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Research Article

ANTIBACTERIAL ACTIVITY OF AMORPHOPHALLUS KONKANENSIS AND AMORPHOPHALLUS BULBIFER TUBER

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ABSTRACT

Objective: Evaluation of antibacterial activity of unexplored, endemic *Amorphophallus konkanensis* (AKT) Hett., Yadav & Patil and *Amorphophallus bulbifer* (ABT) (Roxb.) Bl.

Methods: Acetone and ethanol extracts of tubers of AKT and ABT were evaluated for *in vitro* antibacterial activity against *Bacillus subtilis, Micrococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* using agar diffusion method, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and growth pattern of test organisms. High-performance liquid chromatography (HPLC) analysis was carried out for active phenolic compounds.

Results: AKT and ABT extracts for both solvents showed significant antibacterial activities. The zone of inhibition was ranged from 12-25 mm to 11-23 mm for AKT and ABT extracts respectively. Highest significant activity was observed against *M. aureus*. The MIC values of acetone extracts were ranged from 200-350 µg/ml (AKT) to, 200-400 µg/ml (ABT). Ethanol extracts of AKT and ABT were showed MIC at 250-450 µg/ml and 300-450 µg/ml respectively. MBC value of acetone extracts ranged from 260-460 µg/ml (AKT) to 300-500 µg/ml (ABT) and for ethanol extracts 340-500 µg/ml (AKT) and 380-540 µg/ml (ABT). The effect of extracts on growth of microorganisms was also studied. HPLC analysis showed phenolic compounds *viz.* tannic acid, gallic acid, quercetin, p-coumaric acid, and catechin. This polyphenolic rich extracts of AKT and ABT have demonstrated various degree of microbial growth inhibition.

Conclusion: Antibacterial activity of AKT and ABT implies that, these plants have the potential for preparation of alternative medicines for infections caused by microorganisms that have become resistant to the current therapeutic measures.

Keywords: *Amorphophallus konkanensis*, *Amorphophallus bulbifer*, Antibacterial activity, Minimum inhibitory concentration, minimum bactericidal concentration, High-performance liquid chromatography.

INTRODUCTION

At present, the pharmaceutical drugs available to control antibiotic-resistant bacteria are becoming limited. The discriminate use and abuse of antibiotics have led to the development of antimicrobial resistant strains and toxicity of some drugs to human and animals [1,2]. According to World Health Organization traditional medicines are relied upon by 65-80% of the world's population for their primary health care needs [3]. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [4]. In recent years, attempts have been made to investigate the indigenous drugs against infectious diseases. This may help to develop safer antimicrobial drugs [5].

Many plants of family Araceae have been reported to have antibacterial activities, and a huge number of researchers are going on with the different plants of Araceae for screening of pure compounds responsible for antibacterial activity [6]. The tuberous roots of the species of *Amorphophallus campanulatus* Bl. are used traditionally for the treatment of piles, abdominal pain, tumors, enlargement of spleen, asthma, and rheumatism. The tuberous roots of the plant also have tonic, stomachic and appetizer properties [7]. There are plenty of reports of its traditional medicinal uses. However, there is a little report about the pharmacological properties.

Phenolic compounds were found to have high antibacterial activity [8]. Antibacterial activity of phenolics includes their ability to denature microbial proteins [9], ability to react with cell membrane components which impairs integrity of cells [10].

Amorphophallus konkanensis (AKT) Hett., Yadav & Patil is endemic, widely distributed in the forest area of Western Ghats of Maharashtra. *Amorphophallus bulbifer* (ABT) (Roxb.) Bl. is abundantly present at high elevations, and only species in *Amorphophallus* characterized with bulbils [11]. So far no attempts have been made to evaluate the medicinal properties of both AKT and ABT. Hence, the present study was planned to assess antibacterial potential of AKT and ABT tubers.

Thus, to provide a scientific justification antibacterial activity was studied using acetone and ethanol extracts against some clinically important bacteria with determining the value of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

MATERIALS AND METHODS

Collection of plant material

AKT Hett., Yadav and Patil and ABT (Roxb.) Bl. (Family: *Araceae*) tubers were collected from Ratnagiri and Amboli in Western Ghats of Maharashtra, India and identified with the help of recent floral description [11]. The tubers were cleaned, chopped and kept in hot air oven at 40°C for 48 hrs for drying.

Extraction of plant material

The dried AKT and ABT were made to a fine powder using mortar pestle and were extracted using solvents, acetone and ethanol at solvent to powder ratio of 1:10. Weighed amount of each sample was extracted in known volume of the solvent for 24 hrs with shaking on a rotary shaker. Each extracted material was filtered through Whatman filter paper No. 1. These extracts were again dried and concentrated by evaporating the solvent completely in the water bath at the range of boiling points of solvents. The dried extracts were resuspended in 10% dimethyl sulfoxide (DMSO) [12] and stored at 4°C. The extracts were further used for antibacterial testing.

Test organisms

Six bacterial strains were taken into consideration, viz. *Bacillus subtilis* (NCIM 2045), *Micrococcus aureus* (NCIM 2802), *Pseudomonas aeruginosa* (NCIM 2036), *Escherichia coli* (NCIM 2832), *Salmonella typhi* (NCIM 2501), *Klebsiella pneumoniae* (NCIM 2883). The test organisms were subcultured at 37°C for 24 hrs and maintained on nutrient agar media.

Screening of antibacterial activity

Sensitivity of different bacterial strains to various extracts was measured in terms of zone of inhibition using agar diffusion assay [13]. The plates containing Mueller-Hinton agar media were spread with 0.2 ml of the inoculums equivalent to McFarland 0.5 (15×10^7 CFU/mL) turbidity standard. Wells were cut out from agar plates using a sterilized stainless steel borer and filled with 0.1 ml (500 µg) of the extract. The plates were inoculated with different bacterial cultures and incubated at 37°C up to 48 hrs and diameter of resultant zone of inhibition was measured. The bacteria with a clear zone of inhibition were considered to be sensitive. For each combination of extract and the bacterial strain, the experiment was performed in duplicate and repeated thrice.

Determination of MIC

The MIC of AKT and ABT extracts was determined by agar diffusion assay [13]. The plates containing Mueller-Hinton agar media were spread with 0.2 ml of the inoculums equivalent to McFarland 0.5 (15×10^7 CFU/mL) turbidity standard [14]. Wells were prepared in agar plates using a sterilized stainless steel borer and extracts were loaded. The concentrations acetone and ethanol extracts of AKT and ABT were used in this test ranged from 150 to 550 µg/ml. Each plate contained three wells including control as 10% DMSO. The lowest concentration of the extract that showed zone of inhibition against respective organisms was taken as MIC.

Determination of MBC

The MBC of the AKT and ABT extracts was determined by broth dilution method [12] with some modifications. Both plant extracts were resuspended in 10% DMSO to make final concentration of 1 mg/ml, this was then serially diluted to obtain 580, 540, 500, 460, 420, 380, 340, 300, 260, 220 µg/ml. 10% DMSO was used as control to compare its activity with extracts. Mueller-Hinton broth was prepared and sterilized by autoclave. The sterilized broth was allowed to cool, and 50 µl of broth was added to sterile 1.5 ml Eppendorf tubes which contained plant extracts of different concentrations. Thereafter 50 µl inoculum (0.5 McFerland turbidity standards) was added into each tube. Additional tubes containing only the broth were prepared, in the same way, so as to serve for comparison of growth of the respective organisms. After incubation loopful of the suspension were streaked on Mueller-Hinton agar plate. The plates were then incubated at 37°C for 24 hrs. After the incubation observed the plates for appearance of visible growth. Least concentration of the extract showing no visible growth on subculturing was taken as MBC.

Growth pattern of test organisms

MIC and MBC was followed by checking the effect of the extracts on growth pattern of test organisms [15]. Colonies of the respective bacteria were incubated in nutrient broth, and McFarland 0.5 turbidity standard was obtained. Then 500 µl of bacterial suspension was added to 50 ml of medium along with 250 µl of the respective extract. Similar set of experiments was set, but instead of extracts, the respective solvents were added. This was the solvent control set. The third set of flasks was used where 250 µl of extracts were added, but no organism was inoculated. This set of flasks was used for adjusting zero of the spectrophotometer. The flasks were incubated at 37°C in an incubator shaker. The growth was monitored by measuring absorbance at 540 nm at every 30 minutes interval.

High-performance liquid chromatography (HPLC) analysis

HPLC was done using a Hitachi LaChrome chromatograph fitted with a reversed phase column (Column-C18; 5 mm, 250 mm × 4.6 mm) and an ultraviolet detector set at 240 nm. The column was operated at room temperature. Separations were carried out in a liquid feed pumping system by using acetonitrile (70%) and water (30%) as a mobile phase with a flow rate of 0.5 ml/minutes. The injection volume for all samples was 100 µL. The phenolic compounds were analyzed by matching the retention time and their spectral characteristics against those of standards.

Statistical analysis

All the experiments were repeated 3 times till the data obtained were statistically valid as analysis of variance was carried out for all data using Graph Pad software (GraphPad InStat version 3.00, GraphPad Software, San Diego, CA, USA).

RESULTS

Screening of antibacterial activity

It was observed that both AKT and ABT showed significant antibacterial activity, although the inhibitory activity was strain specific (Table 1). Acetonic extracts of both AKT (25 mm) and ABT (23 mm) showed the highest activity against *M. aureus*, followed against *B. subtilis*. Both the extracts of AKT and ABT showed potent inhibitory zone against *P. aeruginosa*. Acetone extracts of both AKT and ABT were showed relatively significant antibacterial activities against both Gram-positive and Gram-negative bacterial strains tested.

Determination of MIC

The MIC values were, solvent extracts and strained dependent. Better efficacy of both acetone and ethanol extracts of AKT and ABT was further supported by MIC studies. The MIC values of acetone extracts were ranged from 200-350 µg/ml to, 200-400 µg/ml for AKT and ABT respectively. Ethanol extracts of AKT and ABT were showed MIC values ranged from 250-450 µg/ml to 300-450 µg/ml respectively (Table 2). The highest MIC values were observed for acetonic and ethanolic extracts of AKT at 350 µg/ml and 450 µg/ml while of ABT at 400 µg/ml and 450 µg/ml respectively against *P. aeruginosa*.

Table 1: Antibacterial activity of organic solvent extracts of AKT and ABT

Organisms	Zone of inhibition (mm)			
	AKT		ABT	
	Acetone extract	Ethanol extract	Acetone extract	Ethanol extract
B. subtilis	21	16	19	13
M. aureus	25	18	23	15
P. aeruginosa	16	12	14	11
E. coli	18	13	16	13
S. typhi	20	14	17	12
K. pneumoniae	18	13	15	12

B. subtilis: Bacillus subtilis, M. aureus: Micrococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, E. coli: Escherichia coli, S. typhi: Salmonella typhi, K. pneumoniae: Klebsiella pneumonia, AKT: *Amorphophallus konkanensis*, ABT: *Amorphophallus bulbifer*

Table 2: MIC (µg/ml) of AKT and ABT extracts

Organisms	MIC (µg/ml)			
	AKT		ABT	
	Acetone extract	Ethanol extract	Acetone extract	Ethanol extract
B. subtilis	200	300	250	350
M. aureus	200	250	200	300
P. aeruginosa	350	450	400	450
E. coli	300	350	300	400
S. typhi	250	400	300	400
K. pneumoniae	300	350	300	350

B. subtilis: Bacillus subtilis, M. aureus: Micrococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, E. coli: Escherichia coli, S. typhi: Salmonella typhi, K. pneumoniae: Klebsiella pneumonia, AKT: *Amorphophallus konkanensis*, ABT: *Amorphophallus bulbifer*, MIC: Minimum inhibitory concentration

Determination of MBC

Results from the MBC assay supported the data obtained from the agar diffusion assay and the MIC assay (Table 3). Acetone and ethanol extracts of both AKT and ABT showed bactericidal activity. MBC values of acetone extracts ranged from 260-460 µg/ml to 300-500 µg/ml for AKT and ABT, respectively. For ethanol extracts, MBC values ranged from 340-500 µg/ml to 380-540 µg/ml for AKT and ABT, respectively. The least MBC exerted by acetone extract of AKT (260 µg/ml) against *M. aureus*.

Growth pattern of test organisms

The objective of this study was not to just see whether the extracts were inhibiting the growth of the above mentioned organisms, but also to find out exactly what was the response of the organisms growing in presence of the extracts. It can be seen from the Fig. 1 that all the organisms get inhibited and had a reduced growth rate for acetone and ethanolic extracts of both AKT and ABT. In the presence of acetone extract of the AKT and ABT, where it was observed that there was

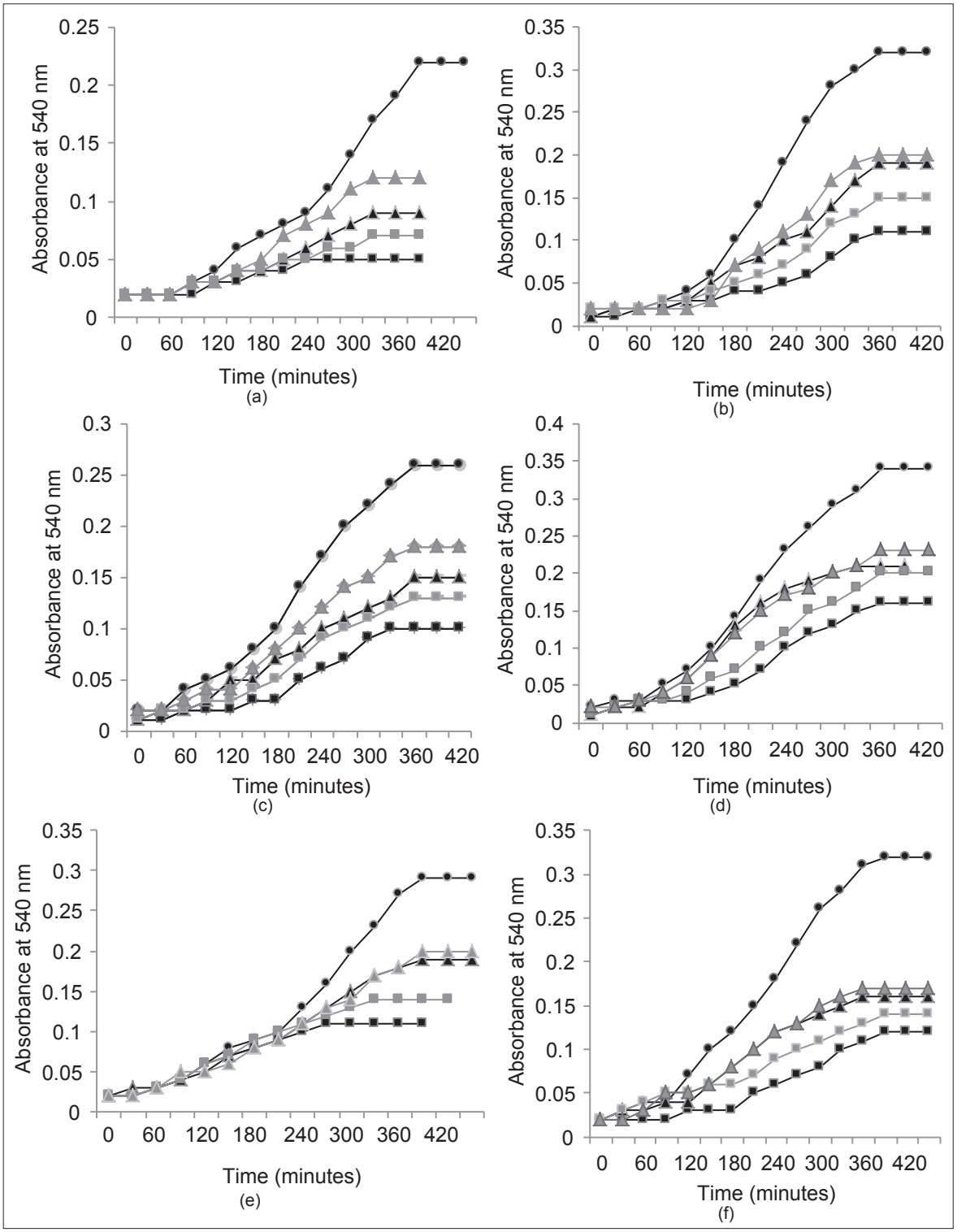

Fig. 1. Growth pattern of microorganisms, (a) Micrococcus aureus, (b) Escherichia coli, (c) Bacillus subtilis, (d) Salmonella typhi, (e) Pseudomonas aeruginosa, (f) Klebsiella pneumoniae in presence of acetonic and ethanolic extracts of Amorphophallus konkanensis (AKT) and Amorphophallus bulbifer (ABT). Control (●), AKT extracts: acetone (■), ethanol (▲); ABT extracts: acetone (■), ethanol (▲)

higher inhibition of growth of all organisms. The results obtained by growth pattern lent further importance to the study and supported the data obtained by screening of antibacterial activity, MIC and MBC.

HPLC analysis

HPLC analysis is the best way for chemical characterization [16,17]. The present study also established HPLC fingerprint for the active phenolic acids that can act as antibacterial, anti-inflammatory, antioxidants. Acetonic extract of both species was used for HPLC analysis that found to be relatively effective as compared to ethanolic extract. Acetonic extract of both AKT and ABT (Fig. 2) showed the presence of tannic acid, gallic acid, quercetin, p-coumaric acid. Catechin was observed only in extract of ABT.

DISCUSSION

The results of the present study are encouraging as all the tested extracts revealed antibacterial potential, although the inhibitory activity was strain specific and concentration dependent. Both AKT and ABT extracts exhibited inhibitory action against both Gram-positive and Gram-negative bacterial strains. Gram-positive bacteria showed more

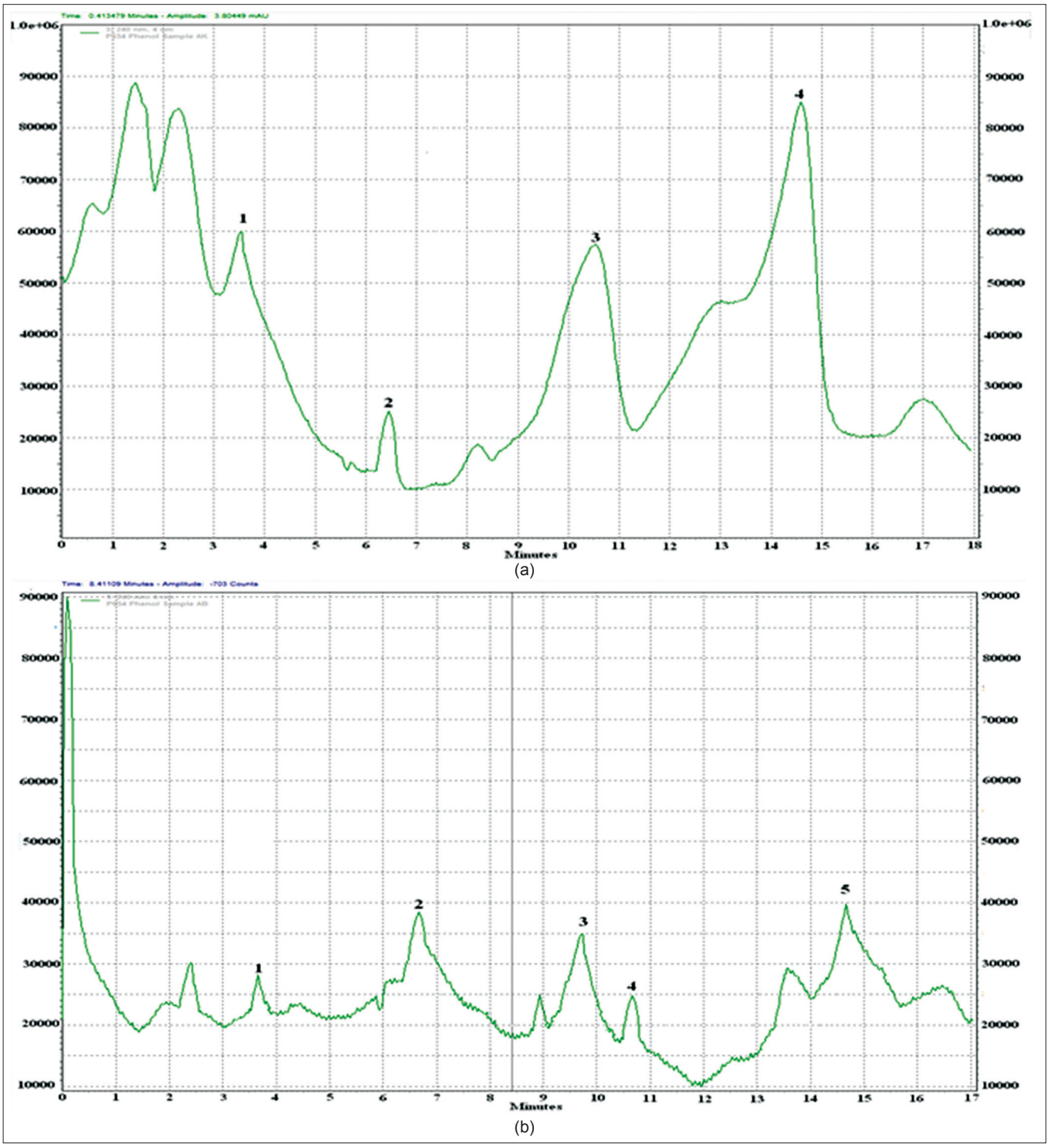

Fig. 2: High-performance liquid chromatography chromatogram for acetone extract of, (a) *Amorphophallus konkanensis*: (1) tannic acid; (2) gallic acid; (3) quercetin; (4) p-coumaric acid, (b) *Amorphophallus bulbifer*: (1) tannic acid; (2) gallic acid; (3) catechin; (4) quercetin; (5) p-coumaric acid

Table 3: MBC (µg/ml) of AKT and ABT extracts

Organisms	MBC (µg/ml)			
	AKT		ABT	
	Acetone extract	Ethanol extract	Acetone extract	Ethanol extract
B. subtilis	300	380	340	420
M. aureus	260	340	300	380
P. aeruginosa	460	500	500	540
E. coli	340	380	380	460
S. typhi	300	460	340	420
K. pneumoniae	340	420	380	460

B. subtilis: Bacillus subtilis, M. aureus: Micrococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, E. coli: Escherichia coli, S. typhi: Salmonella typhi, K. pneumoniae: Klebsiella pneumonia, AKT: *Amorphophallus konkanensis*, ABT: *Amorphophallus bulbifer*, MBC: Minimum bactericidal concentration

sensitivity. Similar results of susceptibility of Gram-positive bacteria to plant extracts have been previously reported [18-21]. It is expected that Gram-positive bacteria are more sensitive to the extracts because of the absence of the lipid layer over the cell wall which is unlike with that of Gram-negative bacteria [13,22]. In this study *M. aureus* was the most sensitive organism to the both AKT and ABT extracts. Both plant extracts are the best source to control penicillin-resistant [22] and methicillin resistant [23] *M. aureus*. The Gram-negative *P. aeruginosa* produced unexpected results, acetone extracts of AKT and ABT showed potent activity even though many studies have shown that the presence of a double membrane makes them less sensitive to extracts or drugs [24].

It has been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants [25]. Natraj *et al*. [26] investigated the presence of secondary metabolites like phenolics and flavonoides in *Amorphophallus paeonifolius*. Amblyone, a triterpenoid isolated from *A. campanulatus* had been reported to show antimicrobial activity [7]. HPLC analysis of AKT and ABT extracts determined the phenolic compounds. Many of them such as gallic acid, catechin, ferulic acid, caffeic acid, salycilic acid possess antibacterial activity [27]. The reducing property of phenolics can influence the redox potential of microbial growth causing growth inhibition [28]. All the tested bacteria had reduced growth rate in the presence of both the plant extracts. The significant results obtained in our study confirm the antibacterial potential of both the species investigated and its usefulness in the treatment of various infections.

CONCLUSION

The demonstrable antibacterial activity of the tuber extracts of the AKT and ABT implies that, these plants have a potential for preparation of alternative medicines for infections caused by microorganisms which have become resistant to the current therapeutic measures, which is an essential requirement of the present medical practice. This is more emphasized by its antibacterial properties against *M. aureus* causes wound infection, skin lesion, food poisoning and equally resistant to many of the common medicines used to treat its infections both in humans and in animals. Hospital acquired nosocomial infections like *P. aeruginosa, E. coli* are also seen to be susceptible to these extracts and this is very significant as an alternative medicine.

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