

Antibacterial Bio-Active Compounds Isolated by GC-MS Analysis of *Tribulus terrestris* L. Fruits

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The genus *Tribulus* belongs to family Zygophyllaceae, comprises about 20 species in the world. Its various parts contain a variety of chemical constituents which are medicinally important, such as flavonoids, flavonol glycosides, steroidal saponins, and alkaloids. Also the Zygophyllaceae family is a source of many biologically active photochemical with tremendous potential for medicinal uses. Secondary metabolites mainly alkaloids, amides and terpenes are reported from the various species of *Tribulus* which are of great economical and medicinal importance. This paper reports the isolation of various bioactive compounds from *Tribulus terrestris*. GC-MS analysis of *Tribulus terrestris* fruits methanol extract revealed the existence of the major compounds cyclotrisiloxane hexamethyl and Cyclotrisiloxane Octamethyl (36.01% and 28.64%). These phytochemicals were interpreted on mass-spectrum GC-MS which conducted using the database of Wiley 275 L. The antibacterial action of AgNPs against human pathogens, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* was recognized.

Key words: Bioactive metabolites, GC-MS, *Tribulus terrestris*, Medicinal plants.

There are more than 35,000 plants species being used in various human cultures around the world for medicinal purpose. Biologically active compounds present in medicinal plants have always been of great interest to scientist working in this field (Koshy Philip et al, 2011). Natural products perform various functions and many of them have interesting and useful biological activities. *Tribulus terrestris* is an annual flowering plant belonging to family Zygophyllaceae and found around the world. Its fruits have been used in traditional Arabian medicine for treatment of eye problems, edema, abdominal distention, emission, morbid leucorrhoea

and sexual dysfunction. In Iraq and other Arabian Gulf countries including Saudi Arabia *Tribulus terrestris* was used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, anti-hypertensive, diuretic lithon-triptic and urinary anti- infectives (Majeed and Mahmood, 1988; Saad Aldein, 1986). Some steroidal saponins have previously been isolated from this plant. Many pharmaceutical preparations and food supplements with these saponins as the active compound have been commercially available. Examples of these are "Tribestane" and "Vitanone", which have been used to treat impotency, as well as "tribusaponins", which have been used for the treatment of cardiovascular disease (Kostova and Dinchev, 2005; Li and Yang, 2006). *Tribulus terrestris*-extract is commonly used in the folk medicine also for control of blood pressure and cholesterol. There are reports showing that this extract decreases blood cholesterol level in humans,

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rats and mice (Chu et al., 2003). Yang *et al.* optimized the extraction condition using orthogonal experiment. Matin Yekta *et al.* (2008) isolated three flavonoid glycosides, viz. quercetin 3-*O*-glycoside, quercetin 3-*O*-rutinoside, and kaempferol 3-*O*-glycoside from the aerial parts of *T. terrestris* L. var. *orientalis* (Kerner) G. Beck in the northeast of Iran. Raja and Venkataraman (2011) identified flavonoids from the petroleum ether and chloroform extracts of fresh fruits of *Tribulus terrestris* from India using ethyl acetate: benzene (1:9) solvent system. These flavonoids were not detected in the fruit extracts of other variety, namely *T. alatus*. To the best of our knowledge, there is no such previous study on the exploration of bioactive characterization of *Tribulus terrestris* in Saudi Arabia. This study will provide base-line data for further detailed investigations of various biological activities of *Tribulus terrestris* and of its use as a functional food.

MATERIALS AND METHODS

Preparation of extract

The fruits of *Tribulus terrestris* L. were collected from different locations in the middle region of Saudi Arabia. Fruits were dried and crushed to fine powder in a mortar. Required amount of the plant powder of *Tribulus terrestris* was weighted, transferred to flask, treated with the Methanol until the powder was fully immersed, incubated overnight and filtered through a Whatmann No.41 filter paper along with Sodium sulphate was wetted with absolute alcohol. The filtrate is then concentrated to 1ml by bubbling nitrogen gas in to the solution. The extract contains both polar and non-polar components of the material and 2µl sample of the solution was employed in GC-MS for analysis of different compounds.

GC – MS analysis

The GC – MS analysis was carried out using a Clarus 500 Perkin – Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbo mass 5.1 spectrometer with an Elite – 1 (100% Dimethyl poly siloxane), 30m x 0.25 mm ID x 1µm of capillary column. The instrument was set to an initial temperature of 110 °C, and maintained at this temperature for 2 min. At the end of this

period the oven temperature was rose up to 280°C, at the rate of an increase of 5 °C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as one ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z).

Identification of phytochemicals

Using the database of Wiley 275 L. library and comparing the spectrum obtained through GC– MS compounds present in the plants sample were identified. Interpretation on mass-spectrum GC-MS was conducted using the database of Wiley 275 L. The spectrum of the unknown components was compared with the spectrum of known components stored in the Wiley25 L. library. The name, molecular weight and structure of the components of the test materials were ascertained.

Microorganisms

The evaluation of antibacterial action was done using various strains. The subsequent microorganisms were used: *Streptococcus pneumoniae* (Thermo Fisher Scientific, AS Polyvalent 2 R671260, Waltham; USA) and *Pseudomonas aeruginosa* (Thermo Fisher Scientific, Set R670372, Waltham; USA). The microbial cultures were maintained by the Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia.

Antibacterial activity study

Antiseptic action of the synthesized AgNPs was performed using the agar well diffusion examine process (Perez *et al.*, 1990). In this technique, disinfected Mueller - Hinton Agar plates were arranged. Pathogenic bacteria used in the current research were widening above the agar plates by sterile cotton wipe down. The plates were allowed to dry and a sterile well - cutter of diameter 5.0 mm was used to bore wells in the agar plates. Subsequently, a 50µl of the synthesized nanoparticle suspension (mass concentration= 0.02µg/µl) was introduced into wells of the inoculated Mueller – Hinton Agar plates. Another two concentrations 10 and 25 µl of the synthesized Ag-nanoparticles were used and introduced into wells of the Agar. The plates containing the bacterial and AgNPs were stand for 1h to allow diffusion to take place and then incubated at 37 °C

for 24 h, and then observed for indication of zones of inhibition, which show as a clear region around the wells (Cheesbrough, 2000). The length of such inhibition zone was calculated using a metre ruler, and the significant value for each type of bacteria was documented and spoken in millimeters.

RESULTS AND DISCUSSION

The active values in the fruit of *Tribulus terrestris* Methanolic extract by GC-MS analysis clearly showed the presence of nine compounds (Table 1). The active principles with their retention

time (RT), molecular formula, molecular weight (MW), and concentration (peak area%) are presented in Table 1. The GC-MS chromatogram of the seven peak of the compounds detected was shown in Fig.1. Chromatogram GC-MS analysis of the methanol extract of *Tribulus terrestris*. Showed the presence of major peaks and the components corresponding to the peaks were determined as follows, Nine compounds were detected in methanolic extracts of *Tribulus terrestris*. The results revealed that cyclotrisiloxane hexamethyl and Cyclotrisiloxane Octamethyl (36.01% and 28.64%) were found as

Table 1. GC – MS analysis of *Tribulus terrestris* fruits. Table. 1 GC – MS analysis of *Tribulus terrestris* fruits.

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Information from Data File:
File       : C:\MSCHEM\1\DATA\ALKAL3.D
Operator   :
Acquired   : 10 Aug 2014  10:35 am  using AcqMethod Flavonoids
Sample Name: Dr. M. Ibrahim ( 6)
Misc Info  :
Vial Number: 1

Search Libraries: C:\DATABASE\WILEY275.L      Minimum Quality: 0
Unknown Spectrum: Apex
Integration Params: flav

Pk#  RT      Area%      Library/ID                                     Ref#  CAS#  Qual
-----
1    3.20    36.01    C:\DATABASE\WILEY275.L
Cyclotrisiloxane, hexamethyl- (CAS 106845 000541-05-9 94
Cyclotrisiloxane, hexamethyl- (CAS 106849 000541-05-9 83
N-ethyl-1,3-dithioisindoline $$ 1 92199 035373-06-9 80

2    3.38    2.85    C:\DATABASE\WILEY275.L
1-METHYLALYL(CYCLOOCTATETRAENE)TE 92376 000000-00-0 80
4' METHYL-2 PHENYLINDOLE 92601 000000-00-0 59
2,3-dimethyl-4-azaphenanthrene 92617 000000-00-0 59

3    4.92    28.64    C:\DATABASE\WILEY275.L
Ethanethioic acid, S-[8-(diethylph 195207 000000-00-0 50
Cyclotetrasiloxane, octamethyl- (C 174366 000556-67-2 50
Cyclotetrasiloxane, octamethyl- (C 174364 000556-67-2 47

4    6.20    3.18    C:\DATABASE\WILEY275.L
Benzaldehyde, 2,5-bis[(trimethylsi 163056 056114-69-3 53
1-phenyl-3-(meta-nitrophenyl)-2-py 150585 077242-45-6 53
2-amino-5-ethoxycarbonylbenzopheno 150645 039695-70-0 52

5    6.63    10.12    C:\DATABASE\WILEY275.L
Cyclopentasiloxane, decamethyl- (C 221467 000541-02-6 50
Silane, [[4-[1,2-bis[(trimethylsil 251988 056114-62-6 38
Benzeneacetic acid, .alpha.,3,4-tr 239721 055268-65-0 38

6    7.64    1.35    C:\DATABASE\WILEY275.L
9H-pyrrolo[3',4':3,4]pyrrolo[2,1-s 206438 095647-39-5 64
4-[o-Chlorophenyl]-2,6-diphenylpyr 206475 000000-00-0 47
methyl 2-trimethylsiloxy-N-(1-trim 213004 000000-00-0 47

7    8.40    3.16    C:\DATABASE\WILEY275.L
Estra-1,3,5(10)-trien-17-one, 2-[( 244783 077883-26-2 43
Morphine, 3-O-[4-trifluoromethylph 244774 000000-00-0 38
MORPHINE, BIS(O-TRIMETHYLSILYL) $$ 244763 055449-66-6 35

8    9.99    1.06    C:\DATABASE\WILEY275.L
2-(bromomethyl)-1,8-dimethoxy-9,10 216630 107960-83-6 30
2-Methylthio-4-(p-aminophenyl)-3H- 162573 085741-24-8 27
Cyclotetrasiloxane, octamethyl- (C 174364 000556-67-2 27

9    13.67    8.04    C:\DATABASE\WILEY275.L
Hexadecanoic acid (CAS) $$ Palmiti 141013 000057-10-3 98
Hexadecanoic acid (CAS) $$ Palmiti 141012 000057-10-3 96
Hexadecanoic acid (CAS) $$ Palmiti 141016 000057-10-3 97

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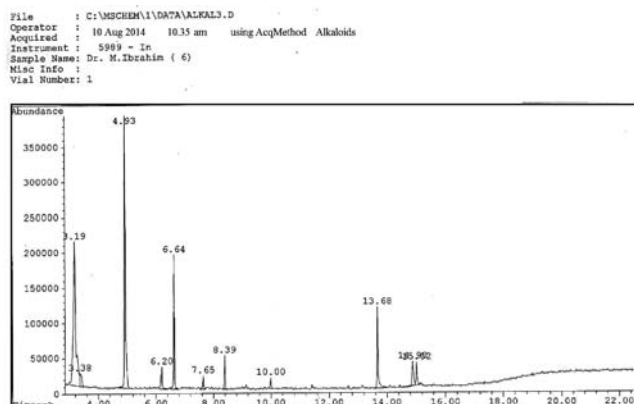


Fig. 1. GC-MS analysis of *Tribulus terrestris* fruits



Fig. 2. Effect of *Tribulus terrestris* fruit extract on the bacterial growth of *S. pneumoniae* and *P. aeruginosa*

the two major component in the methanol extract and the other seven components were considered as minor constituents and ranged between 1.06 % for 2-(Bromomethyl)-1,8-Dimethoxy-9 and 10.12% for Cyclopentasiloxane, Decamethyl- Silane.

Antibacterial activity for *Tribulus terrestris* was done with gram positive bacterial strains like *Streptococcus pneumoniae* and gram negative bacterial strains such as *Pseudomonas aeruginosa*. The inhibition zone caused by 50 μ l of *Tribulus terrestris* fruit extract was reached to about 1.9 cm for gram positive strains *S. pneumoniae* and 1.6 cm for *P. aeruginosa* (Fig. 2).

It could be suggested that *Tribulus terrestris* fruit extract showed effective antibacterial properties owing to their exceptionally antibacterial bioactive compounds, which provides contact with microorganisms and its interactions with bacteria are and localized on the membrane of the organism.

CONCLUSION

In the present study twenty chemical constituents have been identified from Methanolic extract of the whole plant of *Tribulus terrestris* by Gas Chromatogram Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of whole plant various ailments by traditional practitioners. Moreover, these bioactive compounds have a significant antibacterial effect on human pathogenic Gram positive and Gram negative bacteria.

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