Research Article

Evaluation of the antimicrobial efficacy and phytochemical constituents of the extracts of *Andrographis paniculata* against drug-resistant bacterial pathogens

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ABSTRACT

Background: The emergence of drug-resistant bacteria has led to the search for newer plant-derived antimicrobial agents with lesser side effects. *Andrographis paniculata* (Nilavembu), the chief ingredient nilavembu kudineer has been successfully used in the treatment of dengue fever outbreak in India. Hence, this study was proposed to examine the antibacterial activity of *A. paniculata* against multidrug-resistant isolates of Gram-positive cocci (vancomycin-resistant *Enterococcus faecalis* (VRE) and methicillin-resistant *Staphylococcus aureus* [MRSA]) and Gram-negative bacilli (extended-spectrum beta-lactamase [ESBL]-producing *Escherichia coli* and *Pseudomonas aeruginosa*). Standard strains, ATCC 25922 *E. coli*, ATCC 25923 *S. aureus*, ATCC 27853 *P. aeruginosa,* and ATCC 29212 *E. faecalis* were used as controls. **Methods:** Extracts such as methanol, acetone, chloroform, and ethanol of *A. paniculata* (leaf) were prepared by cold percolation method. Agar well diffusion method was employed to assess the antibacterial activity. Minimum inhibitory concentration was determined using microbroth dilution method. **Results:** Results showed methanol extract exhibited maximum antibacterial activity against clinical and control strains. Phytochemical analysis of the methanol extract has confirmed the presence of an array of bioactive compounds. **Conclusion:** Methanolic extract of *A. paniculata* could serve as a potential herbal alternative in the management of infections caused by VRE and MRSA, while the ethanolic extract of *A. paniculata* could be used in the effective therapeutic management of ESBL-positive *P. aeruginosa*.

KEY WORDS: *Andrographis paniculata*, Antibacterial efficacy, Methicillin-resistant *Staphylococcus aureus, Pseudomonas aeruginosa*, Vancomycin-resistant Enterococci

INTRODUCTION

Until the middle of the 20th century, traditional medicines that include the herbal medicines were the only medicines available. According to the WHO, between 70% and 95% of the populations of developing countries currently use traditional medicines for primary care.[1] With the advent of "modern medicines" (i.e., since the production of penicillin, 1943), different classes of antibiotics had been introduced into the market by the pharmaceutical industries; nevertheless, the simultaneous emergence of drug/antibiotic-resistant microorganisms is a cause of global concern. Despite the availability of antibiotics, the treatment of infections caused by methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant enterococci, extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacilli[2] remains an unresolved challenge as there are no promising drugs in the pipeline. This has led to the search for newer plant-derived antimicrobial agents with lesser side effects. Ayurveda (a component of AYUSH), an alternative system of medicine with its historical roots in the Indian subcontinent, is primarily

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based on the plant extracts and other plant derivatives for the treatment of various ailments.[3] The therapeutic potential of hundreds of plant-derived medicines along with the uses, microscopic structure, chemical composition, toxicology, prevalent myths and stories, and relation to commerce in British India was been documented by William Dymock.[4]

Andrographis paniculata commonly known as creat or green chireta (Germplasm Resources Information Network, GRIN)[5] and Siriyanangai (in Tamil) is a native herb of India and Sri Lanka. In Asia, *A. paniculata* has been used in traditional medicine for centuries.[6,7] *A. paniculata* known as Kalmegh or Kalamegha in Ayurveda meaning "dark cloud" belongs to the family *Acanthaceae*. *A. paniculata* called as Nilavembu in Tamil, being the "neem of the ground," "king of the bitters," and "Bile of the Earth" is an annual, erect, branched herb, has a strong bitter taste comparable to that of the large neem tree (*Azadirachta indica*). In earlier reports, antibacterial activity was determined in alcoholic extracts. The objective of this study was to evaluate the antimicrobial potential of the extracts of *A. paniculata* against drug-resistant Gram-positive and Gram-negative bacterial pathogens and to determine phytochemical constituents present in the extracts.

MATERIALS AND METHODS

The leaves of *A. paniculata* (leaves) were shade dried and ground well to a fine powder. The active phytochemical compounds of *A. paniculata* (leaves) were extracted with four different solvents, methanol, acetone, ethanol, and chloroform by cold percolation method. The solvents were evaporated to dryness and stock solution of the respective extracts was prepared in 10% DMSO.[8]

Screening for antibacterial activity of *A. paniculata* (leaves) against the clinical drug-resistant bacterial strains, namely, ESBL-producing *Escherichia coli*, ESBL-producing *Pseudomonas aeruginosa*, methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant *Enterococcus faecalis* (VRE) was assessed by agar well diffusion assay. Standard strains of Gram-positive cocci (*E. faecalis* ATCC 29212 and *S. aureus* ATCC 25923) and Gram-negative bacilli (*E. coli* ATCC 25923 and *P. aeruginosa* ATCC 27853) were included as controls. The ESBL-resistant phenotype of *E. coli* and *P. aeruginosa* was confirmed by combined disk method using ceftazidime (CAZ: 30 µg): Ceftazidime + clavulanic acid (CAC: 30 µg/10 µg) and cefotaxime (CTX: 30 µg): Cefotaxime + clavulanic acid (CEC: 30 µg/10 µg) (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Increase in zone size ≥5 mm in the presence of clavulanic acid was considered as positive for ESBL production.[9] Cefoxitin disk method and vancomycin (6 µg/mL) agar dilution method were adopted to screen for MRSA and VRE, respectively. Further, VRE was confirmed by E test by following Clinical Laboratory Standards Institute guidelines. Minimum inhibitory concentration (MIC) was detected using broth microdilution method.[8] Subsequently, minimum bacterial concentration was measured using spot inoculation onto Mueller-Hinton agar plates. Phytochemical preliminary screening for the presence of steroid, triterpenes, sugars, alkaloids, phenolic groups, flavonoids, saponins, tannins, anthraquinone glycosides, and amino acids content was performed by Liebermann–Burchard, Salkowski, Molisch's method, Dragendorff's, ferric chloride and lead acetate method, Shinoda, chloroform and H_2SO_4, ferric chloride, and Mulish and Milton's test, respectively.[10]

Gas chromatography–mass spectrometry (GC–MS) was performed to determine the phytochemicals present in the extract that exhibited maximum activity. GC–MS-QP2010 ultra GC–MS (Shimadzu Corp) was used and the initial temperature was set to 100°C and maintained for 2 min. Subsequently, the temperature was gradually increased at the rate of 10°C/min until 250°C and maintained for 2 min. Finally, oven temperature was raised to 280°C at the rate of 10°C/ min and maintained for 18 min.

Statistical Assessment

To identify the statistical difference between observed variables, one-way analysis of variance was applied and results were statistically interpreted.

RESULTS

The results of antibacterial activity exhibited by various extracts of *A. paniculata* (leaves) against clinical isolates and control strains of Gram-positive and Gram-negative bacteria are depicted in Table 1. The MIC of the various solvent extracts is indicated in Table 2. Table 3 depicts the phytochemical profile of the extracts of Andrographis paniculata (leaf). GC–MS assay was also performed to find out and segregate potential compounds for the antibacterial activity [Figure 1 and Table 4].

DISCUSSION

In India, herbal drugs have been used in the treatment of several ailments since the prehistoric period. Nearly 60% of the antitumor and antimicrobial medicines currently available in the global market are derived, either directly or indirectly, from medicinal plants, primarily through the application of modern technology to traditional knowledge. *A. paniculata*, the chief ingredient of the polyherbal concoction called nilavembu kudineer has been successfully used in the treatment of dengue fever and chikungunya fever in India.[11] Herein, we investigated the antibacterial efficacy of Nilavembu,

Table 1: Antibacterial activity of the different extracts of *Andrographis paniculata* (leaf)

A. paniculata L. (leaf) extracts against the bacterial isolates tested	Mean diameter of the zone of inhibition (in mm)			
	Methanol	Ethanol	Acetone	Chloroform
S. aureus ATCC 25923	12±1.00	8±0.00	8±0.00	12.67±0.58
Methicillin-resistant *S. aureus* (clinical isolate)	13.33±0.58	8±0.00	8±0.00	11.67±0.58
Enterococcus faecalis ATCC 29212	10.67±0.33	8±0.00	8±0.00	8±0.00
VRE (clinical isolate)	10.33±0.58	8±0.00	8±0.00	8±0.00
E. coli ATCC 25923	8±0.00	8±0.00	8±0.00	8±0.00
ESBL-producing *E. coli* (clinical isolate)	8±0.00	8±0.00	8±0.00	8±0.00
P. aeruginosa ATCC 27853	9.33±0.58	8±0.00	8±0.00	8±0.00
ESBL-producing *P. aeruginosa* (clinical isolate)	9.67±0.58	34.33±0.58	8±0.00	8±0.00

E. coli: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, ESBL: Extended-spectrum beta-lactamase, *S. aureus*: *Staphylococcus aureus*

Table 2: MIC of the extracts of *Andrographis paniculata* (leaf)

Bacteria tested	MIC (µg/mL) of the extracts of *A. paniculata* L. (leaf)			
	Methanol	Ethanol	Acetone	Chloroform
S. aureus ATCC 25923	150	150	150	150
Methicillin-resistant *S. aureus* (clinical isolate)	150	150	150	150
Enterococcus faecalis ATCC 29212	150	150	150	>150
VRE (clinical isolate)	150	150	150	>150
Escherichia coli ATCC 25923	150	75	75	75
ESBL-producing *E. coli* (clinical isolate)	150	75	75	75
P. aeruginosa ATCC 27853	75	37.5	37.5	37.5
ESBL-producing *P. aeruginosa* (clinical isolate)	75	37.5	37.5	37.5

E. coli: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, ESBL: Extended-spectrum beta-lactamase, *S. aureus*: *Staphylococcus aureus*, MIC: Minimum inhibitory concentration

A. paniculata against drug-resistant Gram-positive and Gram-negative bacterial species.

In our study, a significant difference was observed between the activity of the methanol extract of *A. paniculata* (leaf) and the ethanol ($P = 0.0023$) and acetone extracts ($P = 0.0023$) against ATCC *S. aureus*. However, no statistical difference was observed between the antibacterial activity of the methanol and chloroform extracts against ATCC *S. aureus* ($P = 0.37389$). Furthermore, significant difference was observed in the activity of methanol extract of *A. paniculata* (leaf) compared to the other extracts methanol versus ethanol ($P = 00009$), methanol versus acetone extracts ($P = 00009$), and methanol versus chloroform extract ($P = 0.02412$) against MRSA. The methanol extract of *A. paniculata* (leaf) exhibited pronounced antibacterial activity compared to all other extracts against ATCC *E. faecalis* and VRE ($P = 0.00132$; $P = 0.00219$), respectively. None of the extracts of *A. paniculata* L. (leaf) were effective against both the standard strains, i.e., ATCC *E. coli* and the clinical isolate ESBL-producing *E. coli*. Similarly, the methanol extract of *A. paniculata* L. (leaf) exhibited antibacterial activity against the standard strain, i.e., ATCC *P. aeruginosa* and compared to the other solvent extracts ($P = 0.01614$). The methanol extract of *A. paniculata* L. (leaf) exhibited pronounced antibacterial activity compared to the acetone and chloroform extracts against clinical isolate of *P. aeruginosa* ($P = 0.00749$). The ethanol extract of *A. paniculata* (leaf) exhibited pronounced antibacterial activity against the clinical isolate of

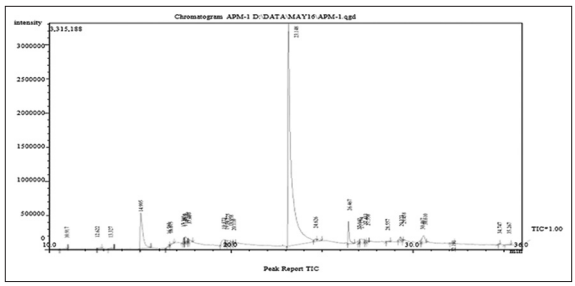

Figure 1: Gas chromatography–mass spectrometry analysis of the methanol extract of *Andrographis paniculata* (leaf)

P. aeruginosa compared to the standard strain ATCC *P. aeruginosa* ($P = 0.000$). Our results are in line with the findings of Zaidan *et al*., 2005, who reported that the aqueous extract of *A. paniculata* (leaf) was effective against ATCC *S. aureus*, MRSA, and *P. aeruginosa,* but no activity was observed against *E. coli*.[12] Our results confirm the potential role of *A. paniculata* L. (leaf) in the treatment of infections caused by drug-resistant bacterial pathogens. This in concurrence with the previous clinical studies that have documented the effect of *Andrographis* preparations in the treatment of patients with common cold, sinusitis, bronchitis, pharyngotonsillitis, lower urinary tract infections, bacillary dysentery, and acute diarrhea.[13-17]

Our study results indicate that methanol (MIC: 75 µg/ mL) and ethanol (MIC: 37.5 µg/mL) extracts of *A. paniculata* were most effective against P. aeruginosa (ATCC 27853 and clinical strain – ESBL-producing *P. aeruginosa*). Furthermore, methanol and ethanol extracts of *A. paniculata* were effective against

Table 3: Phytochemical profile of the extracts of *Andrographis paniculata* (leaf)

Constituents	Methanol	Ethanol	Acetone	Chloroform
Steroids	+	+	+	+
Triterpenes	+	+	+	+
Sugars	+	+	+	+
Alkaloids	+	+	+	+
Phenolic groups	+	−	+	+
Flavones	+	+	+	−
Saponins	+	−	−	+
Tannins	+	+	+	+
Anthraquinone glycosides	−	+	+	+
Amino acids	+	−	+	−

Table 4: Compounds present in the methanol extracts of *Andrographis paniculata* (leaf), GC–MS analysis

APC-1	4-[(4-hydroxy-3,5-dimethylphenyl)methyl]-2,6-dimethylphenol
APC-2	benzene-1,2,3-triol
APC-3	4-(cyclopropylmethyl)benzonitrile
APC-4	2-[(furan-2-yl)formamido]-3-(4-methoxyphenyl)-N-[2-(2-methyl-1H-indol-3-yl) ethyl] propenamide
APC-5	2-(hydroxymethyl)-6-[(naphthalen-1-yl) amino] oxane-3,4,5-triol
APC-6	4,5-dimethoxy-6-(methoxymethyl) oxan-3-yl acetate
APC-7	2-(4-bromophenyl)-3,3,5-trimethyl-6-oxooxan-4-yl acetate
APC-8	5-[(4-chlorophenoxy) methyl] furan-2-carboxylic acid
APC-9	1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid
APC-10	2-ethylhexyl 3-chloropropanoate
APC-11	nonyl propyl carbonate
APC-12	1-(4-chlorophenyl)-2-methylpropan-2-ol
APC-13	1,2-dibromo-3-methylbutane
APC-14	(Z)-(methyl N-hydroxybenzenecarboximidate)
APC-15	1,7-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione
APC-16	4-[(E)-[(2E)-2-[(4-hydroxy-3,5-dimethoxyphenyl) methylidene] hydrazin-1-ylidene] methyl]-2,6-dimethoxyphenol
APC-17	(1E)-2,6,10,14-tetramethylpentadec-1-en-1-ol
APC-18	2-(hydroxymethyl)-5-(2-hydroxypropan-2-yl) cyclohex-2-en-1-ol
APC-19	methyl (2E,4E)-6-(2,2,5-trimethyl-1,3-dioxan-4-yl) hepta-2,4-dienoate
APC-20	methyl (9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trienoate
APC-21	trimethyl (1-phenylethoxy) silane
APC-22	trimethyl-1H,5H,6H,7H-pyrimido[5,4-e][1,2,4]triazine-5,7-dione
APC-23	(2-methyl-1-phenylprop-2-en-1-yl) benzene
APC-24	2-nitropyridine
APC-25	4,5-dinitro-2-(trifluoromethyl)-1H-imidazole
APC-26	1-[4-(acetyloxy)-3-methoxyphenyl]-2-acetamidoethyl acetate
APC-27	1-(4-methoxyphenyl)-2-(3,5,6-trimethylpyrazin-2-yl) ethan-1-ol
APC-28	(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl) nona-2,4,6,8-tetraen-1-ol
APC-29	6-(but-1-en-2-yl)-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-ol
APC-30	Tetramethylpyrazine
APC-31	2-(dichloromethyl)-1,3-dioxolane
APC-32	2-(dichloromethyl)-1,3-dioxolane
APC-33	trimethylsilyl 2,6-bis[(trimethylsilyl) oxy] benzoate
APC-34	6-(4-methoxy-3-methylbutyl)-7,9,13-trimethyl-5-oxapentacycloicosa-6,18-dien-16-ol

E. coli, ATCC 26923 (MIC: 150 µg/mL), and clinical strain ESBL-producing *E. coli (*MIC: 75 µg/ ml). Nevertheless, methanol, ethanol, and acetone extracts of *A. paniculata* exhibited antibacterial activity against the Gram-positive pathogens (*S. aureus* – ATCC 25922 and clinical [MRSA] and *E. faecalis* – ATCC 29212 and clinical [VRE]) at an MIC of 150 µg/ml. The chloroform extract of *A. paniculata* showed least antibacterial activity which is in line with the findings of Premanath and Devi., 2017.[18] The results obtained in the present study indicate that the methanol extract exhibits a broad spectrum of activity against both Gram-positive and Gram-negative drug-resistant pathogenic bacteria. Our results are in line with the previous reports on the methanolic extract against *S. aureus* and *P. aeruginosa.*[19] Of note, we report here the most potent antibacterial activity (MIC 37.5 µg/ mL) of the ethanolic extract of *A. paniculata* against ESBL-producing *P. aeruginosa*.

Phytochemical investigation of the various solvent extracts of *A. paniculata* indicates the presence of soluble nature of phytochemicals in the methanol and acetone fraction. Phytochemicals in the methanolic extract of *A. paniculata* include steroids, triterpenes, tannins, alkaloids, phenolic groups, saponins, flavones, and tannins. GC–MS analysis of the methanol extracts of *A. paniculata* (leaf) revealed the presence of 34 compounds. Our findings are in line with the previous reports which indicate that most of the phytochemical compounds in plants are soluble in methanol.[18,20-21]

CONCLUSION

Due to the presence of various bioactive compounds, the alcoholic extracts of *A. paniculata* (leaf) exhibited pronounced antibacterial activity against both the standard strains (ATCC) and the drug-resistant clinical isolates. Hence, it could be speculated that *A. paniculata* (leaf) could serve as a potential herbal alternative in the therapeutic management of bacterial infections caused by drug-resistant Gram-positive (MRSA and VRE) and Gram-negative (ESBL-producing *P. aeruginosa*) bacteria. However, further studies are required to ascertain the antibacterial potential of the phytocompounds in the control of drug-resistant strains.

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