aqueous. All the extracts obtained were concentrated under reduced pressure at 40°C using a rotary vacuum evaporator and evaporated to complete dryness.

Antimicrobial activity of selected plant extract against the isolated pathogens

Antimicrobial activity test was performed by cup plate method. The organisms isolated from diabetic patients having foot ulcer and UTI were spread plated on sterile Mueller–Hinton agar plates. Wells of 4–5 mm in diameter were made aseptically using cork borer, and 15–20 µl of aqueous and ethanolic extract of plant were inoculated. The result was calculated in terms of the zone of inhibition in millimeters. For every concentration, three sets were performed to confirm the activity and standard drug amoxicillin at 250 mg was made use for the comparison.

Thin-layer chromatography (TLC)

Approximately 4 g of ethanol extract was dissolved in 100 ml of double-distilled water, which showed good solubility, further it was filtered using normal filter paper. Completely dissolved extract was loaded on pre-coated TLC plate, about 1.5–2.0 cm line was drawn with a pencil from the bottom in the plate and capillary tubes were used to load the sample at approximately 1.5 cm distance between them and methanol was used as the solvent system for TLC.

Column chromatography

Gel binding for column chromatography was carried out using 4 g of ethanol extract in double-distilled water with 1 g of silica (mesh size 60–120) and internal diameter – 30 cm; gel loading was done approximately up to 18 cm in an borosil column. Methanol solvent was used as mobile phase.

Fourier-transformed infrared (FT-IR)

FT-IR analysis was performed to investigate the presence of the functional group in the ethanol leaves extract of *A. marmelos* plant, which was carried out and standardized at the USIC Department, Karnatak University, Dharwad. The samples were prepared using spectroscopic pure KBr (5:95); pellets were fixed in the sample holder and analyzed. FT-IR analysis was done on Shimadzu 8400S spectrophotometer (Shimadzu Corporation, Japan) in the mid-IR region of 400–4000 cm^{-1} with 16 scan speeds.

Statistical analysis

The results obtained were expressed as mean±SD. One-way analysis of variance was used to analyze the variation.

RESULTS

Identification of urinary tract infected pathogens and foot ulcer bacterial pathogens

The pus samples of diabetic foot ulcers were found to be *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Enterobacter aerogenes*, whereas *E. coli*, *P. aeruginosa*, *B. subtilis*, *E. aerogenes*, *P. vulgaris*, *S. aureus*, and *Klebsiella pneumoniae* were found in the urine samples of diabetic patients (Table 1).

Antimicrobial activity of leaves of *A. marmelos* plant extracts against foot ulcer and urinary tract isolates

The ethanolic and aqueous extracts of leaves of the plant *A. marmelos* were subjected for the antimicrobial activity against foot ulcer pathogens and urinary tract infected pathogens. In this study, ethanolic extract showed good results for both urinary tract and foot ulcer pathogens to that of aqueous extract when compared with standard drug amoxicillin. Ethanol extract showed the highest zone of inhibition in *P. aeruginosa* (22.5±0.26) and lowest in *E. aerogenes* (14.1±0.31) at the concentration of 200 mg/ml for foot ulcer pathogens, respectively, whereas ethanol extract for urinary tract pathogens showed maximum inhibitory zone for *B. subtilis* (23.5±0.33) and minimum zone of inhibition for *K. pneumoniae* (10.3±0.10) at 200 mg/ml (Tables 2-5).

TLC

In this TLC, ethanolic extract of leaves of the plant *A. marmelos* showed three different bands with Rf value 0.94, 0.81, and 0.72, respectively,

Table 1: Morphological and biochemical characteristics of the isolated pathogens

Tests	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Proteus vulgaris*	*Enterobacter aerogenes*	*Escherichia coli*
Gram's stain	+	–	–	+	–	–	–
Motility	NM	NM	M	M	M	M	M
Catalase	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+w	–
Oxidase	–	–	+	+	–	–	–
Indole production	–	–	–	–	+	–	+
Methyl red	+	–	–	–	+	–	+
Vogues–Proskauer	+	+	–	+	–	+	–
Glucose fermentation	+	+	–	+	+	+	+
Lactose fermentation	+	+	–	+	–	+	+
Starch hydrolysis	+	+	–	+	+	+	+
H_2S production	–	–	–	–	–	+	–
Urease	+	+	–	–	+	+	–

+: Positive, –: Negative, M: Motile, and NM: Non-motile

Table 2: Activity of ethanol extract against foot ulcer pathogens

Isolates	Zone of inhibition (mm)			Amoxicillin (250 mg)
	100 mg/ml	150 mg/ml	200 mg/ml	
Staphylococcus aureus	12.2±0.20	15.3±0.12	19.3±0.10	20.0±0.31
Escherichia coli	9.1±0.51	13.2±0.34	16.9±0.21	18.4±0.24
Bacillus subtilis	11.4±0.21	15.8±0.85	20.6±0.66	22.3±0.11
Pseudomonas aeruginosa	15.1±0.51	19.6±0.13	22.5±0.26	30.1±0.55
Proteus vulgaris	12.1±0.21	14.9±0.15	18.5±0.16	20.2±0.31
Enterobacter aerogenes	9.1±0.30	12.4±0.21	14.1±0.31	19.5±0.40

Values are mean±SD, n=3. Results were analyzed using one-way ANOVA. SD: Standard deviation, ANOVA: Analysis of variance

Table 6: Activity of ethanolic fractions against foot ulcer pathogens

Isolates	Fractions	Zone of inhibition (mm)		
		0.10 mg/ml	0.15 mg/ml	0.20 mg/ml
Staphylococcus aureus	1	4.5	8.8	12.1
	2	3.8	7.2	9.4
	3	3.3	6.5	6.9
Escherichia coli	1	3.5	6.3	9.1
	2	2.8	5.2	7.0
	3	2.0	4.1	6.4
Bacillus subtilis	1	4.9	8.1	11.2
	2	4.1	6.3	9.6
	3	3.5	4.9	7.3
Pseudomonas aeruginosa	1	5.2	10.2	13.5
	2	4.3	8.4	11.3
	3	3.8	6.3	9.1
Proteus vulgaris	1	4.3	8.3	11.0
	2	3.7	6.9	8.9
	3	3.1	5.5	6.4
Enterobacter aerogenes	1	4.0	7.5	10.5
	2	3.5	6.4	8.2
	3	2.9	5.5	7.1

Values are mean, n=3

Table 7: Activity of ethanolic fractions against urinary tract infected pathogens

Isolates	Fractions	Zone of in,.hibition (mm)		
		0.10 mg/ml	0.15 mg/ml	0.20 mg/ml
Escherichia coli	1	3.4	7.3	9.1
	2	2.6	5.9	7.5
	3	2.2	4.8	5.9
Pseudomonas aeruginosa	1	6.9	11.3	15.4
	2	5.4	9.4	13.2
	3	4.6	7.8	12.0
Bacillus subtilis	1	6.1	7.3	12.0
	2	5.2	6.1	10.9
	3	4.6	5.2	9.1
Enterobacter aerogenes	1	4.8	10.2	11.9
	2	4.0	8.8	10.4
	3	3.6	6.5	9.3
Proteus vulgaris	1	6.0	8.4	13.4
	2	5.2	7.2	11.2
	3	4.4	6.9	10.5
Staphylococcus aureus	1	3.8	8.1	11.8
	2	2.9	7.4	10.4
	3	2.4	6.3	9.0
Klebsiella pneumoniae	1	2.5	4.3	6.3
	2	1.8	3.8	5.1
	3	1.2	2.9	4.4

Values are mean, n=3

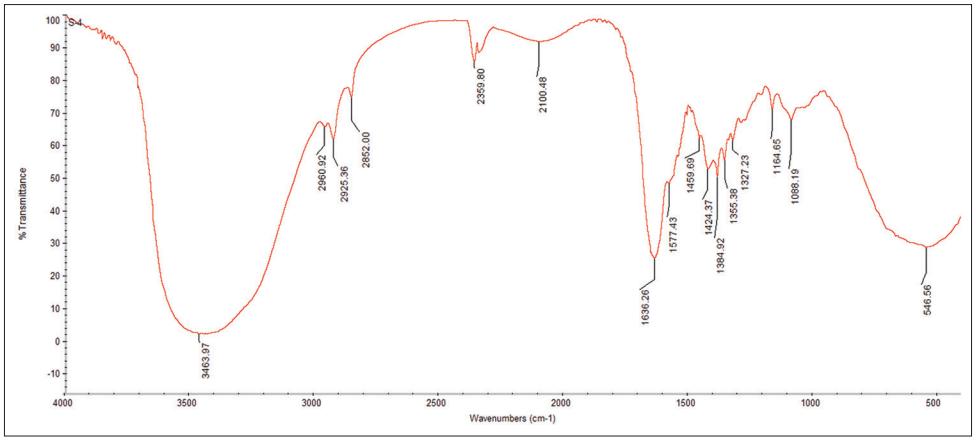

Fig. 1: Fourier-transformed infrared spectra of fraction one ethanolic extract of the leaves of the plant *Aegle marmelos*

Table 8: Characteristics of infrared absorptions

Range cm^{-1}	Type of bond	Functional class
3463.97	O-H stretch, H - bonded	Alcohols, phenols
2960.92	C-H stretch	Alkanes
2925.36	C-H stretch	Alkanes
2852.00	C-H stretch	Alkanes
2100.48	-C = C - stretch	Alkynes
1636.26	-N-H	Primary amines
1577.43	C-C stretch (in ring)	Aromatics
1459.69	C-H bond	Alkanes
1424.37	C-C stretch (in ring)	Aromatics
1384.92	N-O symmetric stretch	Nitro compounds
1355.38 (m)	N-O symmetric stretch	Nitro compounds
1327.23	C-N stretch	Aromatic amines
1164.65 (m)	C-H wag (-CH_2X)	Alkyl halides
1088.19	C-O stretch	Alcohols, carboxylic acids, esters, ethers
546.56	C-Br stretch	Alkyl halides

microbial strains. The previous study has revealed that aqueous and ethanolic extract of plant *A. marmelos* has activity against *P. aeruginosa*, *B. subtilis*, *S. aureus*, and *E. coli*. The ethanolic extract showed more activity to that of the aqueous extract. Elevated antimicrobial activity was shown against *B. subtilis*, followed by *S. aureus*, *E. coli*, and then *P. aeruginosa* [17]. Comparatively, in this study, *P. aeruginosa* (22.5±0.26) exhibited the highest zone of inhibition and lowest was in *E. aerogenes* (16.1±0.31) at the concentration of 200 mg/ml for foot ulcer pathogens, whereas urinary tract pathogens for ethanolic extract showed maximum inhibitory zone for *B. subtilis* (23.5±0.33) and minimum zone of inhibition for *K. pneumoniae* ((10.3±0.10) at 200 mg/ml.

The ethanolic extract of *A. marmelos* was subjected for TLC and column chromatography. The TLC analysis of the methanolic extract of variety in Pant Aparna among all the varieties and accessions of *A. marmelos* in earlier studies detected the occurrence of phenolic compounds such as flavonoids and phenolic acids as the principal contributors to the scavenging effect [18,19]. Similar pattern of results was seen in ethanolic extract of leaves of *A. marmelos* plant with Rf value 0.94, 0.81, and 0.72, respectively, and three different fractions were eluted from the column from which only fraction 1 strongly inhibited the urinary tract pathogens, i.e., *P. aeruginosa* showed the highest zone of inhibition (15.4 mm) whereas the lowest zone of inhibition was seen in *K. pneumoniae* (6.3 mm) at the concentration of 200 mg/ml and comparatively foot ulcer pathogens showed less inhibition.

In the previous study, the FT-IR spectroscopic study of *A. marmelos* L. unripe fruit powder showed the hydroxyl (-OH), carboxyl (-C=O), and amine (-NH) groups of coumarins, alkaloids, or tannins. Identification of functional groups in *A. marmelos* extract and its characterization through FT-IR, X-ray diffractometer visible spectrophotometer and atomic force microscopy [20], in this connection, parallel results were obtained from FT-IR spectroscopy which at different stretching vibrations were found to be alcohols, alkanes, alkynes, amines, nitroamines, phenols, aromatic, carboxylic acids, and ethers. The above reported bioactive compounds may have the potential to reduce the risk and growth of resistant foot ulcer and urinary tract infected pathogens and further, there is need of work to be done on mechanism and action of secondary metabolites to exploit their uses for medicinal purposes.

CONCLUSION

The ethanolic extract of *A. marmelos* plant showed good antimicrobial activity for both foot ulcers and urinary tract pathogens isolated from diabetic patients. Fraction 1 of the ethanolic extract exhibited the highest activity for urinary tract pathogens, i.e., for *P. aeruginosa* at the concentration of 200 mg/ml. Thus, the findings suggest that *A. marmelos* had a potent antimicrobial activity. Further to confirm the activity structural elucidation studies should be carried out to confirm which bioactive compounds are responsible for the antimicrobial activity.

AUTHORS' CONTRIBUTIONS

The author declares that all the named authors have contributed equally to this article.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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