Original Research Article

Antimicrobial activity and GC-MS analysis of bioactive compounds of *Tribulus terrestris*L.

ABSTRACT

Aims: To examine the potential phytoconstituents present in leaves and fruits of*Tribulus terrestris* and its antimicrobial activity against*Enterobacter cloacae*.
Study design: Plant extracts preparation, determination of total phenolic content, total antioxidant activity and antimicrobial activity. GC-MS analysis
Place and Duration of Study: Sample: Fresh plant parts of*Tribulusterrestris*were collected from the campus of Banasthali Vidyapith, Rajasthan, gram-negative bacterial strain, *Enterobacter cloacae*BVDWH38 (Gene bank Acc. No. OQ772170) was used for antimicrobial study.
Methodology: Plant extracts preparation; free radical scavenging of sample extract antimicrobial activity was done by following standard methods. All experiments were repeated at least thrice. The data represented are Mean ± standard deviations (SD) of all the three replicates.
Results: Alcoholic and aqueous leaves and fruits extracts of *T. terrestris* were prepared. Total phenolic compound (TPC) and antioxidant activity was determined and found to be higher in aqueous extract of both plant parts of *T. terrestris*.Antimicrobial activity against water borne pathogenic *E. cloacae*strains was evaluated and found that aqueous extract of both leaves and fruits of *T. terrestris*was more effective. Gas chromatography- Mass spectrophotometer (GC-MS) with alcoholic extract of leaves and fruits of *T. terrestris*identified Digitoxin (6.37%) and Ethyl iso-allocholate (22.90%) respectively among other compounds.
Conclusion: Theleaves and fruits of *T. terrestris* can be used as alternative medicine as it has vital bioactive secondary metabolites.

Keywords: Anti-microbial, Anti-oxidant, GC-MS, Tribulus terrestris, medicinal plant

1. INTRODUCTION

Fresh water plays an essential role in all living organisms for their survival and growth however an ever increasing population, industrialization, urbanization chemical fertilization associated with water-borne diseases [1]. Water associated infectious disease like acute gastrointestinal, cholera, diarrhea, dysentery, typhoid etc. diseases are principally caused by pathogenic microorganisms such as *Enterobacter* sp., *Enterococcus* sp., *Vibrio cholera*, *Escherichia coli*, *Giardiasis* sp., *Shigellosis* sp., *Salmonella* sp. and many more other bacterial pathogens [1, 2].

Enterobacter cloacaeare, the gram negative, rod-shaped bacteria, is majorly found in contaminated water, sewage, hospital environment as well as intestinal tracts of human and animals.*Enterobacter* species were rarely found as pathogens but these group of bacteria progressively encountered and causing urinary tract infection (UTI) and bacteremia[3].

Medicinal plants play an important role in the widely available primary treatment of healthcare remedies. Herbal remedies are in high demand due to their ease of access, low cost, and safer use than modern pharmaceuticals[4].*Tribulusterrestris*commonly known as 'Gokhru' grows in tropical climates and mostly found in Australia, India, and America. In traditional medicine, Gokhru extract is used to control cholesterol and blood pressure, as a tonic, aphrodisiac, analgesic, astringent, stomachic, anti-hypertensive, tonic, aphrodisiac, analgesic, diuretic lithon-triptic and urinaryinfection[5]. The fruits are known for their cooling diuretics and tonic properties, and they are used to treat painful micturition, UTI, and affections. Gokhru fruits have traditionally been used to treat kidney disease, gout, and as diuretics in Punjab. The leaves are used as an aperient and tonic in southern France and Europe [6].

The present work is conducted to evaluate the antimicrobial and antioxidant activity of ethanolic and aqueous extract of *T. terrestris*and characterize bio-active compounds by GC-MS analysis.

2. MATERIAL AND METHODS

2.1 Plant material

Fresh *Tribulus terrestris* plants were collected from the campus of Banasthali Vidyapith, Rajasthan, India and voucher specimen submitted and the herbarium (BURI 1731/2023). The leaves and fruit were properly washed and shade dried. Dried leaves and fruit powder were ground to fine powder and kept in airtight container at room temperature in the dark. Voucher specimen is submitted to the Banasthali Vidyapith Herbarium (BURI).

Figure 1. **A twig and fruits of *Tribulus terrestris***

2.2 Preparation of Plant Extracts

20g of dried powder of T. terrestris leaves and fruits were stored for further use. The extraction was conducted by 250 ml of the solvent (95% ethanol, aqueous) for 72 hours, filtered by Whatmann No.41 filter paper and evaporated to Dryness at room temperature then stored the dried crude extract at 4°C[7].

2.3Determination of Total Phenolic Content (TPC)

Extraction of phenolic compounds was done [8,9].To determine the total phenolic concentration, freshly harvested leaves were used. Total phenolics were determined with Folin-ciocalteau reagent and expressed as "gallic acid (GA) equivalents" [10].

2.4Determination of antioxidant activity

Free radical scavenging of sample extract was determined using DPPH(1, 1 dihydroxy 2-picrylhydrazyl), a stable free radical [11].The total antioxidant activity of the sample extract was determined [11] and Hydrogen peroxide scavenging assay was conducted [12]. Ascorbic acid was used as standard.

2.5Determination of antimicrobial activity

A gram-negative bacterial strain, *Enterobacter cloacae* BVDWH38 (Gene bank Acc. No.OQ772170, was used for studying the antimicrobial activity by broth dilution method [13]. An aliquot of individual bacterial stock of *E. cloacae* was grown in 2ml liquid LB medium overnight at 37ºC.The plant extracts (50-350µg/mL) were added to the bacterial culture. Bacteria were allowed to grow for 24h under standard conditions and absorbance taken at 540nm. MIC_{50} value was determined [14].

2.6 GC-MS Analysis

The ethanolic extract was used for GC-MS analysis. The extract was filtered on a Whatmann no.41 filter paper, evaporated and dried using vacuum concentrator unit and stored at 4°C. GC-MS(Thermo Fischer) with HP-5MS capillary column (film thickness of 0.25m; 30m X 250m) fitted in a split/split less injection system was used. Samples of 1µl aliquot were injected automatically. The temperature variations were: 280°C (injector temperature); 50°C (initial oven temperature) rising up to 300°C at 25°C/min followed by 10 min hold. Carrier gas (Helium) was used with flow rate of 2.1 ml/min at 17.69 psi pressure.

2.7 Statistical analysis

All experiments were repeated at least thrice. The data represented are Mean ± standard deviations (SD) of all the three replicates. Difference between values significantly (P=0.05) are compared using LSD following ANOVA.

3. RESULTS AND DISCUSSION

3.1 Total phenolic content (TPC)

Maximum total phenolic content was observed in aqueous extract of both leaves and fruits of *T. terrestris*. Leaf aqueous extract of *T. terrestris* showed higher TPC value as compared to fruit aqueous extract of *T. terrestris*(Table 1).

Table 1.Total phenolic content of *T. terrestris* leaves and fruit's extracts

Extract/ plant part	Total phenolic content GA equivalent (mM g^{-1}fwt)	
	leaves	fruit
Alcoholic	0.762±0.016[b]	0.557±0.018[a]
Aqueous	1.098±0.011[b]	0.857±0.022[a]
Values followed by the same alphabet in a column are statistically not significantly different at P=0.05 following ANOVA and LSD		

3.2 Antioxidant activity

High free radical scavenging activity and DPPH and phoshomolybdenumactivity was observed in the aqueous of both plant parts followed by alcoholic extract However, both alcoholic and aqueous leaves extract of *T. terrestris* showed higher antioxidant activity as compare to its fruits extract (Figure.2, 3, 4 and 5). Minimum H_2O_2 production was found in leaves and fruits aqueous followed by alcoholic extracts of *T. terrestris* (Fig. 4.1 and 4.2).

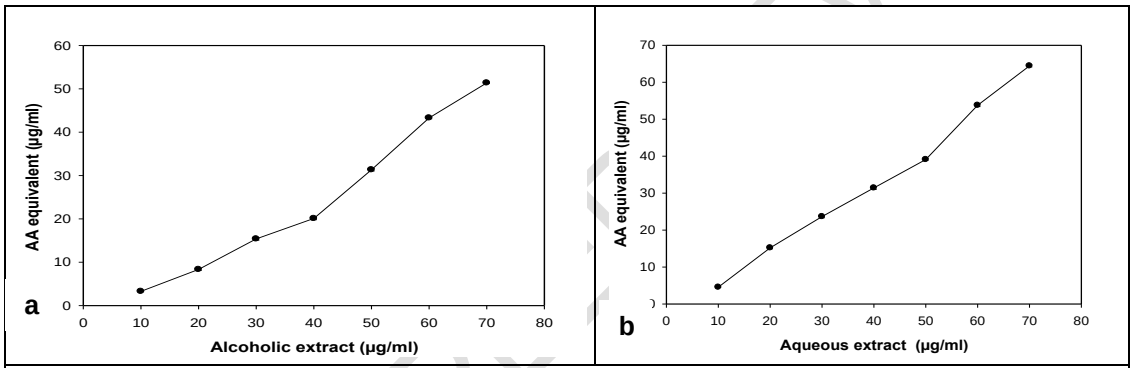

Figure 2Free radical scavenging activity of (a) Alcoholic extract, (b) Aqueous extract of *T. terrestris* leaves extract

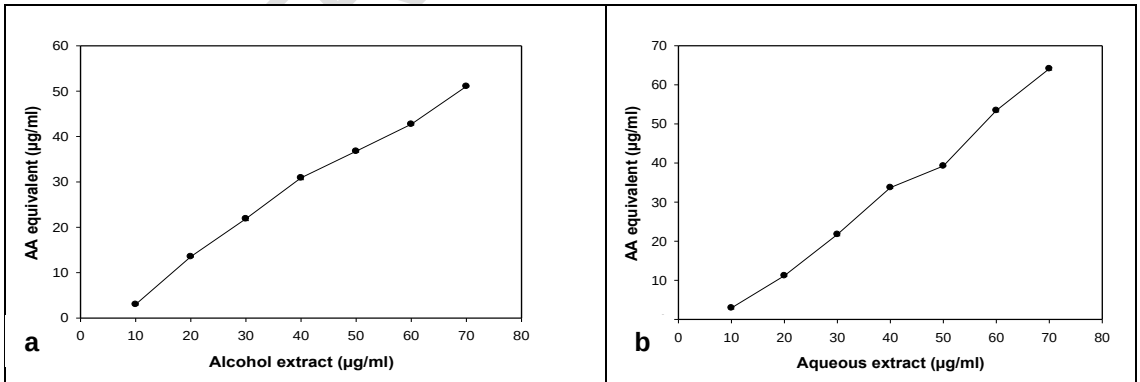

Figure3 Free radical scavenging activity of (a) alcoholic extract, (b) aqueous extract of *T. terrestris* fruit

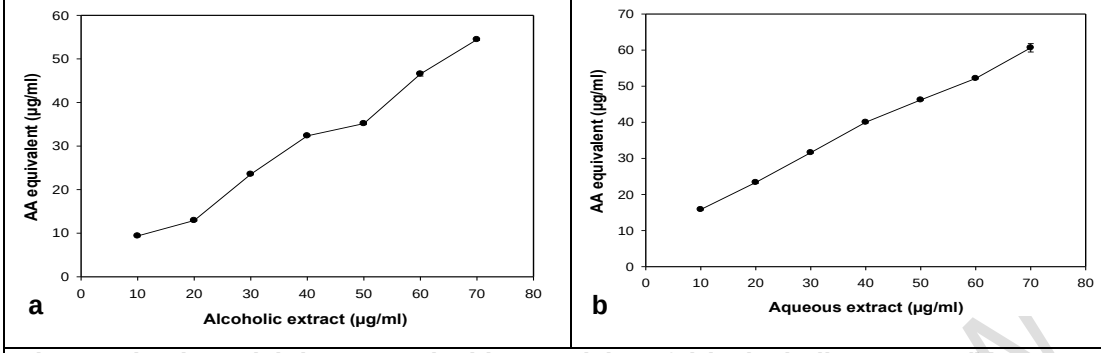

Figure4Phoshomolybdenum antioxidant activity of (a) alcoholic extract, (b) aqueous extract of*T. terrestris* leaves

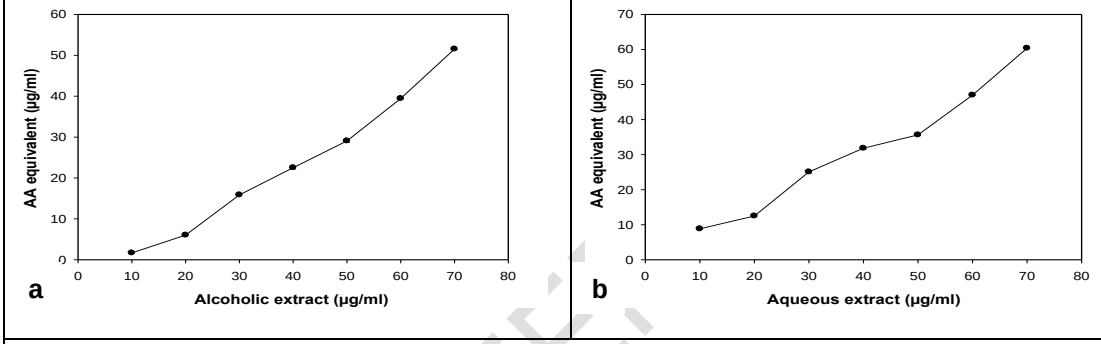

Figure 5Phoshomolybdenum antioxidant activity of (a) alcoholic extract, (b) aqueous extract *T. terrestris*fruit

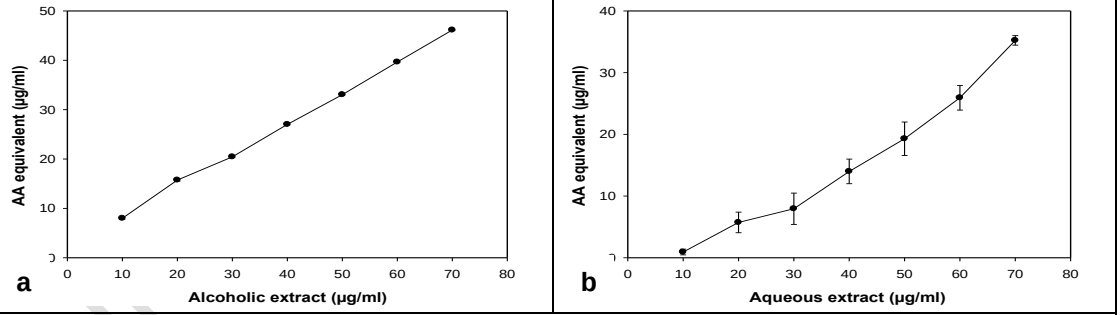

Figure 6 Reducing power of (a) alcoholic extract, (b) aqueous extract of *T. terrestris* leaf

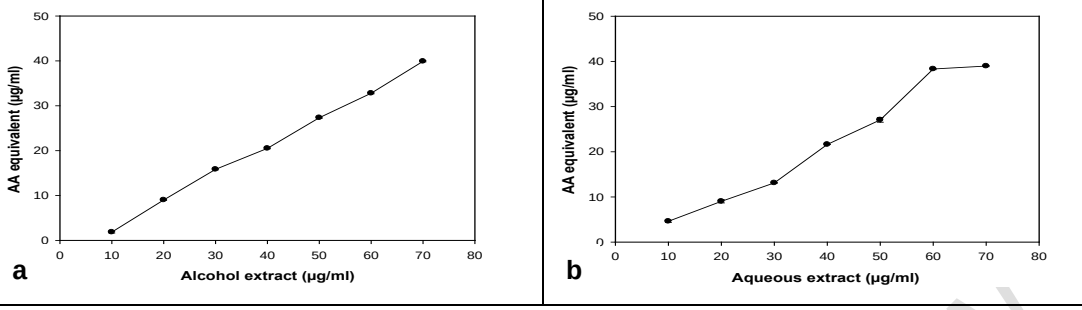

Figure7 Reducing power of (a) alcoholic extract, (b) aqueous extract of *T. terrestris*fruit

3.3 Antimicrobial activity

Antimicrobial activity of plant against *E.cloacae* strainwas evaluated and compared with standard tetracycline. Antibacterial activity of*T. terrestris*fruit extracts was better in comparison to its leaves extracts.

Table2 MIC_{50} and MIC_{90} in different extract of *T. terrestris* leaves and fruits against *E. cloacae*

Plant part	Extract	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
leaves	Aqueous	137.974	302.588
	Alcoholic	133.666	306.826
fruits	Aqueous	109.828	281.502
	Alcoholic	144.106	300.351
Tetracycline		69.83	

3.4 GC-MS Analysis

GC-MS chromatogram (Figure8 and 9) revealed various vital compoundspresent in *T. terrestris* leaves and fruit'sand is represented in Table 3 with their have medicinal properties.

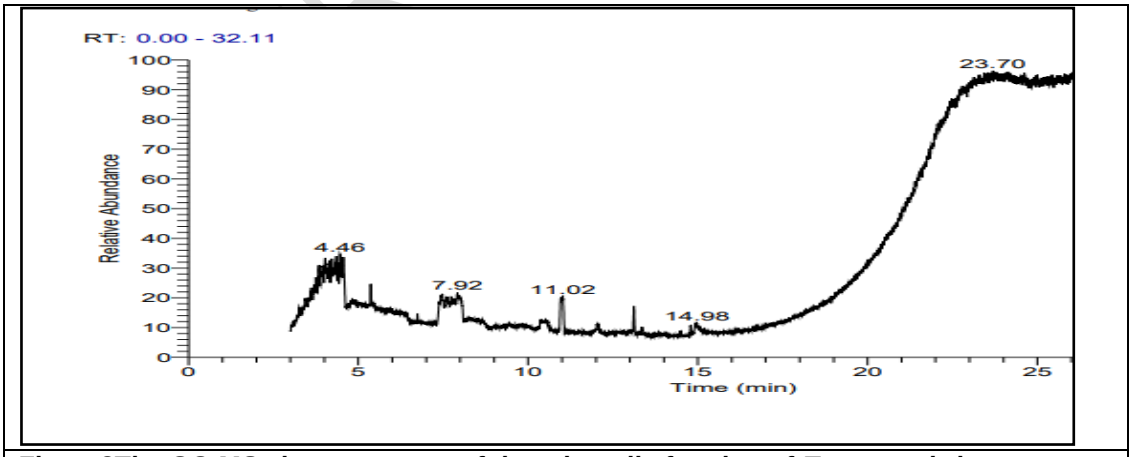

Figure8The GC-MS chromatogram of the ethanolic fraction of *T. terrestris* leaves

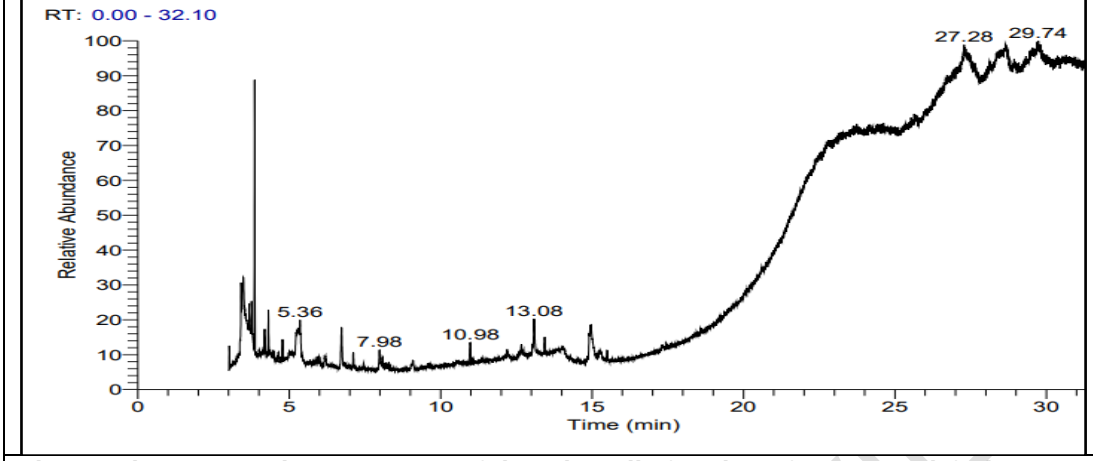

Figure9The GC-MS chromatogram of the ethanolic fraction of *T. terrestris*fruit

Table3.GC-MS analysis of Ethanolic leaves extract of *T. terrestris*

RT (min)	Compound Name	Molecular Formula	MW	Peak Area %	Compound Class	Bioactivity	Ref.
4.02	Digitoxin	$C_{41}H_{64}O_{13}$	764.9	6.37	Glycoside	Treating Heart failure	[15,16]
4.47	2,6 Dihydroxyacetophenone, 2TMS derivative -	$C_{14}H_{24}O_3Si_2$	296.5	4.38	Alkaloids	Antimicrobial, Anti-oxidant, Anti-inflammatory	[17]
4.56	Octadecane, 1,1'-[1,3-propanediylbis(oxy)]bis	$C_{39}H_{80}O_2$	581.1	7.59	fatty acids	hypertension, musculoskeletal disorders, gastrointestinal distress, cough, amenorrhea	[18,19]
5.37	2-Trimethylsiloxy-6-hexadecenoic acid, methyl ester	$C_{20}H_{40}O_3Si$	356.6	13.82	fatty acids	urinary passage, lungand spleen diseases	[20]
7.43	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	$C_{20}H_{13}N_5O_2$	355.3	13.75	Alkaloids	Antimicrobial Antibacteria, Antifungal and Anti-Diabetic	[21,22]
8.00	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis	$C_{28}H_{44}O_4$	444.6	5.76	fatty acids	anti-histaminic, anticoronary, insectifuge, anti-eczemic, anti-acne,	[23,24]
13.12	Glycine, N-[(3à,5á)-24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester	$C_{30}H_{53}NO_4Si$	519.8	11.77	Glycosides, fatty acid	Antimicrobial	[25]

Table 4.GC-MS analysis of Ethanolic fruit's extracts of *Tribulus terrestris*

RT (min)	Compound Name	Molecular Formula	MW	Peak Area %	Compound Class	Bioactivity	Ref.
5.36	Phosphonoacetic Acid, 3TMS derivative	$C_2H_5O_5p$	140.03	6.10	Secondary metabolites	Antiviral agent	[26]
13.09	Oleic Acid	$C_{18}H_{34}O_2$	282.47	24.28	Fatty acid	Prevent heart disease, reduce cholesterol.	[25]
14.96	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298.89	10.60	linoleoyl chloride	Antimicrobial, antioxidant and cytotoxic	[15,20]
27.28	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.6	22.90	Steroids derivative	Antimicrobial activity	[15,18]
28.66	Trilinolein	$C_{57}H_{98}O_6$	879.4	13.32	linoleoyl chloride	Antimicrobial, Antioxidant, anti-ischemic	[27]

4. CONCLUSION

*Tribulus terrestris*plant extract containespolyphenolic compounds, antioxidants and antimicrobials. The presence of bioactive compounds in the ethanolic fractions as detected by GC-MS and their biological roles in *T. terrestris*support the ethnobotanical claims and medicinal value of this plant.

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