Asian Journal of Chemistry; Vol. 35, No. 8 (2023), 1951-1956

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.28061

Antibacterial Activity of *Ampelocissus latifolia* Tuberous Root Extract against Diverse Microbes

KAMAL KISHOR RAJAK, PAVAN M. PAHILANI and HEMANT KUMAR*

Department of Medical Laboratory Technology, Bapubhai Desaibhai Patel Institute of Paramedical Sciences (BDIPS), Charotar University of Science and Technology, CHARUSAT Campus, Changa-388421, India

*Corresponding author: E-mail: hemantkumar.cips@charusat.ac.in

Received: 25 May 2023; *Accepted*: 10 July 2023; *Published online*: 31 July 2023; AJC-21330

Microbial drug resistance is growing every day and novel drugs are need of the hour to counter the future threat of large pandemic. The present work for the first time explores the antibacterial activity of methanol, hydro-alcoholic and *n*-hexane extracts of *Ampelocissus latifolia* (*A. latifolia*) tuberous root against few laboratory and clinical isolates. A total of 10 microbes were screened; out of which 1 is a laboratory strain (acid-fast) and the rest 9 are clinical isolates (5 Gram-positive and 4 are Gram-negative), 2 clinical isolates were drug-resistant (1 Gram-positive and 1 Gram-negative). In this screening, methanol extract showed potential antibacterial activity against Gram-positive, Gram-negative and acid-fast bacteria with zones of inhibition measuring 18 mm, 10 mm and 8 mm, respectively. Hydro-alcoholic and *n*-hexane extract showed a smaller zone of inhibition compared to methanol extract and the positive control. The minimum inhibitory concentration (MIC) of methanol and the hydro-alcoholic extract of powdered tuber root was determined by the micro-broth dilution method. The minimum inhibitory concentration of methanol and hydro-alcoholic extract for Gram-positive and Gram-negative microbes varies from 0.18 mg/mL to 1.5 mg/mL. The methanol and hydro-alcoholic extracts were analyzed with FTIR, which revealed the presence of alcohol, alkanes, alkenes, alkyls, ketones and nitro groups in the methanol and hydro-alcoholic extracts of *A. latifolia* tuberous root. The high-performance thin-layer chromatography (HPTLC) analysis of the methanol extract indicates the presence of at least 5 different compounds with different R_f values.

Keywords: *A. latifolia*, Antibacterial, Micro-broth dilution, HPTLC.

INTRODUCTION

Depending on the host's sensitivity various microorganisms like parasites, bacteria, viruses and fungi may colonize or cause illness in the host. Though bacteria can be found almost everywhere; only a small percentage of them cause infections and diseases that have a significant public health impact [1]. In 2019, approximately 13.7 million of deaths were attributed to infectious diseases [2]. Microbial infection and resistance to various antimicrobial drugs is the most pressing issue in the current situation. Since long the rapid spread of drug-resistant bacteria has been observed by many public health groups and warned about its possible catastrophic effects [3]. A global pandemic of resistant *Staphylococcus aureus* (*S. aureus*) and *Enterococcus* sp. now poses the greatest threat among Gram-positive infections [4,5]. Methicillin-resistant *Staphylococcus aureus* (MRSA) kills more Americans a year than HIV/AIDS, Parkinson's disease, emphysema and homicide combined [6,7]. Members of *Enterobacteriaceae*, especially *Acinetobacter* spp., *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are the most frequent cause of Gram-negative infections in healthcare settings [6,7]. As a result of intensive antibiotic use, resistant bacteria are increasing and multi-drug resistant bacteria are becoming more prevalent, reducing antibacterial drug's efficacy and effectiveness [4,8,9].

The rise of drug-resistant pathogens has prompted the pharmaceutical and scientific communities to focus on exploring the antimicrobial properties of plant-derived substances. These substances, which have long been used in traditional medicine across various countries, represent an underutilized source of potential antimicrobial compounds. In recent years, there has been a growing interest in medicinal plants among these communities, with several studies highlighting the promising antimicrobial effects of plant-derived substances [10]. The use of plant oils and extracts has been widely explored for applications such as food preservation, pharmaceuticals and natural therapies, largely based on their antimicrobial properties.

India has a rich heritage of traditional medicinal systems such as Ayurveda, Siddha and Unani that have roots in ancient Indian scriptures [11]. Ayurveda, which emerged and evolved during 2500-500 BC, is an ancient system of medicine that uses medicinal plants for the treatment and cure of various diseases including infectious diseases [12]. Due to the numerous advantages majority population from the developing world uses plants as a primary medicine [13]. The plants contain thousands of different medically important secondary metabolites such as coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, essential oils, lectin, polypeptides and polyacetylenes which help to survive plant against insects, fungi, herbivores and many diseases [14]. These bioactive compounds are also used as the precursor for the synthesis of different antibiotics [15-17].

Ampelocissus latifolia is an herbaceous plant found in Indian subcontinent and commonly known as Katti-bel, Pani-bel, Junglidrakh, Golind, Dibroli, *etc*. *A. latifolia* belongs to *Vitaceae* family and contains a well-defined shoot, leave and root with tubers. Flowering and fruiting occur between August-November months and the flowers have 5 deep reddish petals inflorescence. The fruit is spherical, black in colour, 6-7 mm in diameter and a single fruit contains 2-3 seeds. This plant is reported to be used to treat a variety of ailments in Indian folk medicine including dental difficulties, ulcers, diarrhea, gout, fractured bones, dyspepsia, indigestion and tuberculosis (TB) *etc*. [18]. Medicinal properties of *A. latifolia* are also mentioned in ayurveda for the treatment of kustha (Leprosy), kamala, sotha and varna [19]. The most routinely used parts of the plant are roots, bark and leaves. *Ampelocissus* leaves are also used as colouring agents. Several recent scientific reports on various parts of *A. latifolia* show the antibacterial, antiproliferative, cytogenotoxic, anti-inflammatory, allelopathic activities and antioxidant activity [18-25]. This study focuses on the antibacterial activity of methanolic, hydro-alcoholic and *n*-hexane extract of *A. latifolia* tuber root against Gram-positive and Gram-negative bacteria and non-pathogenic acid-fast bacteria. This is the first report on the antimicrobial activity of *A. latifolia* tuber root with FTIR and HPTLC analysis.

EXPERIMENTAL

Collection of plant material and extract preparation: The tuber roots of *A. latifolia* (Fig. 1) were collected from a village in Rajnandgaon District, India (21.2056º N, 81.1775º E). According to the locals, the only approach to spot the plant is through its aerial parts, which appear during the rainy season. As a result, this plant was collected in August month only. The plant material was collected by excavating the plant root and immediately washed with running tap water and 0.1% sodium hypochlorite solution, cut into small pieces and dried at room temperature for 3-4 weeks. Any deteriorating piece that was observed in day-to-day inspection was removed immediately. A 150 g of dried plant material was crushed to powder with a sterilized mixer grinder. The crushed material was divided into three parts (50 g each) and extracts were prepared in 300 mL of methanol, *n*-hexane and hydro-alcoholic solvent separately using the Soxhlet apparatus by running the Soxhlet for 24 h.

Fig. 1. Image of *Ampelocissus latifolia* tuber root

The resulting extracts in different solvents were dried on a hot plate and the dried pellets thus obtained were transferred to an Amber-coloured glass bottle and stored at 4 ºC until use.

Tested microorganisms and growth conditions: In this study, some clinically pathogenic Gram-positive and Gram-negative strains, as well as non-clinical and non-pathogenic acid-fast strains were chosen. The clinically isolated, pathogenic, Gram-positive strains used in this study were *M. luteus*, *E. faecium* (drug-resistant), *Enterococcus* spp., *S. epidermidis* and *S. aureus*, while the clinically isolated, pathogenic, Gram-negative strains were *K. pneumoniae* (drug-resistant), *E. coli, S. maltophilia* and *Pseudomonas* spp. The non-pathogenic, non-clinical, acid-fast bacteria used was *M. smegmatis*. All the selected microbes were grown in Mueller Hinton broth (MHB) or agar (MHA) and incubated at 37 ºC for 16 h except for *M. smegmatis.* In this work, *M. smegmatis* was cultivated using Middlebrook 7H9 agar supplemented with 10% oleic acid, bovine serum albumin, dextrose and catalase (OADC) or Middlebrook 7H9 broth supplemented with 10% albumins, dextrose and catalase (ADC) and incubated at 37 ºC for 48 h.

Disk-diffusion assay: The primary antimicrobial screening of crude extracts was performed with disc diffusion assay as described by Patel *et al*. [26] with some modifications. To isolate a single colony for the inoculum preparation, the microbes were grown in their respective agar media (MHA for Gram-positive and Gram-negative and 7H9 agar for acid-fast bacteria). Two to three colonies were taken from the grown bacteria for preparation of the 0.5 McFarland standard (~1.5 × 10^8 CFU/mL) in 10 mL of 1% peptone. The suspension was seeded onto the respective agar plates with the help of sterile cotton swabs. A sterile disc containing 3 mg (20 µL of 150 mg/mL stock) of plant extract was placed on the seeded agar plate. Streptomycin (STM) (10 µg) was used as control for all clinical isolates and isoniazid (INH) (10 µg) for *M. smegmatis*. A sterile disc loaded with dimethyl sulfoxide (DMSO) was used as a solvent control. Later, the plates were incubated at 37 ºC for 16 h for both Gram-positive and Gram-negative bacteria and 48 h for acid-fast bacteria. After incubation, the zone of inhibition was measured and reported in mm.

Minimum inhibitory concentration (MIC): The MIC of the methanol and the hydro-alcoholic extract was determined against those bacteria which have > 10 mm zone of inhibition in disk diffusion assay *i.e.* Gram-positive: *M. luteus*, *S. aureus*, *S. epidermidis* and *Enterococcus* spp. and Gram-negative: *E. faecium*. The MIC was determined by the micro-broth dilution method in a 96-well plate. A two-fold serial dilution (6 mg/mL to 0.046 mg/mL) of plant extract was prepared in 100 µL

of MH broth. The bacterial suspension containing 1×10^8 CFU/mL (0.5 McFarland standard) was diluted to 1:30 in the same sterile broth to achieve a bacterial density of 1×10^6 CFU/mL. A 100 µL diluted culture was added to each well except for the sterility control. Streptomycin (STM) was used as antibiotic control (concentration range 16 µg/mL to 0.12 µg/mL) and 20 µL of 100% DMSO was used as solvent control. After the respective incubation period, which was 16 h for Gram-positive and Gram-negative bacteria and 48 h for acid-fast bacteria at 37 ºC, 20 µL of resazurin solution (0.015%) was added to each well. The change in colour from violet to pink was examined and the last well with no colour change was considered to be the MIC value [27].

FTIR analysis: Dried methanol and the hydro-alcoholic extracts were used for the FTIR analysis. The KBr disc was introduced to the FTIR spectroscope instrument (Thermo Scientific, USA Model: Nicolet 6700) and scanned from the 400 cm^{-1} to 4000 cm^{-1} range with a resolution of 4 cm^{-1} for 32 scans/second. The Omni software was used to analyze the peaks.

High performance thin layer chromatography (HPTLC) analysis: The HPTLC of methanol extract was performed on silica gel 60 F_{254}, 10 × 10 cm TLC plates (Merck, Germany-5642), with methanol:ethyl acetate [2:8 (v/v)] as a mobile phase. A 4 mg/mL stock solution of crude extract was prepared in methanol and applied 10, 20, 30, 40, 50 and 60 µL on tracks 1, 2, 3, 4, 5, 6 respectively, with the help of an automated spray on band applicator. Band size was maintained at 10 mm and the distance between the two bands was 4 mm. The samples were run for 15 min and detection was done under long UV light and short UV light (344 nm and 254 nm, respectively) for image acquisition. The CAMAG TLC Scanner 3 was used to determine the retardation factor (R_f value) of the bands using WIN CATS software (Version 4X) [28].

RESULTS AND DISCUSSION

Antibacterial assay: The introduction to the plant was through a very trivial approach of some ethnobotanical references from locals of the village area of Rajnandgaon district. Locally they know the tubers as "Dote kanda". It has been even understood that the particular specimen are even considered to be pious and have various medicinal properties since ages. It is being used as an immunity booster for humans as well as cattle as a seasonal protection against the infections. The primary antibacterial screening of different extracts of *A. latifolia* tuber root was done against Gram-positive: *M. luteus, E. faecium* (DR), *S. epidermidis*, *S. aureus* and *Enterococcus* spp., Gram-negative: *K. pneumoniae* (DR), *S. maltophilia*, *E. coli* and *Pseudomonas* spp. and acid-fast bacteria: *M. smegmatis* with the disc diffusion assay. According to the zone of inhibition, the antibacterial activities of the studied extracts ranged between 6 to 18 mm (Table-1).

The methanol and hydro-alcoholic extract showed a significant zone of inhibition against Gram-positive, Gram-negative and acid-fast bacteria. Methanol extract formed the largest inhibition zone (18 to 10 mm) against Gram-positive bacteria: *M. luteus, E. faecium*, *S. epidermidis*, *S. aureus* and *Enterococcus* spp. It also exhibited a moderate inhibition zone (10 to 8 mm) for Gram-negative; *S. maltophilia* and *E. coli* and minimum zone of inhibition (8 mm) against acid-fast bacteria: *M. smegmatis*. On the other hand, the hydro-alcoholic extract formed the largest inhibition zone (14 mm to 10 mm) against Gram-positive bacteria mentioned earlier and moderate inhibition zone (8 mm) against Gram-negative bacteria: *S. maltophilia* and minimum inhibition zone (6 mm) against acid-fast bacteria: *M. smegmatis*. The *n*-hexane extract showed a poor activity against all bacterial groups as it formed 8 mm inhibition zone against *E. faecium* and *S. epidermidis* and Gram-negative bacteria (*S. maltophilia*) and an inhibition zone of 7 mm against *M. luteus*. None of the extracts showed zone of inhibition against *K. pneumoniae* and *Pseudomonas* spp. Also, *n*-hexane extracts were unable to inhibit the growth of *Enterococcus* spp. and *S. aureus*, *K. pneumoniae*, *E. coli* and *Pseudomonas* spp. and *M. smegmatis*. To investigate the minimum inhibitory concentration of methanol and hydro-alcoholic extract, Gram-positive bacteria, such as *S. aureus*, *M. luteus*, *S. epidermidis* and *Enterococcus* spp. and Gram-negative bacteria, such as *S. maltophilia* were selected that showed > 10 mm zones of inhibition (Fig. 2).

The methanol and hydro-alcoholic extracts were effective against both Gram-negative and Gram-positive bacteria, with MIC ranging from 0.18 mg/mL to 1.5 mg/mL and 0.37 mg/mL to 1.5 mg/mL respectively, against the selected bacteria. Methanol extract demonstrated the highest antimicrobial activity (minimum MIC value) against Gram-positive bacteria

TABLE-1
PRIMARY ANTIBACTERIAL ANALYSIS OF DIFFERENT EXTRACTS OF *A. latifolia* TUBER ROOT

	Pathogens	Plant extracts			Control
		Methanol	Hydro-alcoholic	*n*-Hexane	
Gram-positive	*Micrococcus luteus*	18	14	7	28
	Enterococcus faecium	18	14	8	00
	Staphylococcus epidermidis	16	12	8	28
	Staphylococcus aureus	15	10	00	19
	Enterococcus spp.	10	8	00	12
Gram-negative	*Klebsiella pneumoniae*	00	00	00	14
	Escherichia coli	8	00	00	12
	Stenotrophomonas maltophilia	10	8	8	20
	Pseudomonas spp.	00	00	00	18
Acid-fast	*Mycobacterium smegmatis*	8	6	00	18

Keys (00) did not show any growth inhibition

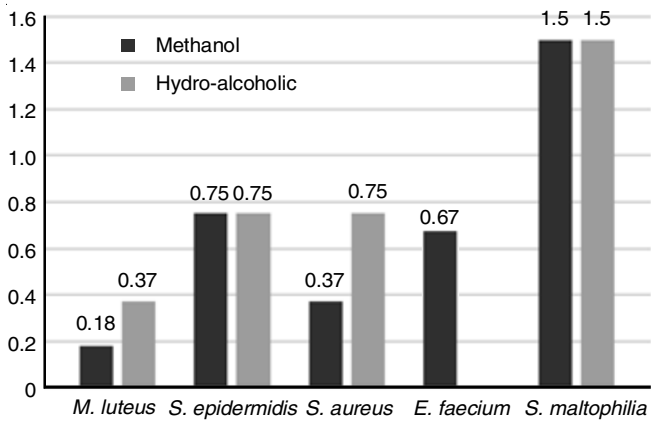

Fig. 2. Minimum inhibitory concentrations (MIC) (mg/mL) of different tuber root extracts of *A. latifolia* methanol extract (dark gray) and hydro-alcoholic extract (light grey)

M. luteus as compared to the hydro-alcoholic extract. The findings of the antibacterial activity of *A. latifolia* tuber root for Gram-positive and Gram-negative bacteria was supported by a previous study that reported the antibacterial activity of the ethanol extract from *A. latifolia* leaves against Gram-positive bacteria, including. *S. epidermidis*, MRSA, *M. luteus* and *Propionibacterium acnes* [18]. The MIC value of STM for both Gram-positive and Gram-negative bacteria was 1 µg, which was validated by earlier reports [29-31]. In brief, Gram-positive, Gram-negative and acid-fast bacteria were susceptible to methanol, hydro-alcoholic and *n*-hexane extract in this order: Gram-positive > Gram-negative > acid-fast bacteria. *A. latifolia* tuber antimicrobial component can be extracted with both methanol and hydro-alcoholic solvents rather than *n*-hexane. There is a significant difference in the antibacterial potential of *A. latifolia* tuber extracts due to the different degrees of solubility of antibacterial constituents in the tuber extract in three solvents used, namely methanol, hydro-alcoholic and *n*-hexane [32]. An interesting observation is that the antimicrobial activity was found against antibiotic-resistant bacteria *E. faecium* but not against *E. coli* both of which are the resident bacteria of human gut microbiota. The drug-resistance making the present generation of antibiotics obsolete and new avenues to treat the infections are need of the hour therefore, further antibacterial evaluation of *A. latifolia* tuber extract in combination with existing antibiotics could offer a potential solution [33].

FTIR studies: FTIR analysis of methanol and the hydro-alcoholic extract was scanned from 4000 cm^{-1} to 400 cm^{-1} and the observed functional group, in their respective wavelengths, type of vibration and bond group assignment as shown in Tables 2 and 3. The FTIR analysis of methanol and hydro-alcoholic extract shows the presence of functional groups like alcohols (O-H), alkenes (C=C), alkanes (C-H), alkynes (C≡C), ketones (C-O) and nitro compounds (N-O) in both extracts. The ethanol extract of *A. latifolia* tuber root has been reported to contain a wide range of functional groups, including alcohols, aromatic compounds, alkanes, aldehydes, ketones, alkenes, amines, amides, nitro compounds, carboxylic acids, ethers, esters and alkyl halides. These functional groups are commonly found in biologically active phytochemicals such as phenolic, flavonoid, terpenoid, glycoside and alkaloid compounds [34]. However, these phytochemicals were also observed in a non-polar solvent [35]. The FTIR spectra of methanol and hydroalcoholic extracts showed a high degree of similarity, suggesting that the two extracts contain similar molecular constituents. However, the methanol extract exhibited additional peaks at 2922.30, 1242.96 and 831.62 cm^{-1}, which are indicative of functional groups such as alkenes and ketones. This suggests that the methanol extract contains additional compounds compared to the hydroalcoholic extract, which may be responsible for the

TABLE-2
FTIR ANALYSIS OF METHANOL EXTRACT OF *A. latifolia* TUBER ROOT

Wavenumber (cm^{-1})	Bond group assignment	Mode of vibration	Functional group	Wavelength range (cm^{-1})
3387.39	O-H	Stretching	H-Bond alcohol	3200-3600
2922.30	C=C	Stretching	Alkenes	2850-2970
1613.59	C=C	Stretching	Alkenes	1600-1680
1515.13	N-O asymmetric stretch	Stretching	Nitro compounds	1475-1550
1445.02	C-H	Bending	Alkanes	1486-1425
1385.45	C-H	Bending	Alkanes	1396-1371
1242.96	C=O, C-H	Stretching; bending	Ketone/alkenes	1290-1211
1077.88	C=O	Stretching	Ketone	1147-1006
831.62	C=C	Wagging	Alkenes	885-802
610.05	C≡C	Bending	Alkynes	700-610

TABLE-3
FTIR ANALYSIS OF HYDRO-ALCOHOLIC EXTRACT OF *A. latifolia* TUBER ROOT

Wavenumber (cm^{-1})	Bond group assignment	Mode of vibration	Functional group	Wavelength range (cm^{-1})
3417.86	O-H	Stretching	H-Bond alcohol	3200-3600
1617.86	C=C	Stretching	Alkenes	1600-1680
1515.82	C=C/N-O asymmetric	Stretching	Alkenes/nitro compounds	1475-1550
1445.28	C-H	Banding	Alkanes	1486-1425
1384.29	C-H	Banding	Alkanes	1396-1371
1074.85	C=O	Stretching	Ketones	1147-1006
636.52	C≡C	Banding	Alkynes	700-610

observed differences in antibacterial activity between the two extracts.

HPTLC studies: HPTLC analysis was used to determine the kind and quantity of phytoconstituents present in the antibacterial active methanol extract of *A. latifolia* tuber root. Different volumes of methanol extract were loaded on the TLC and developed on the polar mobile phase methanol:ethyl acetate [2:8 (v/v)]. The chromatogram was generated by scanning the TLC plate at 254 nm short UV and 366 nm long UV and white light. The results of the HPTLC analysis are shown in Fig. 3. After further analysis the number of the bands, R_f values of starting point of the spot. It was observed that the sample contained different phytoconstituents at varying concentrations, as depicted in figures and tables. When exposed to short-wave UV light (254 nm), UV-active compounds exhibited fluorescence quenching properties, resulting in the observation of dark bands on certain tracks of the plate. Four dark bands with different R_f values were observed on tracks 3 and 6, while five dark bands were observed on tracks 1, 2 and 4. On track 5, six dark bands with varying R_f values were observed. Similarly, when exposed to long UV light (366 nm), the compounds displayed fluorescence properties resulting in the observation of bright bands on certain tracks. Four bright bands were observed on tracks 1, 3, 4 and 6, while five bright bands were observed on tracks 2 and 5 with different R_f values. It is essential to take account of the specific number of bands that may appear on each recording as each has a distinct background. The observed compounds on each track were identical; however, the number of bands detected varied due to the differing contractions loaded on each tract. This result confirms the presence of different phytochemicals with different polarity ranges in the methanol extract of *A. latifolia* [36]. Previous studies done by Tamilarasi *et al.* [25] confirmed the presence of alkaloids in ethanol, acetone and chloroform extract of *A. latifolia* tuber root. However, the phytochemicals were not identified. The R_f values computed for the phytoconstituents present in the tested samples would be useful in identifying new compounds by comparing them to reference standards and the concentration of the compounds could be inferred from the peak area values.

Conclusion

The potential antimicrobial activity of different extracts of *A. latifolia* tuber root against pathogenic Gram-positive, Gram-negative and non-pathogenic acid-fast bacteria indicated

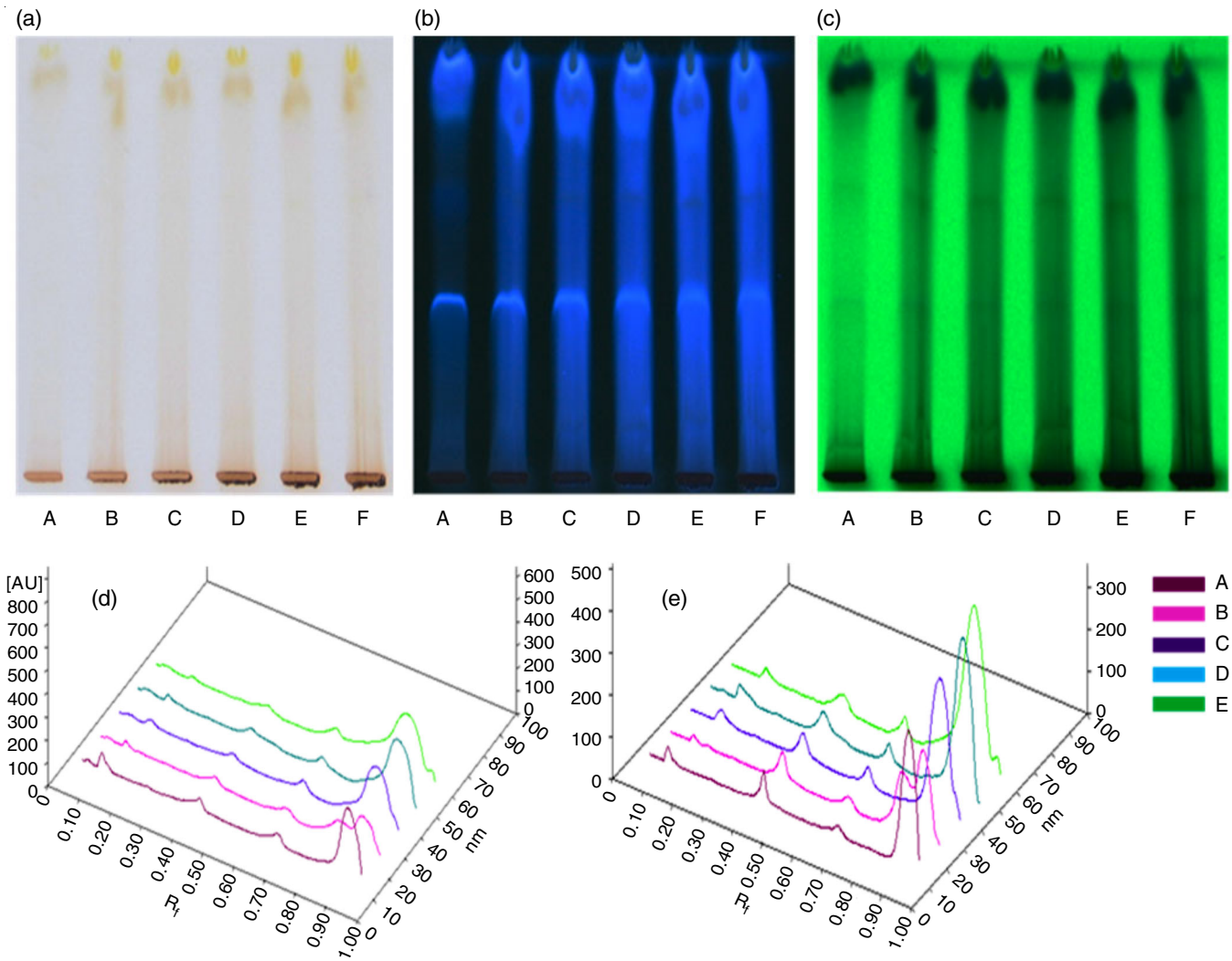

Fig. 3. HPTLC chromatograms of methanol extract of *A. latifolia* tuberous root

that the tuber of *A. latifolia* can be a source of new bioactive substances. The FTIR analysis of the active extract also confirmed the presence of diverse phytocompounds in the tuber root that is further confirmed by the HPTLC analysis of the active extracts in this study itself. The mobile phase used in HPTLC experiment is suitable for further fractionation experiments as it was resulted in well resolved compounds showing a significant difference in the R_f values. The work shows that *A. latifolia* tuber roots are the potential source of broad-spectrum antimicrobials that may be further explored for the antibacterial active principal of the extracts.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Dhara N. Patel, Assistant Prof., MLT Department, Bapubhai Desaibhai Patel Institute of Paramedical Sciences, CHARUSAT University, Changa, India for providing the clinically isolated Gram-positive and Gram-negative pathogenic strains and Prof. Rakesh Bhatnagar, School of Biotechnology, Jawaharlal Nehru University, New Delhi, India, for providing the acid-fast bacteria. One of the authors, Kamal Kishor Rajak acknowledges the Department of Biotechnology (DBT) Government of India for providing the Senior Research Fellowship (award no.: DBT/JRF/BET-18/1/2018/AL/121). The authors are also thankful to Charotar University of Science and Technology (CHARUSAT) University for providing funds to Hemant Kumar (award No.: 1/2021/Prof. Hemant Kumar, Departmental fund for research).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. S. Doron and S.L. Gorbach, in Eds.: H.K. (Kris) Heggenhougen, International Encyclopedia of Public Health, Elsevier, pp. 273-282 (2008); https://doi.org/10.1016/B978-012373960-5.00596-7
2. A. Gray and F. Sharara, *Lancet Glob. Health*, **10**, S2 (2022); https://doi.org/10.1016/S2214-109X(22)00131-0
3. V.K. Viswanathan, *Gut Microbes*, **5**, 3 (2014); https://doi.org/10.4161/gmic.28027
4. C.L. Ventola, *P&T*, **40**, 277 (2015).
5. G.M. Rossolini, F. Arena, P. Pecile and S. Pollini, *Curr. Opin. Pharmacol.*, **18**, 56 (2014); https://doi.org/10.1016/j.coph.2014.09.006
6. Z. Golkar, O. Bagasra and D.G. Pace, *J. Infect. Dev. Ctries.*, **8**, 129 (2014); https://doi.org/10.3855/jidc.3573
7. M. Gross, *Curr. Biol.*, **23**, R1063 (2013); https://doi.org/10.1016/j.cub.2013.11.057
8. A.F. Read and R.J. Woods, *Evol. Med. Public Health*, **2014**, 147 (2014); https://doi.org/10.1093/emph/eou024
9. P. Wikaningtyas and E.Y. Sukandar, *Asian Pac. J. Trop. Biomed.*, **6**, 16 (2016); https://doi.org/10.1016/j.apjtb.2015.08.003
10. D. Savoia, *Future Microbiol.*, **7**, 979 (2012); https://doi.org/10.2217/fmb.12.68
11. M.M. Pandey, S. Rastogi and A.K.S. Rawat, *Evid. Based Complement. Alternat. Med.*, **2013**, 376327 (2013); https://doi.org/10.1155/2013/376327
12. V. Subhose, P. Srinivas and A. Narayana, *Bull. Indian Inst. Hist. Med. Hyderabad*, **35**, 83 (2005).
13. O. Habbal, S.S. Hasson, A.H. El-Hag, Z. Al-Mahrooqi, N. Al-Hashmi, Z. Al-Bimani, M.S. Al-Balushi and A.A. Al-Jabri, *Asian Pac. J. Trop. Biomed.*, **1**, 173 (2011); https://doi.org/10.1016/S2221-1691(11)60021-X
14. F. Chassagne, T. Samarakoon, G. Porras, J.T. Lyles, M. Dettweiler, L. Marquez, A.M. Salam, S. Shabih, D.R. Farrokhi and C.L. Quave, *Front. Pharmacol.*, **11**, 586548 (2021); https://doi.org/10.3389/fphar.2020.586548
15. R. Brun, M. Niehues, T.J. Schmidt, S.A. Khalid, A.J. Romanha, T.MA. Alves, M.W. Biavatti, F.B. Da Costa, S.L. de Castro, V.F. Ferreira, M.V.G. de Lacerda, J.H.G. Lago, L.L. Leon, N.P. Lopes, R.C. das Neves Amorim, I.V. Ogungbe, *Curr. Med. Chem.*, **19**, 2176 (2012); https://doi.org/10.2174/092986712800229087
16. H.O. Edeoga, D.E. Okwu and B.O. Mbaebie, *Afr. J. Biotechnol.*, **4**, 685 (2005); https://doi.org/10.5897/AJB2005.000-3127
17. S. Parvin, M.A. Kader, A.U. Chouduri, M.A.S. Rafshanjani and M.E. Haque, *J. Pharmacogn. Phytochem.*, **2**, 47 (2014).
18. P.A. Pednekar and B. Raman, *Asian J. Pharm. Clin. Res.*, **6**, 157 (2013).
19. P.A. Pednekar, V. Kulkarni and B. Raman, *Int. J. Pharm. Pharm. Sci.*, **6**, 602 (2014).
20. A. Chaudhuri and S. Ray, *J. King Saud Univ. Sci*., **32**, 732 (2020); https://doi.org/10.1016/j.jksus.2018.12.006
21. A. Chaudhuri and S. Ray, *J. King Saud Univ. Sci*., **32**, 1798 (2020); https://doi.org/10.1016/j.jksus.2020.01.046
22. A. Chaudhuri, S. Chatterjee, A. Chaudhuri and S. Ray, *J. Indian Chem. Soc*., **97**, 2874 (2020).
23. C. Anwesa, R. Sanjib, C. Anwesa and R. Sanjib, *Eur. J. Exp. Biol.*, **5**, 1 (2015).
24. A. Chaudhuri and S. Ray, *IOSR J. Agric. Vet. Sci.*, **9**, 90 (2016); 10.9790/2380-09070190100
25. C.T. Tamilarasi, U. Subasin, S. Kavimani and B. Jaykar, *Anc. Sci. Life*, **20**, 14 (2000).
26. J.B. Patel, F.C. Tenover, J.D. Turnidge and J.H. Jorgensen, *Manual of clinical microbiology.*, **2011**, 1122 (2011); https://doi.org/10.1128/9781555816728.ch68
27. M. Elshikh, S. Ahmed, S. Funston, P. Dunlop, M. McGaw, R. Marchant and I.M. Banat, *Biotechnol. Lett.*, **38**, 1015 (2016); https://doi.org/10.1007/s10529-016-2079-2
28. T.A. Maryutina, E.Y. Savonina, P.S. Fedotov, R.M. Smith, H. Siren and D.B. Hibbert, *Pure Appl. Chem.*, **90**, 181 (2018); https://doi.org/10.1515/pac-2017-0111
29. N. Tuyiringire, I. Taremwa Mugisha, D. Tusubira, J.-P. Munyampundu, C. Mambo Muvunyi and Y. Vander Heyden, *J. Clin. Tuberc. Other Mycobact. Dis.*, **27**, 100307 (2022); https://doi.org/10.1016/j.jctube.2022.100307
30. A. Miernik and M. Rzeczycka, *Pol. J. Microbiol.*, **56**, 265 (2007).
31. Y. Hu, L. Liu, X. Zhang, Y. Feng and Z. Zong, *Front. Microbiol.*, **8**, 2275 (2017); https://doi.org/10.3389/fmicb.2017.02275
32. K. Ratananikom and N. Srikacha, *J. Acute Dis.*, **9**, 223 (2020); https://doi.org/10.4103/2221-6189.291288
33. N.L. Maia, M. De Barros, L.L. De Oliveira, S.A. Cardoso, M.H. Dos Santos, F.A. Pieri, T.C. Ramalho, E.F. Da Cunha and M.A. Moreira, *Front. Microbiol.*, **9**, 1203 (2018); https://doi.org/10.3389/fmicb.2018.01203
34. E.S. Teoh, Secondary Metabolites of Plants. In: Medicinal Orchids of Asia, Springer, Cham., p. 59 (2016); https://doi.org/10.1007/978-3-319-24274-3_5
35. K. Snehlata, R. Sheel and B. Kumar, *J. Biotechnol. Biochem.*, **4**, 31 (2018); 10.9790/264X-0405013138
36. B. Spangenberg, A. Seigel and R. Brämer, *J. Planar Chromatogr. Mod. TLC*, **35**, 313 (2022); https://doi.org/10.1007/s00764-022-00176-2