrestris aerial parts (29%); **Gamolenic acid** (10.8%) and **octadecenoic acid** (1.8%). Seven fatty acid components were identified. *T. terrestris* fruits (32%); **Linoleic acid ethyl ester** (6%) and Gamolenic acid (5.2%). Five fatty acid components were identified in *T. terrestris* roots (34.4%); **Linoleic acid** (8.5%) and **9-octadecenoic acid (E)** – (4.7%).

Five fatty acids were identified in *T.pentandrus* aerial parts (32.8%); **Gamolenic acid** (5.2%) and **Linoleic acid** (2%). Ten fatty acid were identified in fruits of *T.pentandrus* (28.5%); **Linoleic acid ethyl ester** (3.96%) and **Gamolenic acid** (3.6%). Six fatty acids were identified in roots of *T.pentandrus* (20.7%); **Gamolenic acid** (4.2%) and **Linoleic acid** (3%).

Fatty acid	***T. terrestris***			***T.pentandrus***			***T.longipetalous***		
	A P	**F**	**R**	**A P**	**F**	**R**	**A P**	**F**	**R**
Pentadecanoic acid	-	-	1.69	-	-	-	-	-	0.26
9,12 – octadecadienoic acid (z,z) - , methyl ester	0.16	0.70	3.31	0.99	-	-	-	-	5.30
9-octadecenoic acid (E) -	0.14	-	4.73	-	0.62	-	-	-	4.99
9,12,15 –octadecatrienoic acid (z,z,z) -	0.42	-	8.54	2.04	0.27	-	8.76	-	5.56
Octadecanoic acid	1.80	2.90	2.33	o.88	3.30	0.83	4.73	4.91	3.00
Dodecanoic acid	0.19	-	-	-	0.19	0.25	-	-	-
Tetradecanoic acid	0.95	-	-	-	1.21	-	-	-	1.94
Hexadecanoic acid methyl ester	0.52	1.42	-	0.52	0.99	0.39	0.53	0.52	0.62
Linoleic acid ethyl ester	1.60	5.94	-	-	3.96	2.96	3.87	-	-
Gamolenic acid	10.73	5.15	-	15.21	3.63	4.23	-	-	-
Oleic acid	-	1.32	-	-	1.32	1.83	10.08	-	-
Butyl 9,12,15 – octadeca trienoate	-	1.03	-	-	-	-	-	-	-
6 – Octadecenoic acid , (z) -	-	-	-	-	-	-	-	1.86	-
Octadeca-9-enoic acid	-	-	-	-	-	-	-	23.35	-

In aerial parts of *T. longipetalus,* five fatty acids were detected (42.3%); **Oleic acid** (10.1%) and **Linoleic acid** (8.8%). In its fruits four fatty acids were detected (42.6%); **Octadeca – 9 – Enoic acid** (23.2%) and **Octadecenoic acid** (4.2%). In its roots seven fatty acid were found (36%); **Linoleic acid** (5.6%) and **9,12–Octadecadienoic Acid (z,z)-, methyl ester (5.3%)**

Table.2 Classes of chemical compounds detected in three Sudanese *Tribulus species*

Chemical class	*T. terrestris*			*T.pentandrus*			*T.longipetalous*		
	A P	**F**	**R**	**A P**	**F**	**R**	**A P**	**F**	**R**
Sugars	55.93	47.72	28.72	50.90	58.32	62.41	43.24	35.75	49.03
Fatty acid	28.94	31.8	34.83	32.83	28.48	20.67	42.33	42.56	35.99
Terpenes	5.60	3.91	-	6.51	2.16	0.31	4.85	4.96	4.56
Steroids	30.30	2.75	-	-	2.54	4.23	-	1.07	1.77
Alkanes	0.91	-	4.99	1.04	1.51	2.12	-	12.03	-
Alcohols	1.59	0.61	4.07	0.75	0.24	0.91	1.14	-	0.84
Steroidal sapogenins	-	-	9.45	3.67	2.30	4.72	-	-	5.04
Alkaloids	-	-	0.57	0.41	0.40	0.95	0.34	-	-

Table.3: Fatty acids in *T. Terrestris, T.pentandrus* and *T. longipetalous*

The Phytochemical compositions of *T. terrestris* were extensively studied including, saponins, flavonoids, alkaloids, lignanamides and cinammic acid amides [17]. *Tribulus pentandrus* is rich in many potential biological substances including, steroidal saponins, flavonoids, alkaloids, amides, lignans, cinammic acid, nitrate and phytosterol [6,20,21,22,23].

From Tribulus longipetalus many scientists identified; steroidal saponins, lignin-amides, alkaloids, spirostane, furostane and cholestane nuclei and flavonoids - flavonol glycosides [20, 21,24,25].

The steroidal sapogenin was identified from the root of the *Tribulus species* (*Tt,Tl,Tp;* 9.5,5.1 and 4.7%) in the present research. Diosgenin- steroidal sapogenin has wide range of pharmacological and medicinal properties and were used for production of steroidal drugs for treatment of hyperlipidemia, inflammation, neurological diseases and as anticancers [20,21,26,27,28]. In this study, *Tribulusterrestris* roots had higher percentage of diosgenin (8.32%), however, all samples of *T.pentandrus* contain diosgenin (1.46 to 2.08%). In China saponins were reported to have cytotoxic activity against in vitro culture [29]. Another study from Korea revealed t *T. terretris* aqueous extract can induce apoptosis in human liver cancer cell [30]. A protective effect of *Tribulus terretris* alcoholic extract was reported before [31,32,33,34]

Many authors noticed reduction in the serum glucose level to normal, and decrease in creatinine level in liver and blood (Amin et al 2006; El-Tantawy and Hassanin, 2007; Bankdaran et al 2008

This study identified Linoleic acid in roots of *T.terrestris* (8.54%) and roots and aerial parts of *T.longipetalous* (5.56-8.76%), respectively.

Linoleic acid (gamma-linoleic acid - Gamolenic acid), is an essential source of omega -6 and 3 polyunsaturated fatty acid. α-Linoleic acid has neuroprotective, anti-inflammatory, antihypertension, anticholestrol and antidepressant properties Various Gamolenic acid formulations were indicated for treatment of rheumatoid arthritis, atopic eczema , acute respiratory distress syndrome, asthma, cardiovascular disease, cancer and insomnia [34,35,36].

Its deficiency in diet may cause neurological and cardiological disorders [34]. Linoleic acid is a popular for preventing and treating diseases of the

heart and blood vessels. It is used to prevent heart attacks, high blood pressure and cholesterol [35,36].

Conclusion:

This study identified pharmaceutically very important bioactive substances from the three *Tribulus species*. These results provide a baseline for preparation of monograph for evaluation and identification. This study recommended the three species of *Tribulus* as an important source of various vital pharmaceutical phytocompone

Tribulosin and β-sitosterol-Dglucoside, the antihelminthic principles of *Tribulus terrestris*. Phytomedicine. 2002; 9: 753-756.

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