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Phytochemical Investigation and GC-MS Analysis of *Tribulus Terrestris* L. Cultivated in Iraq

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Abstract: *Tribulus terrestris L.,Gokshur in Sanskt, is an annual herbaceous, little prostrate, silky from the Zygophyllaceae family. It is found in extensive, arid, and scorching regions of India. is used in traditional folk medicines as an aphrodisiac, astringent, lithotriptic, diuretic, and urinary disinfectant .However, uneven, pinnae, elliptical leaves. Sprinkled, round, compressed, five-cornered, transverse partitioned carpel fruits with many seeds ripen in July. Pieces of the cylindrical-fibrous root are 7-18 cm and 0.3-0.7 cm wide. According to reported studies, the investigations indicated the presence of the saponins ;gitogenin, tigogenin, neogitogenin, hecogenin, and flavonoid tribuloside was kaempferol-3-glucoside. Furthermore, Tribulus terrestris plays a vital role in hepatotoxicity and is greatly reduced by Tribulus extracts. Similarly, T.terrestris plays a remarkable role in reducing the elevated level of serum glucose, triglycerides, VLDL, and cholesterol. This study aims to conduct a preliminary analysis of the phytochemicals of Tribulus leaves cultivated in Iraq and to determine the active constituent of the ethanolic extract of the leaves using Gc-Mass. Qualitative detection of herbal leaves in Iraqi soil, investigated by GC-MASS revealed important constituents. The funding shows a new compound; dihydrocoumarin and other major constituents neophytadiene and alpha-tocopherol.*

Keywords: *Tribulus, Mass, dihydrocoumarin, alpha-tocopherol, phytochemical.*

1. INTRODUCTION

Tribulus terrestris , known as Gokshur (Sanskrit); is distributed along wide dry, and hot geographic regions in India and It is grown in worm regions in southern Eurasia and Africa. The morphological characteristics of the shrub are characterized by a small prostrate, silky hairy shrub, with an aerial part height of 10-60 cm (Tyagi, et al,2023). Leaves are opposite, may be unequal, and pinnae. Furthermore, uneven, pinnae, elliptical leaves. Sprinkled, round, compressed, five-cornered, transverse partitioned carpel fruits with many seeds ripen in July. the root is cylindrical-fibrous 7-18 cm long and 0.3-0.7 cm wide (Chhatre S. et al,2014). Occasionally, investigations reported *T. terrestris* has different bioactivity secondary metabolites of saponins, alkaloids, flavonoids, and tannins. The reported studies revealed the saponins of gitogenin , neogitogenin, and hecogenin (Kostova I, et al,2005). The aerial parts of *T. terrestris* also possess flavonoids; kaempferol and rutin, and its glycosides; tribuloside, kaempferol-3-glucoside, and rutinoside as reported by (Saleh NAM, *et al)*. Harmane alkaloidal compound was also reported in *T. terrestris*. Tian Shung et al. isolated two new compounds;

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Tribulusterine and terrestribisamide, and β-sitosterol, ferulic acid, and vanillin from the dried fruits. *T. terrestris* in folk or ancient medicines, is used mainly as a diuretic,aphrodisiac, and astringent (Akram M,et al,2011). However, Al-Ali, M, et al, reveal good effects of essential oils and elements (high concentration of potassium salts) on their diuretic activity. The *Tribulus* extracts showed incredible effects against drug-induced hepatotoxicity (Kavitha P., et al) and Improvement in fertility(KamenovZ,2015). Furthermore, the herb also exhibited a pro-erectile effect after oral administration on rabbit corpus cavernous smooth muscle (Adaikan PG,2000). Likewise, it has a remarkable t role in reducing the high level of serum glucose and decreasing serum triglyceride, very low-density lipoprotein (VLDL), and serum cholesterol(Li M ,et al,2000). The aim of this study involves the Phytochemical investigation of *Tribulus Terrestris* leaves grown in Iraq and the analysis of the active constituent in the leaves using Gc-Mass.

2. MATERIALS AND METHODS:

Plant material and chemical materials:

The plant material was prepared from the Leaves of *Tribulus terrestris* as shown in Figure 1. They were collected from Al Zahwraa Park Iraq- Baghdad and authenticated by Dr. Ibrahim Salih at the College of Pharmacy-Mustansiriyiha University. The leaves were collected during October/ 2023 and dried under shade conditions at room temperature for 14 days before grinding a commercial electric blender and weighing as a powder**.**

Figure 1: *Tribulus terrestris* cultivated in Iraq

Equipment and Chemicals:

Ethanol (95-97%) was purchased from Merck (Darmstadt, Germany), and A rotary evaporator (BÙCHI Rotavapor R-205, Swiss) was used for the dryness of the extracts. Apparatus ,GC/MS -SHIMADZU -QP-2010 ULTRA-Scan mode -ACQ .

Phytochemical investigations for *Tribulus terrestris leaves*:

The preliminary chemical tests were performed to screen bioactive compounds and GC -MS analysis for ethanolic extract. Qualitative analysis of leaves was performed according to standard procedures toindicate preliminary analysis of chemical constituents (Tawfeeq AA2022, Tawfeeq AA 2018, and Eldalawy R 2021).

1. Test for screening of Saponins (Foam Test):

Approximately one mL of Methanolic extract was mixed with (5mL) of distilledwater in a test tube and then shaken vigorously, the persistent foam at least 1cm in heightindicates the presence of saponin.

2. Test for screening of Terpenoids (Salkowski Test):

About 4mL of alcoholic extract was mixed with chloroform (2mL), then 3mL of concentrated sulphuric acid was carefully added. A reddish–brown color is formed to indicate the presence of terpenoids.

3. Benedict's test:

Benedict’s reagent (5mL) was added in the test tube to the methanolic extract (1mL) and then the mixture was heated in a boiling water bath for 10 minutes. The green precipitate appearance is an indication of the presence of the reducing sugar.

4. Test for screening of Phenols:

Alcoholic extract (0.5 mL) was treated with 3 to 4 drops of ferric chloride. The appearance of an intense green color indicates the presence of phenols.

5. Wagner's reagent for screening of Alkaloids:

Approximately 3 mL of Alcoholic extract acidify with a few drops of hydrochloric acid. Then a few drops of Wagner's reagent were added and the resulting color of the precipitate was reported.

6. Tests for screening of Flavonoids:

Approximately 3mL of alcoholic KOH was added to 1 mL of methanolic leaf extract.

A yellow color indicates a relevant result.

7. Test for screening of Tannins:

About 0.2g of sample was dissolved in (10ml) of distilled water and filtered. Then a Solution of (1% FeCl3) was added and revealed a dark green, dark blue, or black color indicating the presence of tannins.

8. Test for screening of Coumarins:

A few drops of methanol extract from leaves of. *Tribulus terrestris* were placed on filter paper, adding two drops of NaOH (1N) solution, and then the filter paper detected yellow to blue fluorescence under UV light at 366nm.

Extraction of leaves *Tribulus terrestris*:

For the obtaining of extracts, the obtained powdered leaves of *T.terrestris* **(**50 g) were extracted with ethanol 350 mL)(16). Extraction was carried out using a Soxhlet extractor, and the collected extracts were then filtered using a filter paper and concentrated using a rotary evaporator, The obtained extract was stored at 4 °C in dark containers for analysis with Gas Chromatography /Mass.

GC-MS analysis:

Gas Chromatography /Mass was performed and applied to detect phytochemicals in the ethanol extract. The GC-MS conditions were: the carrier gas was helium, the injection volume was 1µL, and the split ratio was 2.0, the Injection temperature was 250˚C, Column temperature was about 80 °C to 310 °C at a rate of 10Cº /min.

3. RESULTS AND DISCUSSIONS

Phytochemicals investigation:

The preliminary tests are essential in predicting different phytochemicals in the plantextracts.

Table 1. Phytochemical screening of Iraqi *Tribulus terrestris*

Chemical Tests	Positive Indicators	Results*
Saponin Test	Foam observed 1cm in high	+ ve
Tannins Test	Dark green, dark blue, or black color	+ ve
Flavonoid Test	Yellow color	+ ve
Alkaloid Test	Reddish-brown precipitate	_ ve

Terpenes Test	Reddish-brown color	+ ve
Coumarin test	yellow to blue fluorescence	+ ve
Benedicts test	green precipitate	+ve

**+ve: positive, -ve: negative*

The results (table 1) showed the presence of different phytochemicals such as flavonoids, tannins, terpenes, coumarins, saponins, and reducing sugar with the absence of alkaloids that are vital in the biological activities of *T.terrestris* plant. These findings were agreed with reported literature in which positive results for flavonoids, carbohydrates, and terpenes tests were obtained (Ibrahim N,2022).

GC/MS Analysis

Analysis by GC-MASS for the qualitative detection of important constituents and determination of bioactive chemical components identified in leaves ethanol extract shows different components and groups of terpenes and steroids as shown in (Figure 2) and (Table 2) shows the major components identified in the ethanolic extract of *T.terrestris* leaves. The GC-MS chromatogram shows different peaks representing chemical constituents. The presence of dihydrocoumarin at a retention time of 17.079 min, neophytadiene (R.time 23.726 min) , alpha-tocopherol at (R.time 24.463), and Silicic acid (R.time 43.28min). The chromatogram can be seen in (Figure 2) with peaks retention time andSimilarity index (SI).

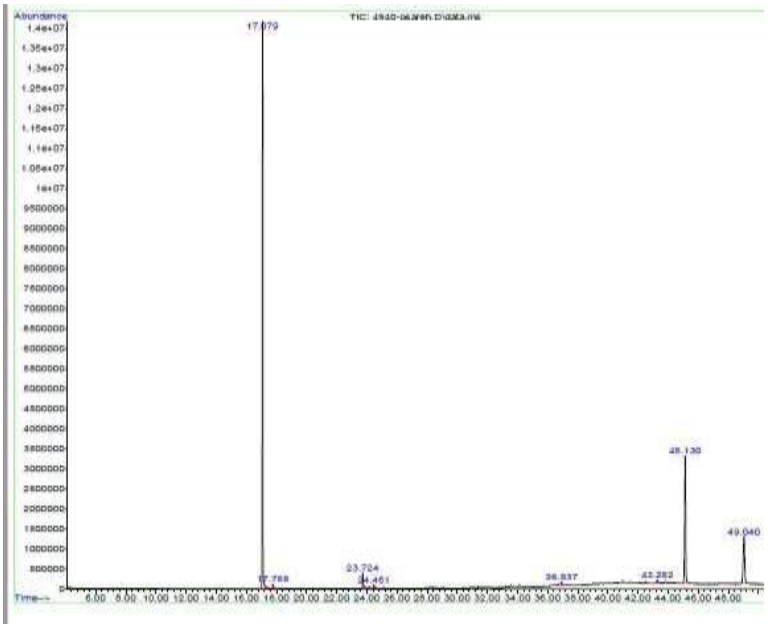

Figure 2: GC/MS chromatogram of methanolic extract of *T.terrestris* leaves

Table 2: GC -MS analysis of main chemical constituents of *T.terrestris* leaves.

Peak NO.	Name	R. time	SI	Area %
1	Di hydrocoumarin	17.079	95	59.07
2	2,4-Di-tert-butylphenol	17.754	97	0.73
3	Neophytadiene	23.726	96	1.71
4	alpha-Tocopherol	24.463	97	0.74
5	4,4'-Ethylenebis(2,6-di-tert-butylphenol)	36.936	93	0.57
6	Silicic acid, diethyl bis(trimethylsilyl) ester	43.282	96	0.77
7	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite	45.129	95	23.06
8	Silane, dimethyl(2-naphthoxy)heptyloxy-	49.041	97	13.35

Dihydrocoumarins, one of the coumarin derivatives, have been reported to influence antioxidant and anti-inflammatory properties. Additionally, they function as an HDAC inhibitor and increase DNA damage sensitivity by inhibiting Rad52(Chen CC,2017). Neophytadiene is a diterpene; 7,11,15-trimethyl-3methylidenehexadec-1-ene. Its role of antimicrobial agent, and anti-inflammatory agent (Rzhepakovsky,et al,2022). Alpha-tocopherol also Enhances the immune system and prevents the formation of blood clots, it aids in the prevention of cellular damage caused by free radicals (antioxidant effect). Vitamin E is currently under investigation in protecting and preventing cancer (Rizvi S, et al,2014). Silicic acid (H4SiO4), an accessible silicon for humans and animals hasbeen disregarded thus far. Moreover, Silicon affects; bone mineralization, collagen synthesis, skin, and hair health, atherosclerosis, Alzheimer's disease, and immune system enhancement (Jurkić LM, 2013).

Conclusions

This study proved that the Iraqi *T.terrestris* contains various secondary metabolites coumarins, flavonoids, terpenes, carbohydrates, and saponin. Dihydrocoumarins is a new compound revealed in GC-Mass analysis that is considered avital as anti-inflammatory and anti-oxidants. other compounds Vitamin E (tocopherol), and Neophytadiene revealed the presence of minor compounds in *T.terrestris* leaves. Outlook, this study is required for further investigations and structural characterizations of phytochemicals in all plant parts.

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