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Commiphora Wightii: A Natural Approach in Control of Urinary Tract Infections

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ABSTRACT

Background: To investigate the extracts of *Commiphora wightii* for the presence of phytoconstituents and screen anti-microbial activity against dominant UTI pathogens. GC-MS and FTIR are used for the identification of functional groups of the bioactive compounds.

Methods: Five different solvents were screened for extraction of compounds from the powdered plant at temperatures ranging from 60 to 80 upto 72 hours. These extracts were used for preliminary phytochemical and antimicrobial analyses. Functional groups were identified by using Fourier-Transform Infrared Spectrophotometer scanning from 450 to 4000 cm^{-1} and a 4 cm^{-1} resolution. The existence of the major compound with the most promising antimicrobial activities was discovered utilising GC-MS analysis.

Result: The results revealed that the methanolic extract of *Commiphora wightii* displayed significant glycosides and flavonoids. Fifty-two functional compounds were identified using GC-MS and FTIR.

Key words: Antibiotic-resistant, Antimicrobial agents, Bioactive compounds, Desert medicinal plant, Urinary tract infections.

INTRODUCTION

Urinary tract infection (UTI) is a widespread infection and they have a significant societal and economic burden (Medina and Pino, 2019; Kenneally *et al*., 2022). Antimicrobial resistance is a global problem, but it is still frequently increasing and the future of antibiotics is yet uncertain; it poses a severe problem in treating bacterial and fungal infections, even though modern medicine has successfully treated and eradicated some infectious diseases and disorders (Willey *et al*., 2011; Nirmala *et al*., 2022). The global incidence of urinary tract infections (UTIs) and the adverse effects of conventional drugs on the protective natural flora of the vaginal canal are of profound significance (Coker *et al*., 2021). Apart from this issue, antibiotics are occasionally connected with adverse side effects and consequences on the host, including hypersensitivity, reduced beneficial gut and mucosal micro-organisms, immunosuppression, allergic responses *etc*. Total expenditure on the treatment of the communicable illness is about 6 billion USD per annum (Prakash and Saxena, 2013). Being the most prevalent infection, every year 10% of women are infected with multiple drug resistance bacteria which is problematic (Peck *et al*., 2021). Despite the development of novel antibiotics against UTI causing pathogens, resistance towards the drugs have been observed in the host. Several studies have showcased that natural anti-microbials derived from plants are effective against the UTI causing multidrug-resistant bacteria (Egharevba *et al*., 2015). Accordingly, appropriate steps are needed to decrease the dilemma of controlling the use of antibiotics, promoting research to explain the genetic mechanisms of resistance adequately and continuing investigations to reveal new medicines (Abuga *et al*., 2021).

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Interestingly, natural compounds isolated from plants and herbs make up to 60% of cancer medicines and 80% of antibacterial, immunosuppressive and cardiovascular pharmaceuticals in the market. As per the reports of the WHO, plants based natural compounds count for 11% of essential pharmaceuticals identified thus far and herbal medicines account for nearly a quarter of all prescribed drugs globally (WHO, 2017). Plant complexes for pharmaceutical reasons have grown in popularity in India; more than 85% of people in urbanised countries use traditional medicine, which includes derived compounds from medicinal plants (Kumar *et al*., 2005). Arid zone plants are also known for medicinal properties such as preventing and treatment of health issues and are low maintenance drugs. Among the nutrients and therapeutic compounds found are vitamins,

minerals, trace elements and active substances with various medical effects.

Commiphora wightii, a member of the *Burseraceae* family, grows in arid regions of India, Bangladesh and Pakistan. Western regions of India such as, Rajasthan and Gujarat are abundant in population of *C.wightii* and are less abundant in southern state Karnataka. Morphologically, this species is a tiny, thorny tree with yellowish gum resin secreted from small ducts in its bark (Bharadwaj and Alia, 2019). *C. wightii* oleo-gum resin is exported in more than 42 countries including developed countries like the United Kingdom and the United States of America, the demand for guggulu is more than its production (Charde *et al*., 2022). It is used to treat a variety of disorders or ailments like COVID-based obesity, hyperlipidemia, inflammation and cancer (Preethi *et al*., 2021). In India's traditional medicine system, Ayurveda has a long history of using the *C. wightii* plant to treat diseases. Atharvaveda one of the four well-known Hindu holy scriptures, has the first reference of its medicinal and therapeutic properties (Vedas) (Satyavati *et al*., 1991). *C. wightii* is one of the nutraceuticals containing plant such as myrecene, dimyrecene and ploymyrecene, guggulosterones like Z-gugglosterone, Eguggulosterone, gugglosterone-I, gugglosterone-II, gugglosterone-III and gugglosterone-IV (Azharhusain *et al*., 2022).

Individual bioactive molecules are known to be produced by desert medicinal plants interacting with the other organisms in the environment and thus, inhibiting bacterial or fungal growth (Jaradat, 2020). Plant phytochemicals and extracts, which are known for antibacterial properties, can be very useful in treatments proven to be effective, providing primary health care to 80% of the rural population (WHO 2017; Osungunna, 2021).

The discovery of the new plant-based antimicrobial compound that will aid in developing new treatments for infectious diseases such as urinary tract infections is common, especially in rural regions. The current work investigates a plant-based regime for combating UTI infections and a phytochemicals investigation of the identified plant uncovering the bioactive ingredients. These compounds could play a significant role in developing new medicines and the future of pharmaceuticals. More research is being conducted to identify various pharmaceutical activities to build a better novel pharmaceutical.

MATERIALS AND METHODS

Collection of plant material

Commiphora wightii was collected from the tribal region of Thar desert (Rajasthan), India.

Extraction of plant sample

Cleaned plants were dried in at room temperature for 7-10 days followed by grinding to powder form. The samples were aliquoted in the sealed containers and stored for further use. Solvents such as methanol and ethanol were used for extraction of compounds for 72 hours at 60 to 80°C. The extracts were filtered using Whatmann filter paper one and stored at 4°C. Extracted samples were concentrated using Rota Vapour and stored in dark bottles for further qualitative phytochemical analysis.

Preliminary phytochemical analysis of plant extract

Standard techniques and chemical tests were used to determine phytochemical constituents in methanol, ethanol and aqueous extracts of *Commiphora wightii*.

Bacterial strains

Escherichia coli (433), *Staphylococcus aureus* (737), *Pseudomonas aeruginosa* (741), *Enterococcus faecalis* (439), *Klebsiella pneumonia* (530), *Candida albicans* (227) were procured from Microbial Type Culture Collection (MTCC). 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 sub-cultured on the same medium (Jaradat, 2020).

Inoculum preparation

Stock cultures were kept 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 hours. This was used to inoculate the plates to be used for disc diffusion (Kavitha and Satish, 2014).

Antimicrobial activity of plant extracts

Kirby-Bauer disk diffusion susceptibility test was used to test antibiotic sensitivity and resistance of plant extracts against uropathogenic bacteria. 100 µl of the test bacterial inoculums from an 18-to-24-hour broth culture were spread on the surface of Muller Hinton agar media plates. On top of which antibiotic discs were positioned. Methanol, ethanol and aqueous extracts were poured onto sterile 6-mm Whatman paper discs, which were placed on inoculation plates in 50 µl (concentration of 100 mg/mL). Antibiotics tested in this study included amoxicillin (30 µg) and nystatin (10 µg). The plates were incubated for 18-24 hours at 37°C after cooling at 4°C for 2 hours. Inhibitory zones were measured in terms of diameter in each plate (Kavitha and Satish, 2014).

FTIR analysis

10 mg of dry extract powder *Commiphora wightii* of was mixed with 100 mg of potassium bromide (KBr) and casted into a pellet. Scanning the disc in a scan range of 450 to 4000 cm^{-1} and a resolution of 4 cm^{-1} 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).

GC-MS analysis

A GC-MS equipment (P-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 of

Commiphora wightii 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°C to 300.0°C) at a rate of 10 minutes before being held for 3 minutes. The compounds spectra were compared to standard spectra from the GC-MS NIST and WILEY libraries (Arulmozhi *et al*., 2018).

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of *Commiphora wightii*

Table 1 shows the findings of preliminary phytochemicals analysis of methanol extract of *C. wightii*. Alkaloids, Glycosides, Steroids, Tannins, Saponins, Carbohydrates and Flavonoids were identified. Compared to aqueous and ethanol extracts, methanol extracts contained higher phytochemical components (Ahmad *et al*., 2015). Because the methanol extract has the maximum number of phytochemical elements, it is used in subsequent research.

Antibacterial activity against *Commiphora wightii*

Based on the results, the antimicrobial activity of the methanolic extract exhibited highest inhibition zone against *S. aureus* (19.3±0.5 mm), *C. Albicans* (17.6±0.52 mm) and *E. faecalis* (16.0±1.24 mm). Inhibition zones developed against gram-negative pathogen were 17.0±0.81 mm in *K. pneumonia*, l5.33±0.94 mm in *E. coli and* 11.0±1.00 mm in *P. aeruginosa*. Compared to gram-negative pathogens, the methanol extract efficiently inhibited gram-positive bacteria. The methanolic extract inhibited both bacteria more effectively than the other ethanolic and aqueous extracts. Except for *P. aeruginosa* and *K. pneumoniae*, the aqueous extract inhibited both strains more efficiently than ethanol extract (Table 2). As a result, the Ethanolic extract displayed the lowest zone of inhibition.

Table 1: Phytochemical analysis of plant extracts.

Phytoconstituents	Ethanol	Methanol	Aqueous
Alkaloid	+	+	+
Flavonoid	+	+	+
Steroids	+	+	-
Tannins	+	+	+
Saponins	-	+	+
Glycoside	+	+	+
Carbohydrates	-	-	-

+: Positive. -: Negative.

Fourier transform infrared spectroscopic analysis

Fig 1 based on the peak values which is shown in FTIR graph, the functional group of methanolic extracts of *C. wightii* was confirmed. The presence of 2947.67, 2841.80, 2522.50, 2046.06, 1647.50, 1454.65, 1412.89, 1021.23, 1111.54, 691.28 and 3402.54 was confirmed by FTIR analysis. At 3402.54 cm^{-1}, major peaks could be assigned to -OH symmetric and asymmetric stretching. As a result of the findings of this investigation, the functional group found in *C. wightii* is O-H symmetric. Fig 1 depicts the other functional groups found in *C. wightii* methanol extracts.

Gas chromatography-mass spectroscopy analysis

According to GC-MS analysis, bioactive mixtures were recognised in the methanolic extract of *C. wightii* and the Graph showing the peak identities of the compound is presented in (Fig 2). Molecular Formula (MF), Retention Time (RT), Concentration (%), Molecular Weight (MW), are presented in (Table 3) Fifty-two 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 Pregna-4,16-diene-3,20-dione (29.02 percentage) and Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(12.62 percentage) was found as the major component is the methanol extract. The bioactive component in the methanolic extracts of *C. wightii* needs to be further investigated to discover a novel antibacterial agent in the fight against global antimicrobial resistance.

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 infectious organisms. It is a severe concern for countries and various industries (Suroowan *et al*., 2019; WHO, 2017). However, evaluating the obtained results are difficult because of the different solvents methods, extraction, microbial pathogens and antimicrobial tests. Aqueous is the most common solvent used by local indigenous people, while other organic solvents are also available. Alkaloids, flavonoids, glycosides, Tannins, saponins and steroids were found in a methanol extract of *Commiphora wightii*. Methanol, Ethanol and the aqueous extract of *C. wightii* contain no carbohydrates. Alkaloid has antidiarrheal, anti-

Table 2: Antimicrobial activity of *Commiphora wightii* Plant extracts against UTI pathogens.

UTI Pathogen	Methanolic extract	Ethanolic extract	Aqueous extract	Amoxicillin/Nystatin
P. aeruginosa	11±1.00	14±0.81	13±0.81	19±3.6
K. pneumonia	17±0.81	10.6±0.47	11.5±0.40	18±1.68
E. coli	15.3±0.97	9.3±1.24	14.3±0.94	17±1.73
E. faecalis	16.3±1.24	12.3±0.84	12.5±1.69	15±2.64
S. aureus	19.3 ±0.57	15.2±1.7	15.2±0.81	19±1.56
C. albicans	17.6±0.52	11±0.81	10.6±1.24	18±3.0

Table 3: Bioactive molecules identified from *Commiphora wightii* by GC-MS peak report TIC.

Peak#	R.Time	Area	Area%	Name
1	5.318	1822586	0.99	Dodecane
2	6.200	2475159	1.34	2-Furanmethanol, tetrahydro-5-methyl-
3	8.303	2246518	1.22	Tetradecane
4	9.884	450775	0.24	Phenol, 2,4-bis(1,1-dimethylethyl)-
5	10.546	318989	0.17	Pentadecane, 3-methyl-
6	10.908	2683806	1.46	Hexadecane
7	12.874	460810	0.25	Heptadecane, 3-methyl-
8	13.193	994102	0.54	Octadecane
9	13.689	579144	0.31	Cyclopentadecanone, 2-hydroxy-
10	13.765	629505	0.34	4,6,6,7,8,8-Hexamethyl-1,3,4,6,7,8-hexahydroc
11	13.912	598572	0.32	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
12	14.158	1388432	0.75	Phenol, 3-pentadecyl-
13	14.599	3047737	1.65	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydr
14	14.947	2479329	1.34	(R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetr
15	15.242	515937	0.28	Eicosane
16	16.306	1157930	0.63	1-Methyl-4-methylene-2-(2-methyl-1-propen
17	16.739	723096	0.39	(3E,7E,11E)-1-Isopropyl-4,8,12-trimethylcyclotetradeca-3,
18	16.897	23265978	12.62	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(
19	17.473	7399997	4.01	Thunbergol
20	17.590	2061455	1.12	Kauran-18-al, 17-(acetyloxy)-, (4.beta.)-
21	18.108	881342	0.48	2-Isopropenyl-5-methyl-6-hepten-1-ol
22	18.464	1130350	0.61	2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15
23	18.595	940735	0.51	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(
24	19.127	1627908	0.88	1,2,3,4-Hexadecanetetrol
25	19.686	1577141	0.86	Acetic acid n-octadecyl ester
26	19.990	2731481	1.48	3-Ethoxy-2-nitro-phenol
27	20.236	609374	0.33	(3-Methylenecyclopentyl)methyl (7,7-dime
28	20.656	1216679	0.66	9-Octadecenal, (Z)-
29	20.754	732095	0.40	1,2,3,4-Hexadecanetetrol, [2R-(2R*,3S*,4S*)]-
30	21.166	1113946	0.60	1-(2-Methylene-3-pentylcyclopropyl)-1-hep
31	21.666	1477739	0.80	Norethandrolone
32	22.022	608005	0.33	5-Pregnen-3.beta.-ol-20-one, trifluoroacetate
33	22.284	3859497	2.09	Pregnan-3.alpha.-ol-20-one
34	22.558	53505531	29.02	Pregna-4,16-diene-3,20-dione
35	22.627	2659997	1.44	5-Pregnen-3.beta.-ol-20-one, trifluoroacetate
36	22.693	825849	0.45	Bis-(4-dimethylamino)-benzophenon
37	22.774	1278784	0.69	Boldenone
38	23.057	7507716	4.07	21-Hydroxyprogesterone, acetate
39	23.341	19550736	10.60	Ethisterone
40	23.510	2740833	1.49	Pregn-4-en-3-one, 20-hydroxy-, (20R)-
41	23.724	913298	0.50	11-Dehydrocorticosterone
42	24.266	1073180	0.58	Silane, diethyl(cis-4-methylcyclohexyloxy)decyloxy-
43	24.417	7136833	3.87	Cholesterol
44	25.460	896298	0.49	Cholesta-3,5-diene
45	25.640	1785915	0.97	Cholest-4-en-3-one
46	26.115	1423094	0.77	Pregna-5,16-dien-20-one, 3-hydroxy-, (3.beta.)
47	26.315	1384502	0.75	.gamma.-Sitosterol
48	26.762	1063604	0.58	(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltr
49	27.439	996313	0.54	Yangambin
50	29.195	1610580	0.87	3-Oxoallobetulane
51	31.329	2942347	1.60	Pregnane-3,20-dione, 11-hydroxy-, (5.alpha.,11.beta.)-
52	32.504	1296859	0.70	1,4-Dimethyl-3-(2-methyl-1-propenyl)-4-viny
		184398418	100.00	

inflammatory, anticancer and anti-diabetic properties and the ability to cure urinary diseases (Singh *et al*., 2016). Flavonoids, commonly known as vitamins, have a variety of therapeutic qualities, including antihypertensive, anti-rheumatism, antidiuretic, antioxidant, antibacterial and anticancer effects (Singh *et al*., 2016). Glycosides and steroids have antimicrobial properties and can help to fight bacterial infections, including UTIs. Steroids can reduce inflammation and swelling in the urinary tract, which can relieve the symptoms of a UTI. 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). Phenols have been shown to inhibit the growth of bacteria associated with UTIs (Mohan *et al*., 2017). This study revealed the presence of these bioactive components in *C. wightii* methanolic extracts. As a result, the use of this plant in traditional medicinal systems is consistent with the findings of previous researchers.

The antimicrobial activity of herbal 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. Antimicrobial activity of *Commiphora wightii* extracts (ethanol, aqueous and methanol) were tested against selected pathogens in this study. Methanolic extracts of *C. wightii* had the best activity against all pathogens tested, with *E. coli, S. aureus and Candida albicans* having the most significant inhibition zones.

By comparing their respective controls, the activities of the zone of inhibition values can be used to estimate the potential of antibacterial activities. Plant extracts containing chemicals with antibacterial properties effectively treat bacterial and fungal diseases (Yabesh *et al*., 2018). *Tribullus terrestris* showed similar antibacterial activity against UTI pathogens (Arulmozhi *et al*., 2018). Similarly, *Capparis zealanica* methanol extracts were also tested for antibacterial activity against *Escherichia coli*, *Streptococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Yasin *et al*., 2015).

The results of this present study coincide with the results of these researchers, because methanolic extract of *C. wightii* was effective against UTI pathogens, such as *Escherichia*

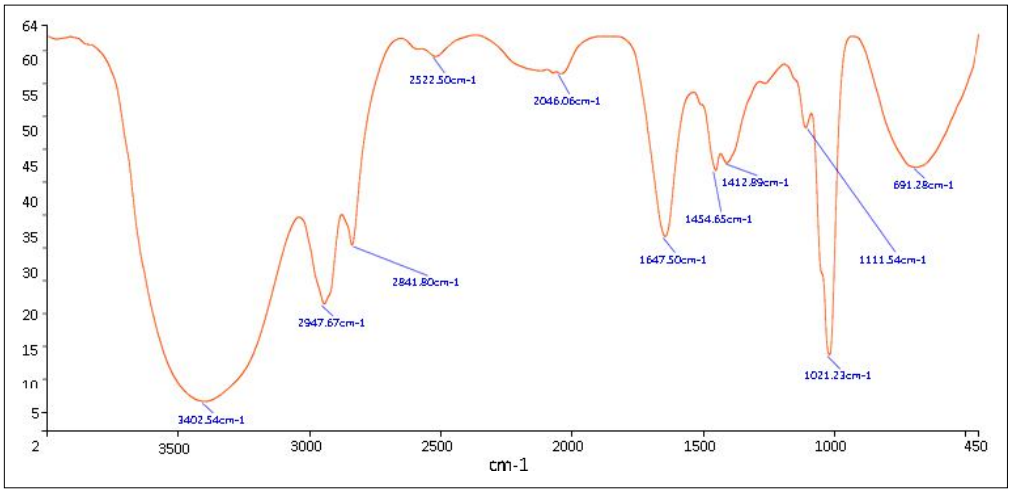

Fig 1: FTIR analysis of *Commiphora wightii.*

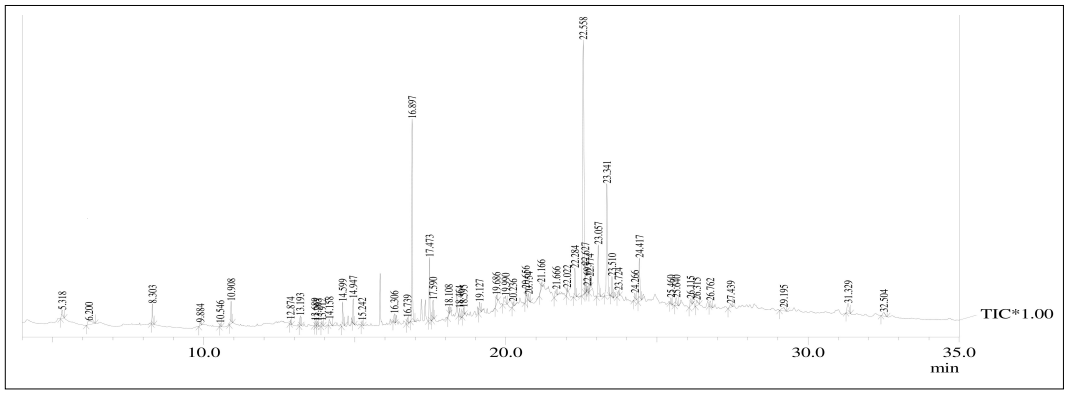

Fig 2: *Commiphora wightii* GC-MS analysis.

coli and *Staphylococcus aureus*. As a result, natural antibacterial agents such as methanolic and aqueous extracts can be used to prevent the infection of these pathogens.

The -OH symmetric and asymmetric stretching was showed by a broad peak in the range of 3402 cm-1 in the FTIR spectra of *C. wightii* fruits extract (Guunzler and Gremlich, 2002). Sharpe peak at 2947 cm^{-1} indicates -CH_2, -CH_3 (Wie *et al*., 2009), peak at 2841 cm^{-1} indicates lipids (Wie *et al*., 2009). peak value at 2522 cm^{-1} indicates S-H stretching, 2046 cm^{-1} indicates N=C=S stretching, 1647 cm^{-1} indicates á-helix protein (Heimburg *et al*., 1999). 1454 cm^{-1} indicates Various ä (C-H) modes (Kamnev *et al*., 2008). The presence of Ionic Phosphate for the peak value of 1412 cm^{-1} (Chauhan *et al*., 2008). The presence of Polyester overlap carbohydrate, various C-O-C and C-C-O vibration results in a value of 1111 cm^{-1} (Kamnev *et al*., 2008). The presence of C-O stretching accounts for the peak value of 1021 cm^{-1}. 691 cm^{-1} is due to presence of C-Br stretching. *C. wightii* main chemical constituent is aldehydes, amine, acid, carbohydrates and halides functional groups 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. As a result, it has a high medicinal value (Ragavendran *et al*., 2011).

GC-MS is a technology that is used to identify Phyto-compounds (Shibula *et al*., 2015). GC-MS has identified fifty-two compounds from the methanolic fruit extract of *C. wightii* in this study. Pregna-4, 16-diene-3, 20-dione (29.02%) compound that was detected a retention time antioxidant, anti-inflammatory properties (Bhatia *et al*., 2015). Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(12.63%) that possesses, antioxidant, antifungal and antimicrobial activities (Perveen *et al*., 2018).

CONCLUSION

Based on the use in ethnobotanical literature, this study evaluated traditionally used medicinal plants for antimicrobial activities. According to the results, methanolic extracts of *Commiphora wightii* have potential to combat UTI pathogens. In addition, the GC-MS analysis revealed several bioactive compounds. Therefore, methanolic fruit extracts of *Commiphora wightii* include bioactive compounds which are responsible for antimicrobial properties. As a result, more research is needed to isolate the effective compound, perform toxicological studies and conduct clinical trials.

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Conflict of interest: None.

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