Phytochemical Investigation and Antioxidant Activity of Iraqi *Tribulus terrestris*

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Abstract

The aim of the present study was to characterize the ***Iraqi Tribulus terrestri***s for the presence of biologically active phyto-chemicals using methanolic extracts of the plant (aerial parts) by Gas Chromatography –Mass spectrometry (GC/MS), while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library , in addition to study the antioxidant activity of plant extract , results confirmed the presence of therapeutically potent compounds in the ***Iraqi Tribulus terrestri***s extract predominantly alkaloids, flavonoids, saponins, tannins and terpenoids. Antioxidant potential of Iraqi *Tribulus terrestris* herbal preparations was evaluated by determination of blood glutathione, serum ascorbic acid and serum superoxide dismutase in rats. The obtained results demonstrated that *T. terrestris* preparations possess a significant antioxidant activity.

Keywords: Iraqi *Tribulus terrestris,* Phytochemical investigation, Anti-oxidant activity.

دراسة المكونات الكيمائية والفعالية المضادة للاكسدة لنبات ذقن الشيخ العراقي

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الخلاصة

الهدف من هذه الدراسة هو تشخيص المواد الكيميائية الفعالة الموجودة في الاجزاء الهوائية للمستخلص الكحولي لنبات عراقي يسمى (دقن الشيخ) او شقشق باستخدام طريقة الفصل الكروماتوجرافي للغازات-مطياف الكتلة GC/MS ومقارنة النتائج مع النتائج المثبتة في المعهد الوطني للمعايير والتكنولوجيا(NIST) بالاضافة الى دراسة الفعالية المضادة للاكسدة لمستخلص النبات .

ان عملية الكشف النوعي التمهيدي للايضات الثانوية المختلفة من قبل كشوفات كيميائية محددة قد تمت على المستخلص الميثانولي للاجزاء الهوائية من النبات واشارت النتائج ان هذه الاجزاء تحتوي على القلويدات, فلافينويدات، صابونيات , مواد دباغية و تربينات.

تضمنت هذه الدراسة ايضا الكشف عن الفعالية المضادة للاكسدة لنبات الشقشق واظهرت النتائج ان المستخلص الكحولي لديه فعالية جيدة ضد الاكسدة.

الكلمات المفتاحية :نبات ذقن الشيخ العراقي ، الدراسة الكيميائية ،الفعالية المضادة للأكسدة .

Introduction

Tribulus terrestris (Family: Zygophyllaceae) is a perennial creeping herb widely distributed in Iraq. it is regarded as an aphrodisiac in addition to its beneficial claims on various ailments such as urinary tract infections, inflammations, oedema and ascites[(1)]. *In Iraq T. terrestris*(figure-1) is used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, anti-hypertensive, diuretic, lithon- triptic and urinary anti- infective [(2)].The aphrodisiac property of this plant extract was examined in rats [(3)]. Administration of *Tribulus* extract to humans and animals improves libido and spermatogenesis [(4)].Clinical studies showed that this plant improved reproductive function, including increased concentration of hormones such as estradiol, with testosterone being very slightlyinfluenced, thereby improving reproductive function, libido and ovulation [(5)].

Free radicals and reactive oxygen species are generated in living cells as a result of different biochemical and physiological processes, they are the causative agents for many chronic diseases, such as cancer, diabetes, aging and other degenerative diseases in humans due to oxidative damage of proteins, lipids and DNA[(6)]. Plants are the valuable sources of natural products for maintaining human health, more than 80%of population across the world use traditional medicine including compounds derived from medicinal plants. Therefore, such plant should be investigated to better understand their properties, safety and efficiency[(7)] . The aim of this work was to investigate the chemical constituents and antioxidant activity of methanolic extract of aerial parts of a newly studied plant widely and wildly distributed in our country Iraq.

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Plant material and Methods

The aerial part of *Tribulus terrestris*(Family: Zygophyllaceae) was collected from Kirkuk, a city in the north of Iraq, 236 kilometers (147 mi) north of Baghdad. The plant was authenticated by the National Herbarium at Abu-Graib, the plant leaves were dried in the shade for several days at room temperature and then grinded as powder and weighed.

Figure(1):Iraqi *Tribulus terrestris*

The experimental work is divided into

• The experimental preliminary phytochemical screening of various secondary metabolites like alkaloids, flavonoids, steroids, tannins, saponins, anthraquinioin and terpenoids in the plant.

• Extraction of different active constituents.

• GC-MS analysis of methanolic extract of the plant.

• Investigation of the antioxidant activity of methanolic extract of aerial parts of plant

Preliminary qualitative phytochemical analysis

Chemical tests were carried out using the methanolic extracts from plant using standard procedures to identify the active constituents [8-10]:

Test for alkaloids

Alcoholic extract (10 ml) was stirred with 5 ml of 1% HCL on a steam bath. Mayer's (1.35gm mercuric chloride in 60ml water + 5gmpotassium iodide in 10ml water)and Wagner's reagents (1.27g of iodine and 2g of potassium iodide in 100ml of water) were added, white and reddish brown color precipitate respectively, were taken as evidence for the presence of alkaloids.

Test for flavonoids

Lead acetate test:

Lead acetate 10% (1 ml) solution was added to 5ml of alcoholic extract, the formation of a yellowish- white precipitate was taken as a positive test for flavonoids.

Tests for steroids

Liebermann-Burchard test:

Extract (3ml) was treated with chloroform, acetic anhydride and drops of sulphuric acid was added. The formation of dark pink or red color indicates the presence of steroids.

Test for tannins

Plant material (10mg) in 10ml distilled water was filtered, and then the filtrate (3ml) + 3ml of FeCl3 solution (5%w/v) were mixed. The formation of a dark green or blue black precipitate was considered an indication for the presence of tannins.

Tests for anthraquinones

Borntrager's test: Alcoholic extract of 3ml was shaken with 3 ml of benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the development of a pink, red or violet color in the ammonical (lower) phase indicates the presence of free anthraquinoin.

Test for terpenoids

Alcoholic extract (2ml) was dissolved in chloroform (2ml) and evaporated to dryness. Concentrated sulphuric acid (2ml) was then added and heated for about 2 min. A grayish color was considered an indication for the presence of terpenoids.

Preparation of extract

Shade-dried coarsely powdered aerial parts of *Tribulus* plant was defatted with hexane for 24 hours then allowed to dry at room temperature. The defatted plant material was extracted with 85% methanol in soxhlet apparatus until complete exhaustion. The alcoholic extract was evaporated under reduced pressure at a temperature not exceeding 40C˚to give a dark-brown residue designated as a crude extract.

Animals

Healthy adult 30 male mice weighing 120-150gm were used in this study. The animals had free access to a standard commercial diet and water; they were kept in rooms maintained at 25-27°C. The animals were divided randomly into three groups; each group consisted of ten male mice:

Group 1: Received 100 mg/Kg body wt. of 85 % methanolic extract of the plant.

Group 2: Received 50 mg/Kg body wt. of85 % methanolic extract of the plant.

Group 3: Served as control group and received only 2% gum acacia (0.2ml).

The extracts were suspended in distilled water using Tween 20, and the dose was orally administered once daily for 4 weeks. At the end of treatment, blood samples were collected centrifuged and serum was separated for the determination of the following:

1- Blood glutathione content according to the method described by Beutler[11].

2- Serum superoxide dismutase activity, the method was carried out according to the pyrogallol method of Marklund[12].

3- Serum ascorbic acid was estimated by the method of Jagota[13].

GC-MS analysis

Instruments and chromatographic conditions

GC-MS analysis was carried out on GC-MS-QP2010 Shimadzu system comprising a gas chromatographinterfaced to a mass spectrometer instrument employing the following conditions : column VF-5MS fussed silica capillary column (30.0m x 0.25mm x 0.25µm, composed of 5% phenyl/95% dimethylpolysiloxane), operating in electron impact mode at 70ev; helium (99.999%) was used as carrier gas at a constant flow of 1. ml/min and an injection volume of 0.5µl was employed (split ratio of 10:1) injector temperature 240 0C ion-source temperature 200 C°. The oven temperature was programmed from 100 C° (isothermal for 3 min), with an increase of 10C°/min, to 240 C°, ending with a 9min isothermal at 270 C°. Mass spectra were taken at 70ev; a scan interval of0.5 seconds and fragments from 40 to 440Da. Total GC running time is 30min.

Results and Discussion

The results of preliminary qualitative phytochemical of Iraqi *Tribulus terrestris* are given in table-1.The results of preliminary phytochemical screening of plant extracts showed the presence of alkaloids, flavonoids, tannins, saponins and terpenoids and the absence of steroids and anthraquinoin. Many researchers reported that the concentration of secondary metabolites are varying from plant to plant belong to the same genus and even in the different parts of the same plant[14] , this is due to many factors like environmental heterogeneity, since the effect of environmental heterogeneity is highly scale-dependent. It may create high niche diversity and hence allow species to coexist at a large spatial scale[15], also the high complexity and heterogeneity of soil, like(soil structure, texture and depth, moisture retention characteristics, aeration) create a big variation in the chemical constituents even in the same country [16].

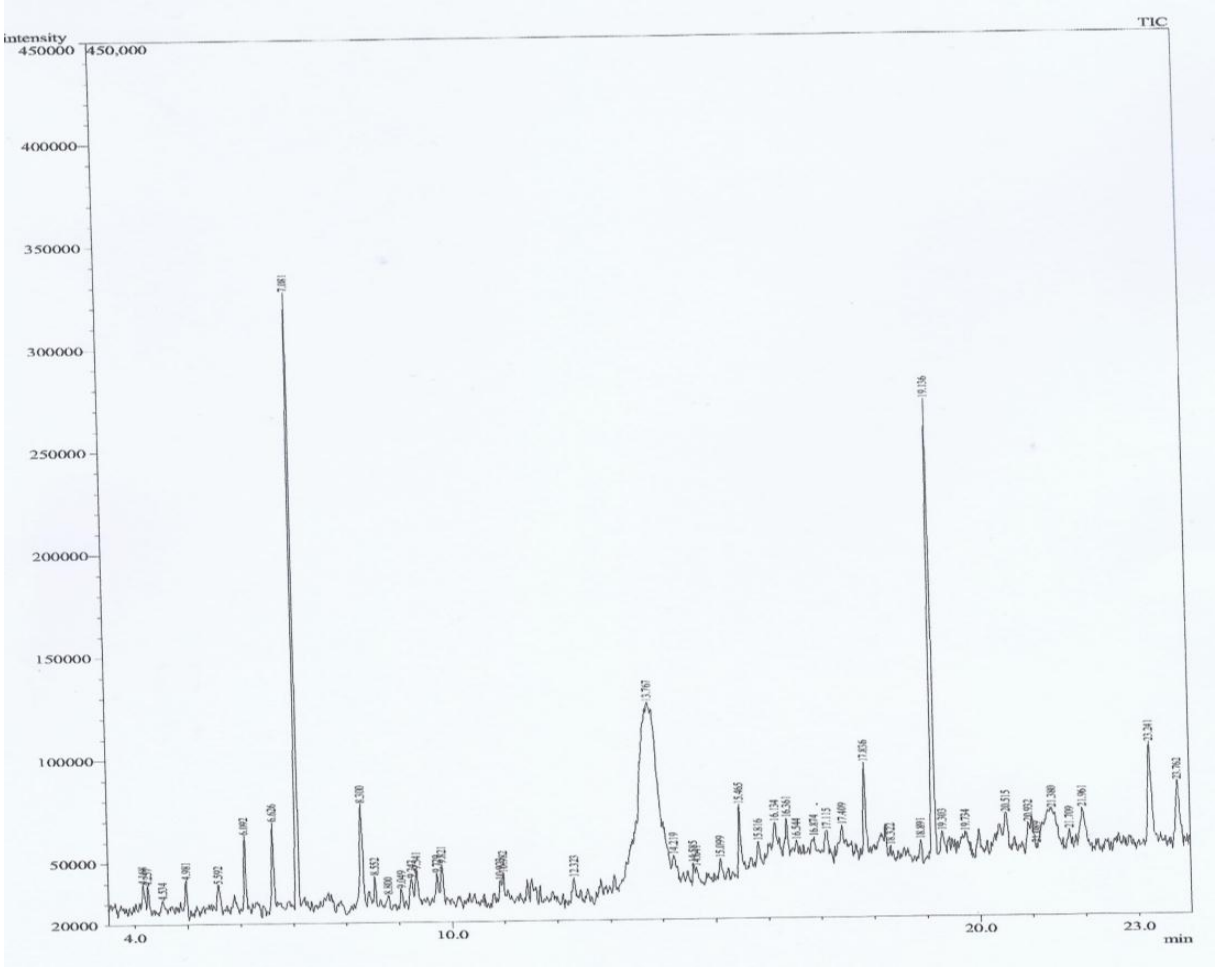
Identification of components by GC-MS:

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library . The results of GC-MS analysis led to the identification of number of compounds from the methanol extract of ***Iraqi Tribulus*** plant. GC-MS chromatogram showed 46peaks, indicating the presence of 46 compounds (figure-2) and (table- 2).many of these components reported in this plant for the first time like monoterpene [example Terpineol] sesquiterpenes: [2,3,8,8-Tetramethyltricyclo-2ene], [1 ,4 – dimethyl – 8 - isopropylidenetricyclodecane, alkaloids like -(3-Methoxy-2-pyrazinyl)-2-methyl-1-propanol and Thiazole, Saturated fatty acid[example Myristic acid]Coumaran and many phenolic compounds, Coumaric acid, Linoleic acid, Arachidic acid and oleic acid.

Table(1): Phytochemical screening of *Tribulus* extract

Alkaloids	Flavonoids	Steroids	Tannins	Saponins	Anthraquinoin	Terpenoids
+	+	-	+	+	-	+

+, - represent presence and absence of phytoconstituents respectively.



Figure(2): GC-MS Chromatogram of methanolic extract of *Iraqi Tribulus terrestris*

Table(2): Phytocomponents identified in the methanolic extracts of*Tribulus terrestris*

Peak#	R.Time	Area%	Name
1	4.168	0.56	3-Penten-2-one, 4-methyl-
2	4.257	0.39	1-Butanol
3	4.534	0.24	Hexanal dimethyl acetal
4	4.981	0.68	Glycerin
5	5.592	0.97	Trimethylsilylmethanol
6	6.092	1.54	4-Hexenoic acid, 2-(phenylsulfonyl)-, methyl ester, (E)-
7	6.626	1.50	Coumaric acid
8	7.081	12.75	2-Hexanol, 2-methyl-
9	8.300	2.29	Archidic acid
10	8.552	0.30	2-Pentanone, 4-hydroxy-
11	8.800	0.19	Nonanal dimethyl acetal
12	9.049	0.48	1-Hexanol, 2-ethyl
13	9.242	0.77	Linoleic acid
14	9.341	0.74	Silane, [2-(2-methoxyethoxy)ethoxy]trimethyl-
15	9.729	0.32	Propanoic acid
16	9.821	0.81	Myristic acid
17	10.923	0.27	(R)-(-)-2,2-Dimethyl-1,3-dioxolane-4-methanol
18	10.982	0.26	1,4-dimethyl-8-isopropylidenetricyclodecane
19	12.323	0.35	Cyclohexanol, 1-methyl-4-(1-methylethylidene)-
20	13.767	38.72	Stearic acid
21	14.219	0.27	(2-Benzyloxy-2-oxiran-2-ylethoxy)-t-butyldimethylsilane
22	14.585	0.30	Cyclononasiloxane, octadecamethyl-

Table(2): Contained phytocomponents identified in the methanolic extracts of *Tribulus terrestris*

Peak#	R.Time	Area%	Name
23	14.641	0.07	N-Cbz-glycylglycine p-nitrophenyl ester
24	15.099	0.62	Phenylethyl Alcohol
25	15.465	1.59	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
26	15.816	0.58	Dodecanal
27	16.134	0.68	D-Mannotridec-6-ene-1,2,3,4,5-pentaol
28	16.361	0.42	Heptacosanoic acid, methyl ester
29	16.544	0.23	Heptanoic acid, 3-buten-1-yl ester
30	16.874	0.55	Thiazole, 4-ethyl-2,5-dimethyl
31	17.409	0.33	Methyl(methyl 3,4-di-O-methyl-.alpha.-D-mannopyranoside)uronate
32	17.409	2.24	2-Pentadecanone, 6,10,14-trimethyl-
33	18.322	0.15	Ethanone, 1-phenyl-2-(phenylsulfonyl)-
34	18.891	0.48	Z-25-Tetratriaconten-2-one
35	19.136	12.43	Hexadecanoic acid, methyl ester
36	19.303	0.46	2,3,8,8-Tetramethyltricyclo-2ene
37	19.734	0.18	Phenol, 3,5-bis(1,1-dimethylethyl)
38	20.515	0.99	Methyl 10-methyl-hexadecanoate
39	20.932	0.22	1,4-dimethyl-8-isopropylidenetricyclodecane
40	21.089	0.03	Hexanedioic acid, bis(2-ethylhexyl) ester
41	21.709	0.2	Diethyl Phthalate
42	21.961	1.9	Benzofuran, 2,3-dihydro-
43	23.241	3.59	Octadecanoic acid, methyl ester
44	23.762	1.73	9-Octadecenoic acid, methyl ester
45	24.231	0.77	3-Methoxy-2-pyrazinyl)-2-methyl-1-propanol
46	25.211	0.43	Oleic acid

The groups treated with alcoholic extracts of aerial part of Iraqi *Tribulus* showed a significant increase in blood glutathione level, serum superoxide dismutase activity and serum ascorbic acid level comparing with control group

Table(3): Mean blood glutathione content, serum ascorbic acid and serum superoxide dismutase among group of rats treated alcoholic extracts of *T. terrestris*.

Tested parameter	Control group	Group (1)	Group (2)
Blood glutathione (mg/ gm Hb)	1.92 ± 0.052	8.53 ± 0.317*	4.15 ± 0.21*
Superoxide dismutase (µg/ml)	10.23 ± 0.6	30.2 ± 2.06*	17.5 ± 1.03 *
Ascorbic acid (µg/ml)	3.23 ± 0.43	12.09 ± 0.46*	6.54 ± 1.03*

Group (1): treated with100 mg/Kg body wt. of 85 % methanolic extract of the plant
Group (2): treated with 50 mg/Kg body wt. of 85 % methanolic extract of the plant.
* Significantly different from control value.

Discussion

In much of the developing countries, 70–95% of the populationrely on traditional medicines for primary care, and between70% and 90% of populations in industrialized world use traditional medicines under the titles "complementary", "alternative",or "nonconventional"[17].

Plants have formed the basis for traditional medicinal systems for thousands of years, with the first records dating from about 2600 BC in Mesopotamia. Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs. In the present study, methanolic extract of the *Iraqi Tribulus terrestris*was analyzed for the first time for the presence of different secondary metabolites which could be of medicinal & economic value and study antioxidant activity of crude extract of this Iraqi plant. The comparison of the mass spectrum with the NIST database library gave more than 90% match as well as a confirmatory compound structure match. This work will help to identify the compounds, which may be used in body products, drugs, pharmaceutical and therapeutic value since

many components isolated from this plant reported for the first time, also the present study results were confirmed the traditional uses of this plant as an antioxidant, anti-inflammatory, antispasmodic agent due to different secondary metabolites constituents like flavonoids, essential oil, alkaloids , saponins and others . The characteristic antioxidant properties of *T.terrestris* may cause significant increase in blood glutathione level, serum superoxide dismutase activity and serum ascorbic acid level.

Based on the results obtained in this study, it could be said that *T.terrestris* plant powder contains chemical constituents of pharmacological and nutritional significance. However, it is recommended that further work be carried out to isolate and purify the bioactive constituents in this plant powder using various extraction solvents with a view to characterizing their molecular structure, formula, weight as well as evaluating their safety or otherwise (toxicity) for human and other animal use.

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