



ISSN NO. 2320-5407

Journal homepage: <http://www.journalijar.com>

INTERNATIONAL JOURNAL
OF ADVANCED RESEARCH

RESEARCH ARTICLE

GC-MS Analysis and Antimicrobial Screening of Non-Polar Fraction of *Tribulus Terrestris*

*Syed Tariq Ali¹, Syed Kashif Ali¹, Syed Abdus Subhan², Abdul Wahab², Muneera Naz Baloch²,
Tanveer Abbas², and Sobiya Perwaiz³

1. Department of Chemistry, University of Karachi, Karachi Pakistan.

2. Department of Microbiology, University of Karachi, Karachi Pakistan.

3. Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan.

Manuscript Info**Manuscript History:**

Received: 11 January 2015

Final Accepted: 25 February 2015

Published Online: March 2015

Key words:

Twigs, Antimicrobial, Non-polar fraction, Retention indices

***Corresponding Author**

Syed Tariq Ali

Abstract

Traditionally *Tribulus terrestris* commonly utilized for the treatment of renal dysfunction. In present study, the phytochemical composition of non-polar fraction of ethanol extract of *Tribulus terrestris* twigs have been evaluated with the help of GC followed by GC-MS along with its antimicrobial activity. A total of eleven compounds were identified mainly fatty acid and their methyl esters. In order to support the identifications Kovat's Retention Indices were also calculate of these compounds. The n-hexane fraction did not showed significant antimicrobial activity against several bacterial strains, only *Micrococcus luteus* was inhibited by one of the non-polar fraction. However, the distinctive chemical composition of *T. terrestris* twigs indicate its potential as a source of natural antimicrobial compounds.

Copy Right, IJAR, 2015.. All rights reserved

INTRODUCTION

One of the famous traditional medicinal plant *Tribulus terrestris*; commonly known as Gokharu, is belongs to zygophyllaceae family. *T. terrestris* is widely distributed in warm region of Asia, Africa, Europe, America and Australia. In traditional medicine system *T. terrestris* used for the

treatment of variety of diseases including cardiac disease, eye trouble, edema, skin itching, and impotency, especially treatment of renal dysfunction [Chu et. al. 2003; Conrad et. al 2004; Dymock et. al. 1972; Nasroallah et. al. 2013; Sahelian 2003]. A saponin isolated from *T. terrestris* is found to dilatation effect on coronary artery [Bowen et.al. 1990]. The diuretic properties are related to the presence of nitrates and alkaloid [Chopra et. al. 1958; Bose et. al. 1963]. aims of present study to identify the phytochemicals in non-polar fraction of twigs ethanol extract of *Tribulus terrestris* along with the therapeutic value of this fraction. It may be noted that the identification of compounds of the non-polar fraction of the ethanol extract of twigs has not been reported prior.

Material and Methods**Plant material**

The aerial part of *T. terrestris* was collected near from Karachi. The authentication of plant was accomplished by Prof. Dr. S. I. Ali from Department of Botany, University of Karachi and a voucher specimen deposited in the herbarium.

Preparation of Extraction

The twigs of *Tribulus terrestris* (3Kg) were extracted by the following cold maceration method at room temperature with ethanol for two days. The extraction process was repeated twice to ensure complete extraction. The combined

extracts were filtered and subject to remove solvent under vacuum which ultimately afforded concentrated extract (1.5kg). The concentrated extract was partitioned between 90% MeOH and n-hexane. The n-hexane fraction (TTH) was dried over sodium sulphate and concentrated under vacuum (250g). The TTH small fraction (150mg) was treated with MeOH and HCl for methylation. After work-up the methylated fraction (TTH-M; 120mg) was obtained. The TTH and methylated fraction TTH-M was subjected to GC-FID and GC-EIMS.

GC/GCMS Conditions

Both fractions were subject on Shimadzu GC-17 gas chromatograph equipped with split/split-less injector, flame ionization detector (GC-FID) and less-polar capillary column SPB-5 (45m x 0.53mm ID with 0.50 μ m film thickness of 5% phenyl and 95% methyl silicone), hooked with Shimadzu workstation Class GC-10 used for GC-FID analysis.

The nitrogen is used as carrier and make-up gas with the flow rate 1.4 and 40 mLmin⁻¹ respectively. The split injector with a splitting ratio of 1:30 was set at 250°C and the FID was set at 270°C. The analysis was performed with an initial temperature 60°C for 1min, and then ramped at a rate of 8°C/min to a final temperature 240°C with holding time 30min. Kovats retention indices were also calculated for identified phytochemicals.

The gas chromatography electron impact mass spectrometer (GC-EIMS) studies was performed on a Hewlett-Packard 5890 gas chromatograph, equipped with HP-5 (25m x 0.22mmID and 0.25 μ m film thickness, an equivalent of SPB-5) was joint with a Jeol, JMS-HX 110 mass spectrometer operating in EI mode. The temperature of ion source was set at 250°C and the energy of bombardment electron was set at 70 eV, while helium was used as carrier gas at 1.4kg/cm² pressure. The fraction was dissolved in chloroform (1:20). The injection volume was 1.0 μ L.

GCMS Analysis

The mass spectral survey (NIST Mass Spectral Search Program, ver. 2.0g, GC-Library NIST-11) with Automated Mass Spectral Deconvolution and Identification System (AMDIS) were used for the characterization of phytochemicals by means of comparison of mass spectrum of the unknown component with known component stored in library.

Activity

Antimicrobial activity of non-polar fraction TTH was determined against five different bacterial strains which include, *M. leutus*, *B. subtilis*, *S. aureus*, *S. epidermidis* and *Salmonella typhi* and *Enteropathogenic E. coli* (EPEC) using agar well diffusion method described by National Committee for Clinical Laboratory Standard (NCCLS) [NCCLS, 1993].

Result and Discussion

The study active non-polar fraction of twigs ethanol extract of *T. terrestris* revealed the identification of eleven compounds in which majority belongs to fatty acid and their methyl esters. The presence of carboxylic acid in fraction TTH was further confirmed by methylation. The identifications were further supported by Kovat's Retention Indices reported in literature and NIST Library.

For calculation of Kovat's retention indices available pure n-alkanes C6, C8, C10, C12 and C22 were used as primary reference and cover the range from C8 to C25 by spiking pure n-alkanes in kerosene oil and diesel, used as secondary standard. All these were analyzed on GC-FID followed GC-EIMS under the identical conditions as for the fractions and further confirmed by mass spectral survey (NIST Mass Spectral Search Program, ver. 2.0g, GC-Library NIST-11) with Automated Mass Spectral Deconvolution and Identification System (AMDIS).

It has been observed that n-hexane fraction (TTH) was effective against bacteria culture *M. leutus* whereas other strains were not inhibited by these non-polar fractions (Figure 1). However, further investigations are recommended to compare the antimicrobial activity of *T. terrestris* extracts with commercially available antibiotics.

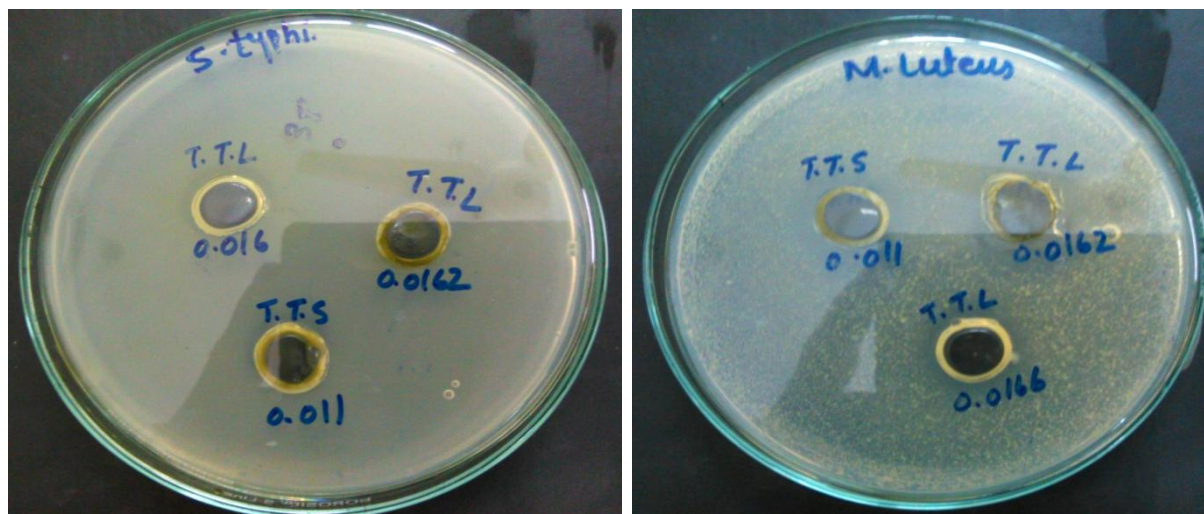


Figure 1: Screening for antimicrobial activity of *T. terrestris* non-polar fraction against *Salmonella typhi* and *Micrococcus luteus*.

Table 1: Qualitative analysis of TTH

S. #	Retention Time (min)	Constituent Name	Chemical Formula	Molecular Weight	Calc. R. I. ^a
1	27.83	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (1)	C ₂₀ H ₄₀ O	296	1920
2	29.27	Methyl hexadecanoate (2)	C ₁₇ H ₃₄ O ₂	270	1924
3	29.91	n-Hexadecanoic acid (3)	C ₁₆ H ₃₂ O ₂	256	1968
4	31.34	Methyl octadeca-9,12-dionate (4)	C ₁₉ H ₃₄ O ₂	294	2015
5	31.53	Phytol (5)	C ₂₀ H ₄₀ O	296	2020
6	31.71	Methyl octadecanoate (6)	C ₁₉ H ₃₈ O ₂	298	2028
7	31.82	Octadeca-9,12-dionic acid (7)	C ₁₈ H ₃₂ O ₂	280	2145
8	32.03	n-Octadecanoic acid (8)	C ₁₈ H ₃₆ O ₂	284	2157
9	35.23	Glycerol-1-palmitate (9)	C ₁₉ H ₃₈ O ₄	330	2460
10	48.57	23,28-Cyclostigmasta-5-en-3 β -ol (10) ^b	C ₂₉ H ₄₈ O	412	-
11	50.74	4,4-Dimethylcholestan-3-one (11) ^b	C ₂₉ H ₅₀ O	414	-

a) On capillary column with 5% phenyl 95% methyl silicone as stationary phase (DB-5)

b) Standard not available

Conclusion

This study represents qualitative analysis of non-polar fraction of twigs ethanol extract as well new natural antimicrobial source. Total eleven compounds have been identified from mentioned fraction through gas chromatography mass spectrometry (GC-MS). The presence of bioactive constituents indicates the use of this fraction as potential candidate for natural antimicrobial source and further antimicrobial spectrum against other gram-negative and gram-positive bacteria might be useful in determining the potential of these compounds. Also,

the use of advance technique GC/MS ensures the chemical constituents and promotes the systematic studies of plant.

-

References:

- Bose B. C., Saifi A. Q., Vijayavargiya R. and Bhatnagar J. N., Some aspects of chemical and pharmacological studies of *T. terrestris*, *Indian. J. med Sci.*, 1963, 17, 29.
- Bowen, W., Long'em M., Tong ku L., Climical observation on 406 cases of angina pectoris of coronary heart disease treated with saponin of *T. terrestris*, *Chinese J. of Inte. Trad. And West. Med.*, 1990, 10, 85-87.
- Chopra, R. N., Chopra, I. C., Handa, K. L., Kapur, L. D." Indigenous Drugs of India" Academic publishers, Calcutta. 2nd Ed., 1958, 431.
- Conrad J, Dinchev D, Klaiber I, Mika S, Kostova I, Kraus W., A novel furostanol saponin from *Tribulus terrestris* of Bulgarian origin., *Fitoterapia*, 2004; 75, 117-122
- Dymock W, Waden C J H and Hopper D., *Pharmacographia Indica*, Institute of health and TB Research, Karachi: 1972,pp. 306.
- Ivan A. Ross, *Medicinal Plants of the World*, Published by Humana Press. 2001, Vol-2, p411-426
- Nasroallah M. K., Hamid M. T., Tahereh A., *Journal of Biology and today's world*, 2013, 2, 31-40.
- NCCLS. 1993. Performance Standards for Antimicrobial Disc Susceptibility Tests. Approved Standard NCCLS Publication M2- A5, Villanova, PA, USA.
- Sahelian, R., Experimental *Tribulus terrestris* poisoning in sheep: clinical laboratory and pathological findings, *Veterinary Research Communications*, 2003, 27, 53-62.