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Antimicrobial potential of selected arid and semi-arid plants against urinary tract infection causing pathogens through GC-MS and FTIR analysis

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ABSTRACT

The purpose of this study was to investigate the presence of phytoconstituents in the extracts of *Convolvulus microphyllus*, *Acacia nilotica, Withania somnifera, Tribullus terrestris,* and *Citrullus colocynthis,* and to test their anti-microbial activity against the most common strains causing bacterial urinary tract infection (UTI). The dried powdered plant parts were extracted using five different solvents in a Soxhlet apparatus for 48 h at a temperature ranging from 60 to 80°C. These extracts were used for preliminary phytochemical and antimicrobial analyses. Fourier-transform infrared spectroscopy (FTIR) with a scan range of 450 to 4000/cm and 4/cm resolution and GC-MS analysis were carried out to analyse the samples for the presence of phytochemicals having antimicrobial activity. The methanolic extract of *Tribulus terrestris* was found to have the most promising antibacterial properties. The results revealed that the methanolic extract of *Tribullus terrestris* displayed significant antimicrobial activity against the uropathogens, especially against *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. Phytochemicals like steroids, glycosides and alkaloids were found in the methanolic extracts of *T. terrestris*. FTIR analysis and GC-MS revealed the presence of 50 compounds. We conclude that the methanolic extract of *T. terrestris* fruit has more potent antimicrobial activity than other extracts and standard antibiotics used in this study. In the future, additional investigation will be required to isolate the active compound, undertake toxicological investigations, and start a clinical trial.

Keywords: Antibiotic-resistance, Anti-microbial agents, Bioactive compounds, Desert medicinal plants, Herbal regime, Urinary tract infections.

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1. INTRODUCTION

Urinary tract infections (UTI) are the most widespread microbial infection and impose a significant financial and social cost on society (Peck et al., 2021). It is the most prevalent bacterial infection in women, infecting 10% of them each year and 60% of them at least once in their lives. It is becoming an even bigger problem due to the development of multi-drug resistance in pathogens against antibiotics, leading to serious consequences (Willey et al., 2011; Kenneally et al., 2022; Singh et al., 2022). Apart from this, antibiotics are occasionally connected with adverse consequences on the host, including hypersensitivity, reduced beneficial gut and mucosal micro-organisms, immunosuppression, allergic responses etc. The global incidence of urinary tract infections (UTIs) and the adverse effects of conventional drugs on the protective natural flora of the vaginal canal have compelled researchers to look for antimicrobial compounds which are harsh on the microbes, can overcome microbial resistance while being mild on the host (Prakash et al., 2013; Coker et al., 2021). Several studies

have found that natural antimicrobials derived from plants are effective against multidrug-resistant bacteria. As a result, research needs to be conducted to reveal plant based novel treatment strategies (Egharevba et al., 2015; Abuga et al., 2021). Interestingly, natural compounds account for over 80% of antibacterial, immunosuppressive, cardiovascular drugs and 60% of cancer treatments in the market (WHO, 2018). An advantage of the plant based ethnomedicine is that they can operate on numerous targets at the same time (Gupta et al., 2012). Up to 80% of the rural population in India uses herbal medicine as primary form of health care. A number of desert medicinal plants contain bioactive compounds that interact with other environmental species to prevent bacterial or fungal development (Jaradat, 2020). The current study aims to screen arid and semi-arid dessert plants for treatment of urinary tract infection and phytochemical analysis of their extracts to find the bioactive compounds responsible for their anti-microbial action.

2. METHODS AND MATERIALS

2.1 Collection of Desert Plant Samples

A total of 5 plants and their parts were collected from Rajasthan, India and identified by the taxonomist B. C. Tayal, S. Sharma, A. Kumar, I. Talreja, from Department of Botany, Rajasthan university. The voucher specimen number is *Convolvulus microphyllus* (root) (RUBL5774), *Acacia nilotica* (leaves) (RUBL1615), *Withania somnifera* (root) (RUBL2208), *Tribullus terrestris* (fruit) (RUBL1409), *Citrullus colocynthis* (fruit) (RUBL3653), deposited in Rajasthan University.

2.2 Preparation of Plant Extracts

Plant samples were cleaned with distilled water and dried in shade for 7–10 days at room temperature. After drying the plant components were pulverised in a mixer grinder. The powdered samples were kept in sealed containers until needed. Twenty- gram powder of each plant sample was extracted with different organic solvents (methanol, ethanol, chloroform, and petroleum ether (300 mL each) in addition to water for 72 h at 60 to 80ºC (not more than the solvent's boiling point). The extracts were filtered using Whatman filter paper number 1. The extracts were collected after incubation and stored in refrigerator at 4°C. The yield of dried extract was 2–3% of the starting dried plant materials. The extracts were concentrated at a reduced pressure using a rota vapour and stored in brown, amber vials until needed for qualitative phytochemical analysis (Satish et al., 2007).

2.3 Bacterial Strains

The Microbial Type Culture Collection (MTCC), Chandigarh, India provided all pure cultures of *Escherichia coli* (MTCC 433), *Staphylococcus aureus* (MTCC 737), *Pseudomonas aeruginosa* (MTCC 741), *Enterococcus faecalis* (MTCC 439), *Candida albicans* (MTCC 227). Muller Hinton agar was used for bacterial cultures, and Sabouraud dextrose agar (SDA) was used for pure fungus cultures. Each bacterial and fungal culture was preserved at 4°C and regularly subculture on the same medium.

2.4 Inoculum Preparation

Stock cultures were stored at 4°C in Muller Hinton agar slants. A loopful from stock cultures was transferred to sterile muller Hinton broth media containing tubes and incubated at 37°C for 24 h (Kavitha and Satish, 2014). This was used to inoculate the plates to be used for disc diffusion.

2.5 Antimicrobial Activity of Plant Extracts Against Uropathogenic Bacteria

Kirby-Bauer disk diffusion susceptibility test was used to test antibiotic sensitivity and resistance of plant extracts against uropathogenic bacteria (Bauer et al., 1996). 100 µL of the test bacterial inoculums from an 18–24 h broth culture were spread on the surface of Muller Hinton agar media plates. On top of which antibiotic discs were positioned. Petroleum ether, methanol, chloroform, and ethanol extracts were poured onto sterile 6-mm Whatman paper discs, which were placed on inoculation plates in 50 µL (concentration of 100 mg/mL). Some antibiotics were also tested in this study included amoxicillin (30 µg), norfloxacin (l0 µg), gentamicin (l0 µg), and kanamycin (10 µg). The plates were incubated for 18–24 h at 37°C after cooling at 4°C for 2 h. Inhibitory zones were measured in terms of diameter in each plate (Kavitha and Satish, 2014).

2.6 Preliminary Phytochemical Analysis of Plant Extract

Standard techniques and chemical tests were used to determine phytochemical constituents in chloroform, ethanol, methano1, petroleum ether, and aqueous extracts of *Convolvulus microphyllus, Acacia nilotica, Withania somnifera, Tribullus Terrestris,* and *Citrullus colocynthis* (Ghani, 2003).

2.7 Fourier-Transform Infrared Spectrophotometer Analysis

10 mg of dry extract powder of *Tribulus terrestris* was mixed with 100 mg of potassium bromide (KBr) and cast into a pellet. Scanning the disc in a scan range of 450 to 4000/cm and a resolution of 4/cm in a FTIR spectroscope (FTIR, Perkin Elmer Shelton, CT, USA Spectrum IR Version 10.6.0.) revealed the functional groups present in the extract (Arulmozhi et al*.,* 2018).

2.8 Gas Chromatography Mass Spectrometry Analysis

A GC-MS equipment (QP-2010 series Ultra Shimadzu company, Tokyo, Japan) equipped with detector and Elite-5 capillary column, length (30 m × 0.25 mm ID × film thickness 0.25 µm) was used to analyse the methanolic extract with the most promising antimicrobial activity. Helium gas was used as the carrier gas (flow rate: 1

mL/min). Both the injector and the interface reached a temperature of 270°C. The column oven temperature was programmed to rise from (100.0 to 300.0°C) at a rate of 10 min before being held for 3 min. The compounds spectra were compared to standard spectra from the GC-MS NIST and WILEY libraries (Arulmozhi et al., 2018).

2.9 Statistical Analysis

All the experiments were performed in triplicate. The mean ± and standard deviation data were expressed and the comparison of the antibacterial activity of the samples with standard antibiotics. Two different statistical software were used for descriptive statistics R and JMP. Fig. 6 was made on R (version 4.2.0), and Figs. 1 to 5 on JMP 16 pro.

3. RESULTS

The ethnomedicinal knowledge on desert medicinal plants is well documented. Most plants are used in herbal treatment by local ethnic Aborigine communities for various diseases such as for bronchitis, asthma, diarrhoea, diabetes, fever, dysuria, syphilis, gonorrhoea, diuretic, and urinary issues etc as described in Table 1.

The antibacterial results revealed that the methanolic extract of *Tribullus Terrestris* Showed significant antimicrobial activity against the tested pathogens, especially for *Escherichia coli* (20 mm), *Pseudomonas aeruginosa* (23 mm) and *Candida albicans* (19 mm), as compared to the other plant extracts like (*Convolvulus microphyllus*, *Acacia nilotica*, *Withania somnifera* and *Citrullus colocynthis*). The inhibition zone was interpreted under Clinical and Laboratory Standards Institute (CLSI) standards (Figs. 1–5).

The presence of phytochemicals test, the plants contained bioactive agents which relate to antimicrobial properties in the extracts are shown in Fig. 6. of *Convolvulus microphyllus* (root), *Acacia nilotica* (leaves), *Withania somnifera* (root), *Tribullus terrestris* (fruit), *Citrullus colocynthis* (fruit). Alkaloids and flavonoids were found in all solvent extracts (chloroform, methanol, petroleum ether, ethanol, and aqueous) in *Convolvulus microphyllus,* whereas carbohydrates and saponin were found only in the aqueous extract, and glycosides and all solvent extracts were devoid of tannins. All solvent extracts showed tannin and saponin from *Acacia nilotica*. However, flavonoids from chloroform and aqueous extracts were only present in methanol extract, and alkaloids, carbohydrates, and glycosides were not present in all solvent extracts. Saponins and alkaloids were observed in all solvent extracts of *Withania somnifera*, whereas carbohydrates were found in all solvent extracts except methanol and aqueous, tannins were found in all solvent extracts except methanol, flavonoids, and glycosides were found in all solvent extracts except methanol and chloroform extracts. All solvent extracts of *Tribullus Terrestris* contained alkaloids, flavonoids, glycosides, tannins and saponins, whereas aqueous extracts contained, and solvent extracts contained no carbohydrates. In all solvent extracts of *Citrullus colocynthis* contained alkaloids, carbohydrates, saponins, and glycosides. However, all solvent extracts contained flavonoids except the methanol extract, and all solvent extracts were devoid of tannins.

Table 1. Medicinal properties of desert medicinal plants used in urinary tract infections

S. no	Plant name	Part used	Local name	Family	Ethnomedicinal uses
1	*Convolvulus microphyllus*	Root	Shankpushpi	Convolvulaceae	Shankhapushpi leaves were once used to cure chronic bronchitis and asthma. The oil increases hair growth, and the root was used to treat infantile fever and uterine issue while also improving strength and digestive power (Sharma, 1983).
2	*Acacia nilotica*	Leaves	Babool	Mimosaceae	Cholera, hair loss, syphilis, gonorrhea, leucorrhoea, diarrhea, dysentery, diabetes, and antifungal are traditional uses for this plant. Eye complaints, headaches, throat infections, ulcers, antiscorbutic, urinary difficulties, and gonorrhea are treated with the leaves (Sharma et al., 2014).
3	*Withania somnifera*	Root	Ashwagandha	Solanaceae	Fevers and severe swelling are treated with leaves. Flowers are aphrodisiac, astringent, depurative, diuretic, and aphrodisiac. Urinary issues are treated with roots (Ahmad et al., 2017).
4	*Tribullus terrestris*	Fruit	Bindii	Zygophyllaceae	In folk medicine *Tribullus terrestris* used as tonic, aphrodisiac, analgesic, astringent, stomachic, antihypertensive and in urinary tract infections (Usman et al., 2007).
5	*Citrullus colocynthis*	Fruit	Desert gourd	Cucurbitaceae	Gastrointestinal diseases, constipation, dysentery, enteritis, and bacterial infections are among the traditional therapeutic uses (Hussain et al., 2014).

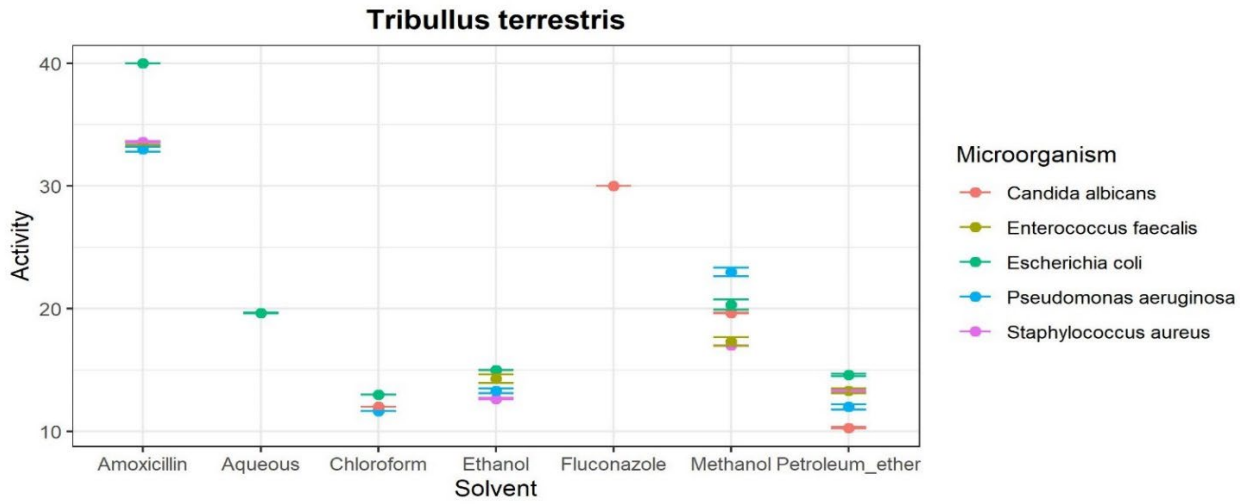

Fig. 1. *Tribullus terrestris* solvents mixed Fruit extracts and commercial drugs shown the antimicrobial activity against UTI pathogens

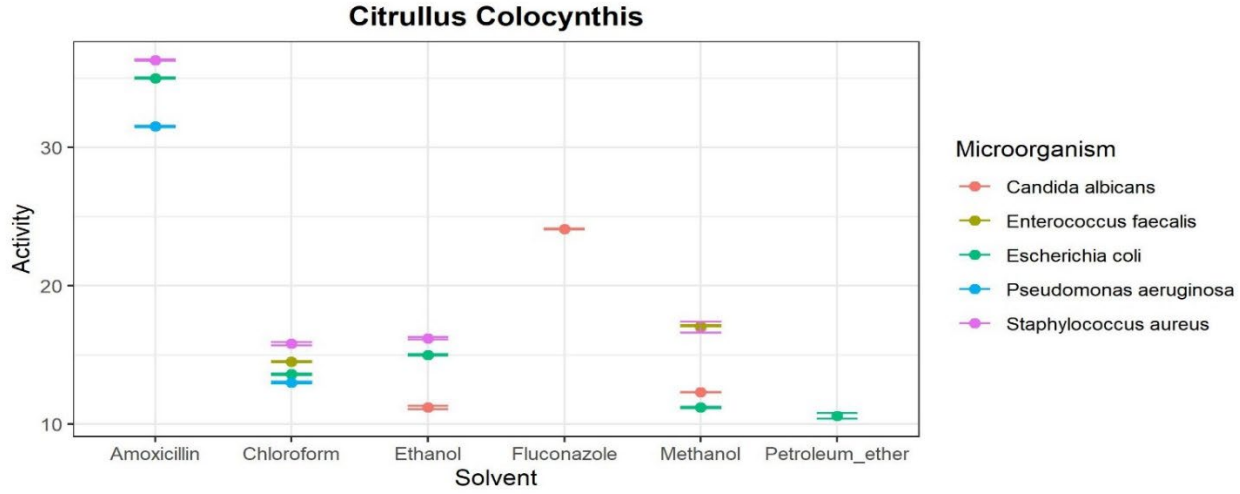

Fig. 2. *Citrullus colocynthis* solvents mixed Fruit extracts and commercial drugs shown the antimicrobial activity against UTI pathogens

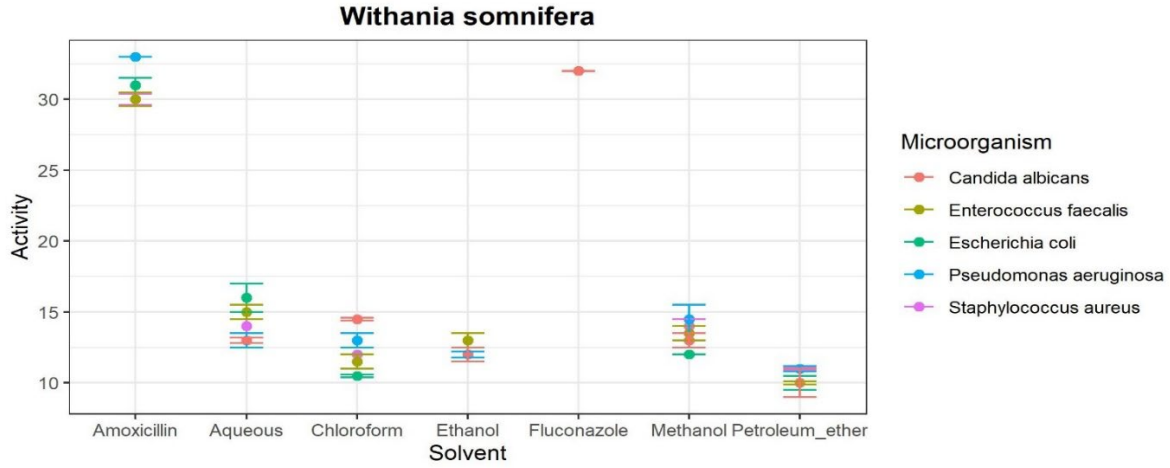

Fig. 3. *Withania somnifera* solvents mixed root extracts and commercial drugs shown the antimicrobial activity against UTI pathogens

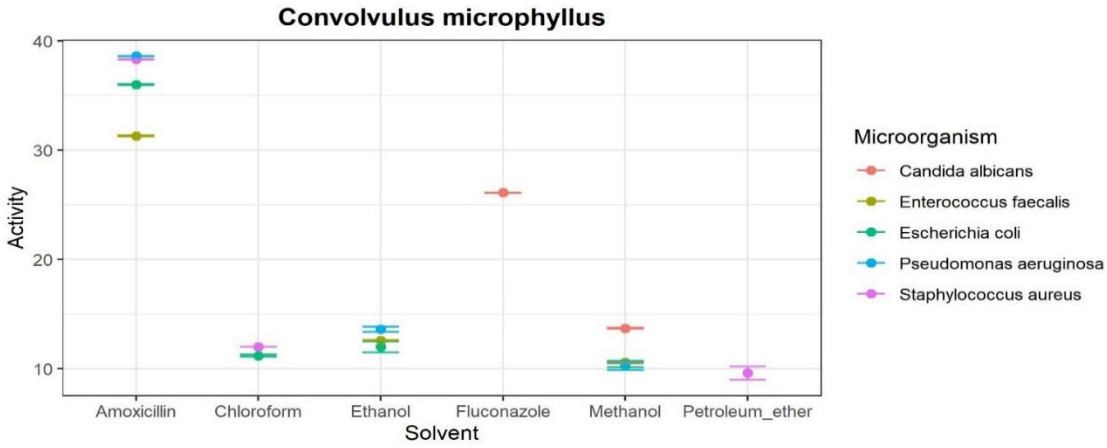

Fig. 4. *Convolvulus microphyllus* solvents mixed root extracts and commercial drugs shown the antimicrobial activity against UTI pathogens

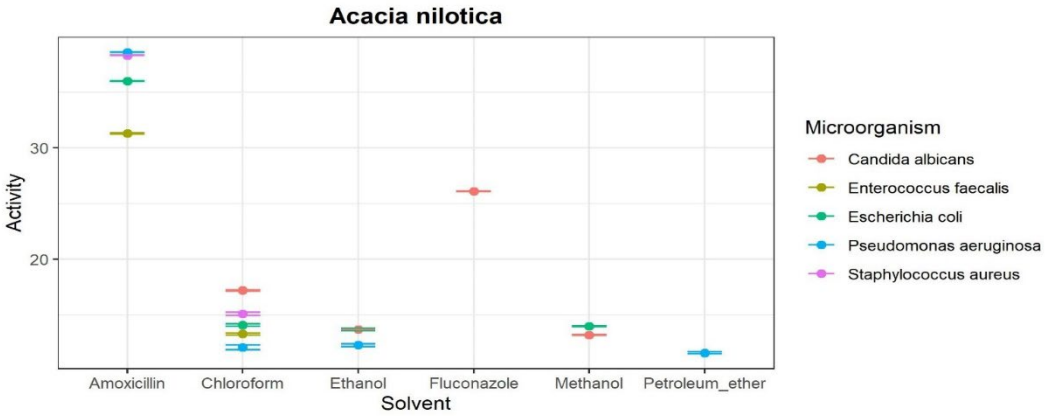

Fig. 5. *Acacia nilotica* solvents mixed leaves extracts and commercial drugs shown the antimicrobial activity against UTI pathogens.

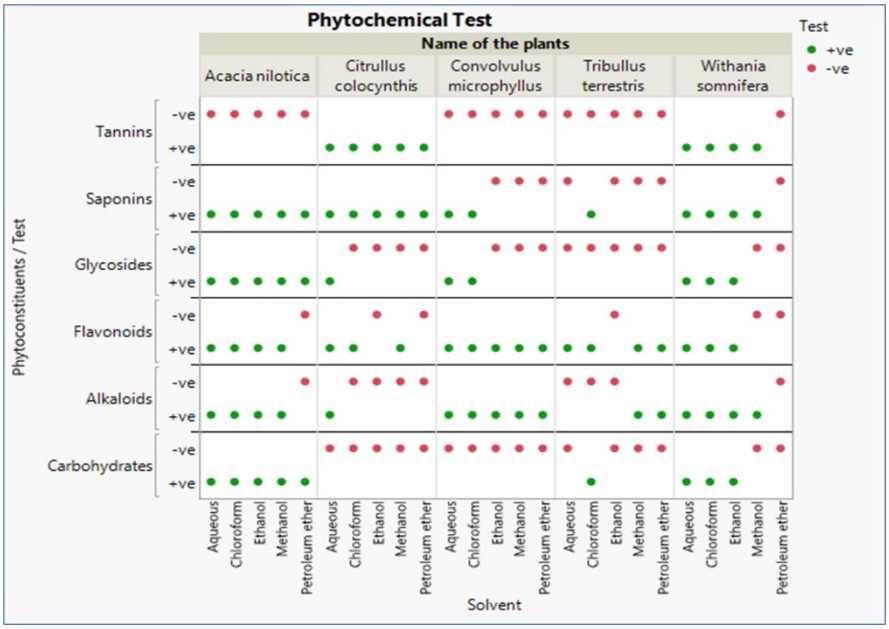

Fig. 6. Phytochemical analysis of plant extracts
(●) Positive, (●) Negative

Table 2. Zone of inhibition (mm; in diameter) against different UTI pathogens by plants extracts

S. No.	Name of the plants	Solvent	UTI Pathogens				
			Escherichia coli	*Staphylococcus aureus*	*Enterococcus faecalis*	*Pseudomonas aeruginosa*	*Candida albicans*
1.	*Tribullus terrestris*	Aqueous	19.66 ± 0.05	-	-	-	-
		Petroleum ether	14.6 ± 0.11	13.3 ± 0.05	13.3 ± 0.2	12 ± 0.2	10.3 ± 0.05
		Ethanol	15 ± 0	12.66 ± 0.05	14.3 ± 0.37	13.3 ± 0.2	-
		Methanol	20.33 ± 0.41	17 ± 0	17.33 ± 0.34	23 ± 0.34	19.66 ± 0.05
		Chloroform	13 ± 0	12 ± 0	12 ± 0	11.66 ± 0	12 ± 0
		Amoxicillin	40 ± 0	33.6 ± 0.05	33.3 ± 0.05	33 ± 0.2	-
		Fluconazole	-	-	-	-	30 ± 0
2.	*Citrullus colocynthis*	Aqueous	-	-	-	-	-
		Petroleum ether	10.6 ± 0.21	-	-	-	-
		Ethanol	15 ± 0.06	16.2 ± 0.09	-	-	11.2 ± 0.11
		Methanol	11.2 ± 0.06	17 ± 0.41	17.1 ± 0.06	-	12.3 ± 0.01
		Chloroform	13.6 ± 0.06	15.8 ± 0.12	14.5 ± 0.04	13 ± 0.08	-
		Amoxicillin	35 ± 0.05	36.3 ± 0.06	31.5 ± 0.04	31.5 ± 0.05	-
		Fluconazole	-	-	-	-	24.1 ± 0.04
3.	*Withania somnifera*	Aqueous	16 ± 1	14 ± 0.5	15 ± 0.5	13 ± 0.5	13 ± 0.2
		Petroleum ether	10 ± 0.5	11 ± 0.1	10 ± 0.1	11 ± 0.2	10 ± 1
		Ethanol	12 ± 0.5	-	13 ± 0.5	12 ± 0.2	12 ± 0.5
		Methanol	12 ± 0	14 ± 0.5	13.5 ± 0.5	14.5 ± 1	13 ± 0.5
		Chloroform	10.5 ± 0.1	12 ± 0	11.5 ± 0.5	13 ± 0.5	14.5 ± 0.1
		Amoxicillin	31 ± 0.5	30 ± 0.4	30 ± 0.05	33 ± 0	-
		Fluconazole	-	-	-	-	32 ± 0.01
4.	*Convolvulus microphyllus*	Aqueous	-	-	-	-	-
		Petroleum ether	-	9.6 ± 0.61	-	-	-
		Ethanol	12 ± 0.5	-	12.6 ± 0.02	13.6 ± 0.23	-
		Methanol	10.3 ± 0.17	10.6 ± 0.07	10.6 ± 0.05	-	13.7 ± 0.05
		Chloroform	11.2 ± 0.08	12 ± 0	-	10.3 ± 0.42	-
		Amoxicillin	36 ± 0.05	38.3 ± 0.02	31.3 ± 0.05	38.6 ± 0.02	-
		Fluconazole	-	-	-	-	26.1 ± 0.03
5.	*Acacia nilotica*	Aqueous	-	-	-	-	-
		Petroleum ether	-	-	-	11.6 ± 0.08	-
		Ethanol	13.7 ± 0.11	-	-	12.3 ± 0.13	13.7 ± 0.01
		Methanol	14 ± 0.03	-	-	-	13.2 ± 0.05
		Chloroform	14.1 ± 0.12	15.1 ± 0.14	13.3 ± 0.09	12.1 ± 0.21	17.2 ± 0.07
		Amoxicillin	36 ± 0.05	38.3 ± 0.02	31.3 ± 0.05	38.6 ± 0.02	-
		Fluconazole	-	-	-	-	26.1 ± 0.03

(-) Not detected

Based on the peak values which is shown in FTIR graph, the functional group of *T. Terrestris* fruit extract was confirmed (Fig. 7). FTIR spectra of *Tribulus terrestris* revealed peaks at 3422.41, 2946.38, 282.92, 2527.09, 2045.40, 1649.29, 1454.66, 1112.21, 1410.93, 1051.56, 1016.68, 655.15/cm respectively.

According to GC-MS analysis, bioactive mixtures were recognised in the methanolic extract of *Tribulus Terrestris* and the Graph showing the peak identities of the compound is presented in Fig. 8. Molecular Formula (MF), Retention Time (RT), Concentration (%), Molecular Weight (MW), are presented in Table 3. Fifty compounds were identified in this extract. The appearance of prominent peaks, as well as the components that correlate to them, were determined. The results revealed that Benzene propanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (14.602%) and Hexadecane (10.910%) was found as the major component is the methanol extract and the Forty-eight minor components. The bioactive component in the methanolic extracts of *Tribullus terrestris* fruits needs to be further investigated to discover a novel antibacterial agent in the fight against global antimicrobial resistance.

4. DISCUSSION

As UTI continues to affect our ever-growing population, emerging countries are unable to cope with allopathy medicine because to its long-term effects on the human body. According to WHO studies, antimicrobial resistance (AMR) is a public health hazard that impacts a wide range of

Table 3. Bioactive molecules identified from *Tribulus terrestris* by GC-MS analysis

Peak #	R. Time	Area	Area %	Name
1	7.626	413067	0.90	Cyclohexanol, 1-methyl-4-(1-methylethylidene)-, acetate
2	7.884	397064	0.86	Pentadecane, 3-methyl-
3	8.200	209829	0.46	Cyclohexane, 1,2,3-trimethyl-
4	8.307	3536462	7.67	Tetradecane
5	8.715	283957	0.62	Caryophyllene
6	9.391	427656	0.93	(3e)-3-Methyl-4-(2,6,6-trimethyl-2-cyclohexe
7	9.496	1032604	2.24	2-Buten-1-ol, 2-ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-y
8	9.657	162939	0.35	cis-Z-.alpha.-Bisabolene epoxide $$ 4-[(1Z)-1,5-Dimethyl-1,4-hexadienyl]-1-methyl-7-oxabicyclo
9	9.768	753017	1.63	4H-Inden-4-one, 1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamet
10	9.890	290628	0.63	Phenol, 2,4-bis(1,1-dimethylethyl)-
11	10.131	231546	0.50	Lily aldehyde
12	10.233	1216377	2.64	Carbonic acid, methyl octadecyl ester
13	10.394	4948906	10.74	2-d,2-pentadecyl-1,3-dioxepane
14	10.547	471820	1.02	Pentadecane, 3-methyl-
15	10.828	319212	0.69	1-Hexadecene
16	10.910	4186522	9.08	Hexadecane
17	11.381	344512	0.75	Chrysantenyl 2-methuylbutanoate
18	11.614	4183424	9.08	Methyl (3-oxo-2-Pentylcyclopentyl) acetate
19	11.772	1209053	2.62	1-(4-Isopropylphenyl)-2-methylpropyl acetate
20	11.957	210637	0.46	Cyclopentane acetic acid, 3-oxo-2-pentyl-, methyl ester
21	12.159	406145	0.88	Tricyclo[4.3.0.0(7,9)]nonane, 2,2,5,5,8,8-hexamethyl-, (1.a
22	12.411	540552	1.17	Benzene, (1-pentylheptyl)-
23	12.780	755129	1.64	Octanal, 2-(Phenyl methylene)-
24	12.878	816568	1.77	*Heptadecane*, *3-methyl-*
25	13.129	383969	0.83	1-Heptadecene
26	13.196	1905891	4.14	Octadecane
27	13.325	443951	0.96	Malonic acid, hexadecyl 2-hexyl ester
28	13.560	309590	0.67	Benzene, (1-butylnonyl)-
29	13.692	839443	1.82	Cyclopentadecanone, 2-hydroxy-
30	13.766	276345	0.60	4,6,6,7,8,8-Hexamethyl-1,3,4,6,7,8-hexahydroc
31	14.412	328004	0.71	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione
32	14.527	434595	0.94	Hexadecenoic acid, methyl ester
33	14.602	5195506	11.27	Benzene propanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester
34	14.958	334626	0.73	2-Bromotetradecane
35	15.183	120398	0.26	Hexadecane dinitrile
36	15.244	650767	1.41	*Eicosane*
37	15.410	249922	0.54	Tridecane, 4-cyclohexyl-
38	16.166	622816	1.35	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
39	16.223	1763801	3.83	9-Octadecenoic acid, methyl ester, (E)-
40	16.460	614991	1.33	Methyl stearate
41	18.676	409137	0.89	9-Octadecenamide, (Z)-
42	19.301	312873	0.68	Hexadecenal, 2-methyl-
43	20.033	76592	0.17	Methyl 7,11,14-eicosatrienoate
44	20.071	411214	0.89	trans-9-Octadecenoic acid, pentyl ester
45	20.814	468825	1.02	10-Octadecenoic acid, methyl ester
46	21.530	513239	1.11	*9-Octadecenoic acid (Z)-, tetradecyl ester*;
47	21.863	560739	1.22	13-Docosenamide, (Z)-
48	22.230	660461	1.43	Oleic acid, eicosyl ester
49	25.819	356151	0.77	*Tetracontane*
50	26.329	499946	1.08	. gamma. -Sitosterol

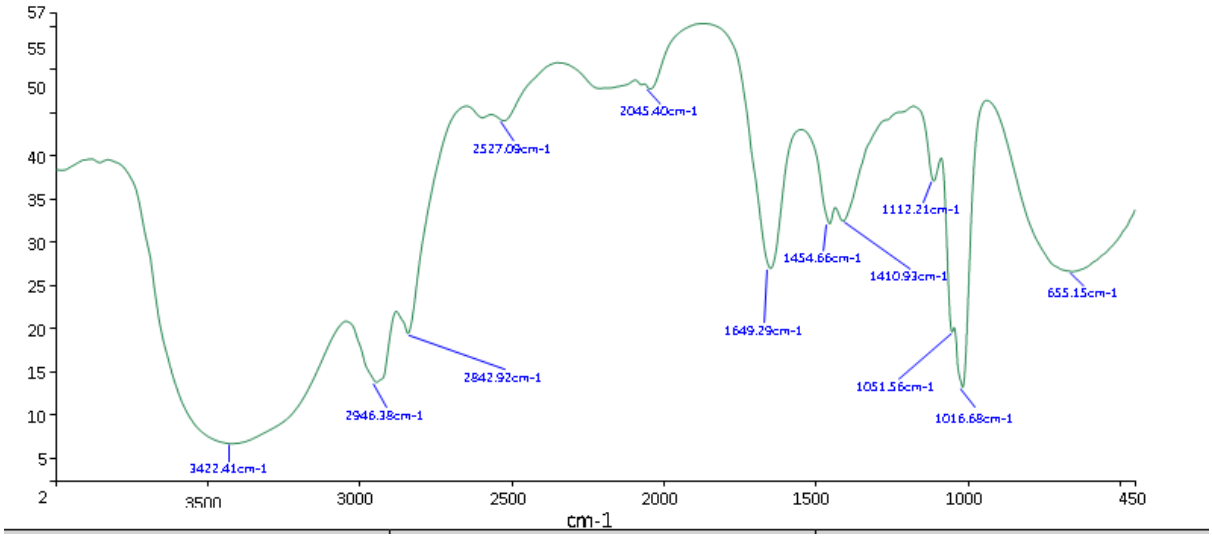

Fig. 7. Fourier-transform infrared spectroscopy (FTIR) analysis of *Tribulus terrestris*

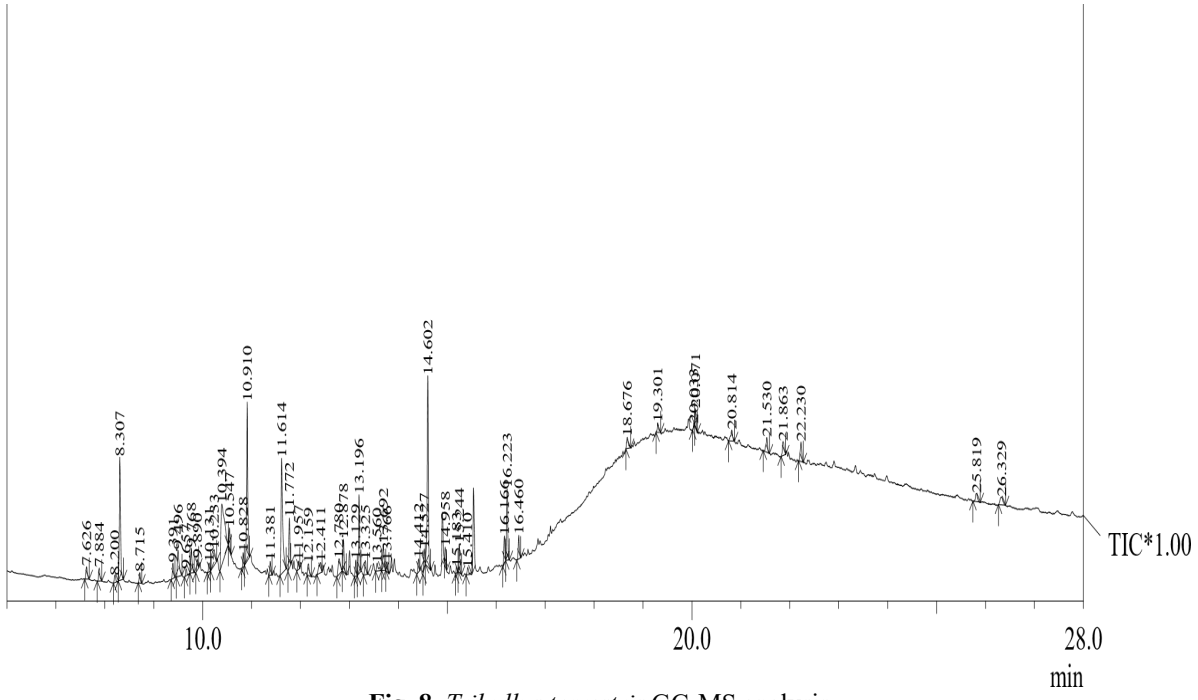

Fig. 8. *Tribullus terrestris* GC-MS analysis

infectious organisms. It is a severe concern for countries and various industries (Jaradat, 2020; Osungunna, 2021). However, repeated use of allopathy drugs can lead to dysbiosis of the normal flora in the vaginal and intestinal tracts and antibiotic resistance due to bacteria's high mutation capacity and parallel gene transfer abilities, thus, it is high time to seek alternative methods for the prevention and treatment of UTIs (Das, 2020). In the current target-rich, lead-poor challenge, ethnopharmacology and natural product drug development remain a primary hope (Sharma et al., 2009). Antimicrobial activity of herbal medicinal plant extracts against various infections has already been documented in the literature based on ethnobotanical data. However, evaluating the obtained results are difficult because of the different solvents methods, extraction, microbial pathogens, and antimicrobial tests (Suroowan et al., 2019). Here, the antimicrobial properties of five medicinal plants used by local medicine men of tribal region of Rajasthan for various treatments were evaluated against UTI pathogens. After preliminary analysis through disc diffusion and phytochemical testing, we found that the *Tribullus terrestris* methanolic extract showed highest antibacterial activities against the uropathogens. The maximum inhibition was obtained against *Pseudomonas*

aeruginosa. At the same time, *Enterococcus faecalis* and *Staphylococcus aureus* was found to be not very effective.

T. terrestris extracts showed activity in different ways against different UTI pathogens. However, we found *e. coli* was more susceptible among other tested pathogens as all extracts exhibited activity against it. This observation conflicts previous research that indicated *E. coli* had the lowest sensitivity to several plant extracts among other pathogens (Batoei et al., 2016). The extract showed maximum inhibition among other extracts against *E. coli*. Methanolic extract also exhibits tremendous activity against *Candida albicans*, with the mean zone of inhibition of 19.66 mm.

Methanol extract of *Citrullus colocynthis* show significant antibacterial activity against all pathogens except *Pseudomonas aeruginosa*. Maximum zone of inhibition was found against *Enterococcus faecalis*. All four extracts were active, but the chloroform extract was observed as most active extract. It shows considerable activity against the pathogens except *Candida albicans*. The maximum zone of inhibition is shown against *S. aureus* (Table 2).

The methanolic extract of *Withania somnifera* shows moderate activity, with the inhibition zone range is 12–4.5 mm. We observed a maximum zone of inhibition against *P. aeruginosa* and a minimum inhibition in *E. coli*. The extract also showed an excellent inhibition zone against *S. aureus* and a moderately inhibited zone (13.3–13 mm) against *E. faecalis* and *C. albicans*. The activity of the chloroform extract showed the same level (10.5–14.5 mm) against all pathogens to similar to the methanol extract. Ethanol and Petroleum ether extracts showed average inhibition, while aqueous extract proved most effective among all extracts showing maximum inhibition zone against *E. coli*.

The methanolic extract of *Convolvulus microphyllus* was effective against all the pathogens except *P. aeruginosa*. Maximum zone of inhibition was observed against *C. albicans*. Moderate inhibition zone was observed against *S. aureus*, *E. coli*, and *E. faecalis*. Chloroform and ethanol extracts of *C. microphyllus* showed average activity against three uropathogens, while petroleum ether extract showed the least inhibition against *S. aureus*.

Except for all the pathogens, methanolic extract of *Acacia nilotica* was active against *E. coli* and *C. albicans*. *A. nilotica* extracts indicated the presence of phytoconstituents in ethanolic and chloroform extract. The most active solvent was chloroform extract, which showed excellent activity against all pathogens. The maximum inhibition zone was found against *C. albicans*. *P. ether* extract showed mild activity only against *P. aeruginosa*. According to the antimicrobial activity results, the solvent extract compared to the aqueous extract showed significant antibacterial efficacy against UTI pathogens. Organic solvents contain potential antibacterial and antifungal compounds due to the presence of antimicrobial compounds. In the present investigation secondary metabolites such as flavonoids, tannins, alkaloids, phenols, steroids, saponins, and glycosides may explain this activity in the solvent extracts. The presence of tannin and saponins compound have been shown to potential therapeutic activities in plants for the treatments of various diseases (Al-bayati et al., 2008). Saponins have exhibited a broad spectrum of physiological actions, including anthelminthic and antibacterial capabilities in the past (Banothu et al., 2017). These findings were supported by the current study, which found that the methanolic fruit extract of *T. Terrestris* had better antimicrobial activity, possibly because of the existence of steroids, tannins, and glycosides (Fig. 6).

Based on the preliminary results, we conducted further evaluation of *T. terrestris* to determine the functional groups. The O-H stretching was showed by a broad peak in the range of 3422/cm in the FTIR spectra of *Tribulus Terrestris* fruits extract. Sharpe peak at 2946/cm indicates the absorption C-H stretching, peak at 2842/cm indicates C-H stretching, peak value at 2527/cm indicates S-H stretching, 2045/cm indicates N=C=S stretching, 1649/cm indicates C=C stretching, 1454/cm indicates C-H bending. The presence of S=O stretching accounts for the peak value of 1410/cm. The presence of C-O stretching accounts for the peak value of 1112/cm. The presence of C-O stretching results in a value of 1051/cm. The C-F stretching causes 1016/cm and 655/cm is due to presence of C-Br stretching. *T. Terrestris* main chemical constituent is a carboxylic acid, which is used as a pharmaceutical product to treat ulcers, stomatitis, fever, liver ache, edema, and rheumatic joint problems. The extract is also rich in alkanes, alcohols, and aromatics, all of which have therapeutic potential high medicinal value (Ragavendran et al., 2011). GC-MS has identified fifty compounds from the methanolic fruit extract of T. Terrestris in this study. 3,5-bis(1,1-dimethylethyl)-4-hydroxybenzene propanoic acid (11.27%) methyl ester is a unique compound that was detected a retention time antioxidant, anti-inflammatory properties (Mujtaba et al., 2020). Hexadecane (10.91%) is a hydrocarbon that possesses, antioxidant, antifungal & antimicrobial activities (Egbung et al., 2017). *Tribullus terrestris* plant extract could be an effective solution to combat establishment of UTI infections. Nevertheless, numerous plants have been demonstrated to be harmful at different dosages and with variable impacts on biological systems. Despite the effectiveness of conventional medications, clear information regarding the ideal dosing range and manufacturing techniques for many plants is hard to come by. Although the ethnobotanical data on the medicinal plants of the desert indicates that they have great potential, only a small number of them have been studied to demonstrate their efficacy in treating infections that are resistant to multiple drugs and the development of drugs to treat such infections. To develop safe and distinctive drugs to combat drug-resistant microbes for sustainable therapy of urinary tract infections utilising novel botanicals, thorough ethnobotanical, pharmacological, toxicological, and clinical studies on new medicinal plants are urgently required.

5. CONCLUSION

Based on their use in ethnobotanical literature, this study evaluated traditionally used medicinal plants for antimicrobial activities. According to the results, methanolic extracts of *T. Terrestris* have potential to combat UTI pathogens such as *E. coli*, *P. aeruginosa* and *C. albicans*. In addition, the GC-MS analysis revealed several bioactive compounds which imparts the antimicrobial function to the plants therefore, more research is needed to isolate the effective compound, perform toxicological studies, and conduct clinical trials.

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