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Antibacterial Activity of *Alpinia galanga* (L) Willd Crude Extracts

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Abstract Methanol, acetone and diethyl ether extracts of *Alpinia galanga* have been evaluated against pathogens viz. *Bacillus subtilis* MTCC 2391, *Enterobacter aerogene, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli* MTCC 1563*, Klebsiella pneumoniae, Pseudomonas aeruginosa* MTCC 6642, *Salmonella typhimurium, Staphylococcus aureus* and *Streptococcus epidermis* using Agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of all the extracts were determined using the macrodilution method. Methanol extracts have shown excellent activity towards all the pathogens with MIC and MBC values ranging from 0.04–1.28 mg/ml and 0.08–2.56 mg/ml, respectively. The GC–MS analysis of methanol extracts have yielded compounds like 5-hydroxymethyl furfural (59.9%), benzyl alcohol (57.6%), 1,8 cineole (15.65%), methylcinnamate (9.4%), 3-phenyl-2-butanone (8.5%) and 1,2 benzenedicarboxylic acid (8.9%), which could be responsible for its broad spectrum activity. So, *A. galanga* can be quite resourceful for the development of new generation drugs.

Keywords *Alpinia galanga* · Antimicrobial activity · MIC · MBC · GC–MS analysis · Pathogens · Plant extracts

Introduction

Plants have been used throughout the world as drugs and remedies for various diseases since time immemorial. According to pharmacologists, developed countries are turning to the use of traditional medicinal systems, with about 1,400 herbal preparations being in use [1]. Plants are invaluable sources of pharmaceutical products that have drawn the attention of ethno-pharmacologists from around the world [2]. Many plants have been used because of their antimicrobial constituents, which are due to compounds synthesized in the

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secondary metabolism of the plant. These products are known by their active substances, e.g. the phenolic components which are part of the essential oils [3], as well as in tannins [4]. Essential oils from aromatic and medicinal plants receive particular attention as potential natural agents for food preservation due to their effectiveness against a wide range of microorganisms [5–8]. In recent years, several reports have been published concerning the composition and biological properties (antimicrobial, antioxidant, anticancer and the stimulated effect on the immune system) of Zingiberaceae extracts [9–15]. Even though a number of antimicrobials have been isolated and studied, there is a growing need for the discovery of new antimicrobials as there is an ever increasing crisis of bacterial resistance towards the existing drugs.

Alpinia galanga (L) Willd syn. *Languas galanga* commonly called greater galangal, belonging to the family Zingiberaceae is a rhizomatous herb distributed in various parts of India and throughout Southeast Asia. *A. galanga* has been used as food additive in Thailand and other countries in Asia for a long time. The rhizome is used against rheumatism, bronchial catarrh, bad breath and ulcers, whooping colds in children, throat infections and fever. 1′-Acetoxychavicol acetate, a component of *A. galanga,* was found to have very good antimicrobial activity [16, 17]. The essential oil of *A. galanga* rhizome has been found to have inhibitory activity against certain dermatophytes, filamentous fungi and yeast [18]. Suganya and Sombat have reported that higher potential in antioxidant and antimicrobial activities of *A. galanga* oil was supposed to be due to the composition of certain constituents viz. 1, 8-cineole, 4-allylphenyl acetate and β-bisabolene within the essential oil [19].

The objective of the present study is to evaluate the active components responsible for antimicrobial activity with different solvent systems like methanol, acetone and diethyl ether in different parts (root, rhizome and leaf) of *A. galanga* and to compare their activity towards the human pathogens. *A. galanga* constituents when extracted in acidic pH had pronounced activity towards the pathogens. The extracts were shown to have broad-spectrum activity towards microorganisms. The activities of all the extracts were compared and the best solvent, methanol was subjected to gas chromatography–mass spectroscopy (GC–MS) analysis.

Materials and Methods

Chemicals and Reagents

Amikacin, Mueller–Hinton agar (MHA) and Mueller–Hinton broth were supplied by Himedia, India. Methanol, acetone and diethyl ether were of analytical/HPLC grade supplied by Merck.

Plant Extracts

The plants of *A. galanga* (L) Willd were collected from AG biotek, Hyderabad. The plants were thoroughly washed and separated into three different parts, i.e. rhizome, root and leaves. They were oven dried at 60 °C for 24 h to remove the moisture and finely ground into a powder using an electric blender. Eighteen extracts were obtained with methanol, acetone and diethyl ether as the solvent systems using a soxhlet apparatus. The phytoconstituents were extracted in acidic (5.5) and neutral range. The pH was adjusted using 0.1 N HCl. The extracts were concentrated under reduced pressure using rotavapour (Heidolph Rotacool, Germany).

Bacterial Cultures

A combination of gram+ve and gram−ve pathogenic microorganisms were used for the present study. Gram positive bacteria: *Bacillus subtilis* MTCC 2391, *Enterococcus faecalis, Staphylococcus aureus* and *Streptococcus epidermis*. Gram-negative bacteria: *Enterobacter aerogene, Enterobacter cloacae, Escherichia coli* MTCC 1563*, Klebsiella pneumoniae, Pseudomonas aeruginosa* MTCC 6642 and *Salmonella typhimurium*. The MTCC cultures were obtained from IMTECH, Chandigarh and other test microorganisms were collected as clinical isolates from Global Hospitals, Hyderabad, India. All the cultures were tested for purity by standard microbiological methods. The bacterial cultures were maintained on Mueller-Hinton agar (Himedia, India) slants at 4 °C with a subculture period of 15 days. Each bacterial strain was reactivated by transferring from these stored slants into Mueller-Hinton broth (Himedia, India) and cultured overnight at 37 °C before the antimicrobial assay.

Determination of Antibacterial Activity

The Antimicrobial activity of plant extracts was investigated by the Agar well diffusion method [20]. The Mueller–Hinton agar was poured onto the Petri plates with an inoculum size of 10^6 colony forming units (cfu)/ml of bacteria. The wells were made in the MHA plates with the help of a borer, with a diameter of 8 mm. Each well was dispensed with different plant extracts at a concentration of 500 µg in the respective solvents with amikacin as the positive control. Amikacin is a broad spectrum antibiotic that acts by inhibiting protein synthesis. The concentration of amikacin used was 50 µg. The zone of inhibition around the wells was measured after 24 h of incubation at 37 °C. The sensitivity of the microbial species to the plant extracts was determined by measuring the diameter of the inhibitory zones around the wells (including the diameter of the well). All the experiments were performed in triplicate and the results embodied.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The minimum inhibitory concentration (MIC) was determined by broth dilution method [21]. Twofold serial dilutions of the crude extracts as well as the positive antibiotic control (Amikacin) were prepared in Mueller–Hinton broth [22]. A direct suspension of bacteria was prepared in 5 ml sterile distilled water from a 24-h-old suspension in Mueller–Hinton broth. The turbidity of the suspension was adjusted to match a 0.5 McFarland standard [23] which corresponds to 1.5×10^8 cfu/ml. For broth dilution tests, 50 µl of standardized suspension of bacteria was added to each tube containing crude extracts at a final concentration of 0.005–5.120 mg/ml and incubated at 37 °C. The lowest concentration that did not show any visible growth after macroscopic evaluation was considered as MIC.

After the determination of MIC, the tubes which did not show any visible growth were diluted 100-fold with drug-free Mueller–Hinton broth and incubated at 37 °C for 48 h. The lowest concentration of the tube that did not show any visible growth was considered as the minimum bactericidal concentration (MBC). The assays were performed in triplicate.

Statistical Analysis

Results calculated from triplicate data were expressed as means±standard deviations. The data was compared by least significant difference test using Statistical Analysis System (ver. 9.1).

Analysis by GC–MS

For GC–MS analysis, the samples were injected into a HP-5MS capillary column (30 m length×0.25 m i.d×0.25 μm film thickness), Agilent Technologies, USA GC–MS model, consisting of 6,890 N gas chromatograph coupled with 5973 insert Mass Selective Detector. The injector was set at 250 °C and the detector at 280 °C. The stepped temperature program was as follows: held at 50 °C for 2 min, then, from 50–280 °C at the rate of 10 °C/min, held for 5 min. The total run time was of 30 min. The GC–MS interface temperature at 280 °C. The injection volume was 1 μl. The solvent delay was 2 min and injected in a split ratio of 1:10. The MS scan range was from 35–6,000 Da. Compound identification was obtained by comparing the retention times with those of authentic compounds and the spectral data obtained from library data of corresponding compounds.

Results and Discussion

Results

The antimicrobial principles were extracted using three different solvent systems viz. methanol, acetone and diethyl ether. The phytoconstituents of leaf, rhizome and root were extracted separately. Ten different bacterial species were used to evaluate antimicrobial activity of *A. galanga* (L) Willd. The microorganisms used for the study include food borne, intestinal and respiratory pathogens viz. *S. aureus,* which can cause a host of infections from simple skin infections to the persistent types like pneumonia and endocarditis.

The extracts of all the plant parts of *A. galanga* viz. leaves, rhizomes and roots have shown good activity towards all the pathogens, the results of which are given in Table 1. The activity of plant extracts diminished with decrease in solvent polarity, i.e methanol> acetone>diethyl ether. The results clearly indicate that among the three solvents used for the study, the activity of methanol extracts at acidic pH (5.5) were excellent. The activity of acetone and diethyl ether were almost similar with acetone taking a slightly higher edge. The antimicrobial principles were extracted at acidic as well as neutral range. The activity of the plant samples extracted at acidic pH (5.5) was superior when compared to the samples at neutral pH (7.0). On further lowering the pH, the activity slowly diminished. Amikacin, a broad-spectrum aminoglycoside antibiotic was used as positive standard reference that displayed good activity towards the entire set of pathogenic microorganisms, irrespective of the gram's reaction. The respective solvents were checked for antimicrobial activity that served as the negative controls.

The inhibition zones for methanol extracts ranged from 30.72±0.30 mm (*B. subtilis*) to 14.16±0.29 mm (*P. aeruginosa*). In the case of *S. epidermis* (27.50±0.36 mm) and *S . aureus* (28.19±0.37 mm), the diameter of inhibition zone for methanol extract was equivalent to the positive antibiotic standard (28.45±0.38 mm and 29.03±0.17 mm, respectively). The inhibition zone of methanol root extract towards *S. typhimurium* (29.08± 0.13 mm) was more, compared to the inhibitory zone of the positive standard (27.74± 0.15 mm) displaying the potent broad spectrum activity of the extracts. *K. pneumoniae* and *P. aeruginosa* were less susceptible to methanol extracts but were inhibited by acetone and diethyl ether extracts. The leaf extracts of *A. galanga* in acetone were more efficient in inhibiting *K. pneumoniae* and *P. aeruginosa* with the inhibition zones of 24.59±0.06 mm and 21.89±0.32 mm, respectively. The response for plant parts was different towards each of the pathogens. In all the cases of gram+ve bacteria, rhizome was showing the best result.

In the present study, *B. subtilis* was found to be the most sensitive and *P. aeruginosa* the most resistant.

MIC and MBC Values of A. galanga Towards the Pathogens Different extracts were studied under a wide range of concentrations ranging from 0.005–5.120 mg/ml. The methanol extract was excellent in showing very low values of MIC, in the range of 0.040–0.640 mg/ml with an exception of *P. aeruginosa* showing a value of 1.28 mg/ml. The MBC values were in the range of 0.080–2.56 mg/ml. The values for methanol extracts were comparable to the positive standard reference used in the study. The acetone and ether extracts also had significant MIC and MBC values in the range of 0.16–2.56 and 0.32–>5.12 mg/ml and 0.32–2.56 and 0.32–>5.12 mg/ml respectively. The MIC and MBC results of *A. galanga* leaf, rhizome and root are given in Table 2.

GC–MS Results of A. galanga in Methanol In rhizome and leaf, 15 compounds each have been detected where as in root 21 compounds have been detected. In case of rhizome and root, 5-hydroxymethyl furfural and benzyl alcohol were present in higher quantities, i.e. 59.975% and 57.665%, respectively. Both these compounds were not detected in the leaf extract which was replaced by 1, 8-cineole in considerable amount (15.65%). Compounds like 3-phenyl-2-butanone, methyl cinnamate, phenylpropionaldehyde and 1, 2-benzene dicarboxylic acid were also present in noticeable quantities in the leaf. The difference in activities of the extracts was justified by the detection of varied phytoconstituents in different parts of the plant. The results of methanolic extract of leaf, rhizome and root (Figs. 1, 2 and 3) have been analyzed using GC–MS and tabulated (Table 3).

Discussion

The emergence of bacterial super resistant strains is a result of the currently used antibiotic agents, failing to end many bacterial infections [24]. Plants readily synthesize substances for defense against attack by insects, herbivores and microorganisms [25]. The main advantage of natural agents is that, the crude extracts contain a mixture of compounds like phenols, acids, esters, aldehydes etc., for which it is difficult to develop resistance by bacteria unlike the synthetic antibiotics that contain a single compound. Farnsworth and Bunyapraphatsara in 1992 reported that essential oils from both fresh and dried rhizomes of galangal have antimicrobial activities against bacteria, fungi, yeast and parasites [26]. In addition to the sesquiterpenes, essential oils and phenylpropanoids; *A. galanga* also contains phenols, esters, aldehydes etc. which makes it an alternative choice for developing new drug candidates.

A. galanga plant parts like the root, rhizome and leaf in methanol, acetone and diethyl ether have shown good activity towards the pathogens. Among all these methanol extract has proven to be the best in acidic pH (5.5). The activity of the extract at acidic pH has been proved by Syed et al., in a study on the activity of *Raphanus sativus* against pathogenic bacteria in varied pH range [27]. However, Soma Roy et al. (2009) have reported that during the antimicrobial evaluation of *Andrographis paniculata* extract against pathogens, the activity of the extract at neutral pH was much better when compared to that at acidic pH [28]. The root and rhizome extracts were almost similar in their activity with all the solvents, but the leaf extracts in both methanol and acetone were showing slightly higher activity against *K. pneumoniae* and *P. aeruginosa.* Whereas the ether extract of the leaf was good against *P. aeruginosa* only. In both the cases, acetone leaf extracts was much better when compared to the methanol extracts. The reduced activity of methanol extract against

Table 1 Inhibition zones of plant extract in different solvents systems.

Name of the organism	Plant parts	Diameter of inhibition zone (mm)						
		Methanol		Acetone		Diethyl ether		Antibiotic
		Acidic (5.5)	Neutral (7.0)	Acidic (5.5)	Neutral (7.0)	Acidic (5.5)	Neutral (7.0)	
Gram positive								
B. subtilis	Leaf	27.79±0.44	26.32±0.60	22.08±0.15	16.16±0.32	20.06±0.19	14.86±0.26	34.12±0.23
	Rhizome	30.72±0.30	27.10±0.20	20.93±0.25	17.14±0.32	21.06±0.18	17.44±0.45	
	Root	27.94±0.20	26.05±0.07	21.19±0.20	18.98±0.10	17.02±0.25	13.94±0.34	
E. faecalis	Leaf	24.56±0.39	21.46±0.10	20.46±0.49	18.26±0.28	17.27±0.39	14.37±0.76	32.09±0.24
	Rhizome	27.83±0.22	22.53±0.80	18.14±0.44	15.27±0.04	19.27±0.27	15.54±0.14	
	Root	25.93±0.13	22.48±0.14	19.67±0.13	14.19±0.19	20.72±0.28	18.22±0.28	
S. aureus	Leaf	22.08±0.24	18.28±0.36	16.32±0.65	15.11±0.36	14.07±0.18	10.65±0.39	29.03±0.17
	Rhizome	27.21±0.21	21.88±0.21	17.70±0.19	14.32±0.32	13.34±0.49	9.34±0.28	
	Root	28.19±0.37	20.52±0.01	14.90±0.23	12.14±0.23	11.49±0.54	9.72±0.36	
S. epidermis	Leaf	24.31±0.35	20.88±0.21	15.61±0.46	12.65±0.29	14.58±0.36	11.22±0.67	28.45±0.38
	Rhizome	27.50±0.36	22.30±0.55	19.60±0.42	15.27±0.16	16.79±0.18	12.17±0.20	
	Root	21.41±0.12	17.47±0.23	21.97±0.30	13.99±0.03	19.25±0.29	15.15±0.39	

Gram negative								
E. aerogene	Leaf	16.46±0.11	14.18±0.54	19.25±0.60	16.72±0.43	14.85±0.27	11.93±0.58	30.63±0.51
	Rhizome	20.81±0.26	18.89±0.29	15.97±0.32	11.37±0.75	16.04±0.50	13.92±0.27	
	Root	19.69±0.43	15.05±0.52	15.55±0.25	11.99±0.06	19.64±0.35	15.86±0.34	
E. cloacae	Leaf	21.01±0.28	14.73±0.47	15.89±0.38	10.93±0.53	11.84±0.38	8.91±0.32	29.86±0.47
	Rhizome	19.73±0.78	15.27±0.39	14.30±0.24	10.05±0.34	13.09±0.42	11.19±0.23	
	Root	24.16±0.34	17.22±0.34	15.19±0.21	12.24±0.29	16.14±0.25	13.21±0.19	
E. coli	Leaf	24.29±0.20	20.94±0.12	20.43±0.63	18.84±0.49	23.43±0.54	21.14±0.19	31.72±0.89
	Rhizome	29.93±0.25	27.26±0.10	25.74±0.56	19.99±0.32	20.43±0.51	19.26±0.59	
	Root	27.15±0.17	24.43±0.32	26.78±0.22	24.90±0.25	23.49±0.46	20.56±0.58	
K. pneumonia	Leaf	22.23±0.30	19.58±0.30	24.59±0.06	18.34±0.10	21.24±0.12	12.45±0.12	26.83±0.42
	Rhizome	17.19±0.32	14.48±0.21	20.23±0.09	15.40±0.21	16.46±0.13	14.51±0.17	
	Root	18.25±0.28	14.27±0.13	21.32±0.67	16.48±0.12	17.45±0.58	16.50±0.47	
P. aeruginosa	Leaf	16.75±0.43	13.84±0.25	21.89±0.32	16.02±0.30	20.66±0.19	13.14±0.28	25.92±0.64
	Rhizome	18.09±0.24	14.86±0.29	18.56±0.45	14.83±0.24	15.00±0.38	11.19±0.25	
	Root	14.16±0.29	10.36±0.41	18.92±0.39	14.02±0.21	13.03±0.17	10.24±0.42	
S. typhimurium	Leaf	25.56±0.10	21.47±0.16	17.17±0.21	15.16±0.42	14.60±0.25	10.74±0.28	27.74±0.15
	Rhizome	28.65±0.08	23.71±0.28	18.97±0.34	16.52±0.40	13.49±0.06	11.14±0.13	
	Root	29.08±0.13	25.44±0.23	21.36±0.32	18.48±0.07	16.32 ± 0.12	14.48±0.23	

Borer size is 8 mm

Table 2 The MIC AND MBC values of plant extracts towards the pathogens.

Name of the organism	Plant parts	Concentration (mg/ml)							
		Methanol		Acetone		Diethyl ether		Antibiotic	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram positive									
B. subtilis	Leaf	0.08	0.32	0.16	0.32	0.64	1.28	0.08	0.16
	Rhizome	0.04	0.16	0.16	0.64	0.32	0.64		
	Root	0.08	0.16	0.32	1.28	0.64	1.28		
E. faecalis	Leaf	0.08	0.64	0.16	0.64	0.64	2.56	0.02	0.04
	Rhizome	0.04	0.32	0.32	1.28	0.64	1.28		
	Root	0.08	0.32	0.32	1.28	0.64	1.28		
S. aureus	Leaf	0.16	0.64	0.64	1.28	0.64	2.56	0.08	0.16
	Rhizome	0.04	0.32	0.64	1.28	1.28	2.56		
	Root	0.04	0.16	2.65	>5.12	1.28	>5.12		
S. epidermis	Leaf	0.16	0.64	0.64	1.28	1.28	2.56	0.16	0.32
	Rhizome	0.04	0.16	0.32	0.64	1.28	2.56		
	Root	0.16	0.64	0.16	0.64	0.64	2.56		
Gram negative									
E. aerogene	Leaf	0.64	1.28	0.16	0.32	0.64	2.56	0.08	0.16
	Rhizome	0.16	0.32	0.16	0.64	0.64	1.28		
	Root	0.16	0.64	0.16	0.64	0.32	1.28		
E. cloacae	Leaf	0.16	0.32	1.28	2.56	2.56	>5.12	0.08	0.16
	Rhizome	0.16	0.64	2.56	5.12	1.28	5.12		
	Root	0.08	0.32	1.28	2.56	1.28	2.56		
E. coli	Leaf	0.08	0.32	0.16	0.32	0.32	0.64	0.005	0.01
	Rhizome	0.04	0.08	0.08	0.16	0.32	1.28		
	Root	0.08	0.16	0.04	0.16	0.16	0.32		
K. pneumonia	Leaf	0.16	0.64	0.16	0.32	0.64	1.28	0.08	0.32
	Rhizome	0.32	1.28	0.32	1.28	0.64	1.28		
	Root	0.32	0.64	0.32	1.28	0.32	0.64		
P. aeruginosa	Leaf	0.64	2.56	0.16	0.64	0.32	1.28	0.04	0.08
	Rhizome	0.64	2.56	0.32	1.28	1.28	2.56		
	Root	1.28	2.56	0.32	1.28	2.56	>5.12		
S. typhimurium	Leaf	0.08	0.64	0.64	2.56	1.28	>5.12	0.08	1.28
	Rhizome	0.04	0.16	0.64	1.28	1.28	>5.12		
	Root	0.04	0.08	0.32	1.28	0.64	2.56		

the above microbes could be due to the permeability of the compounds and resistance mechanisms displayed by the organisms towards the extracts. The outer membrane of the gram-negative bacterial cell wall appears to act as a barrier to many substances including antibiotics [29].

The MIC and MBC values of the extract even though in a crude form and at higher concentration (with reference to the standard), were comparable to that of the standard antibiotic. In case of *E. cloacae*, *E. aerogene* and *S. epidermis* the MIC and MBC values

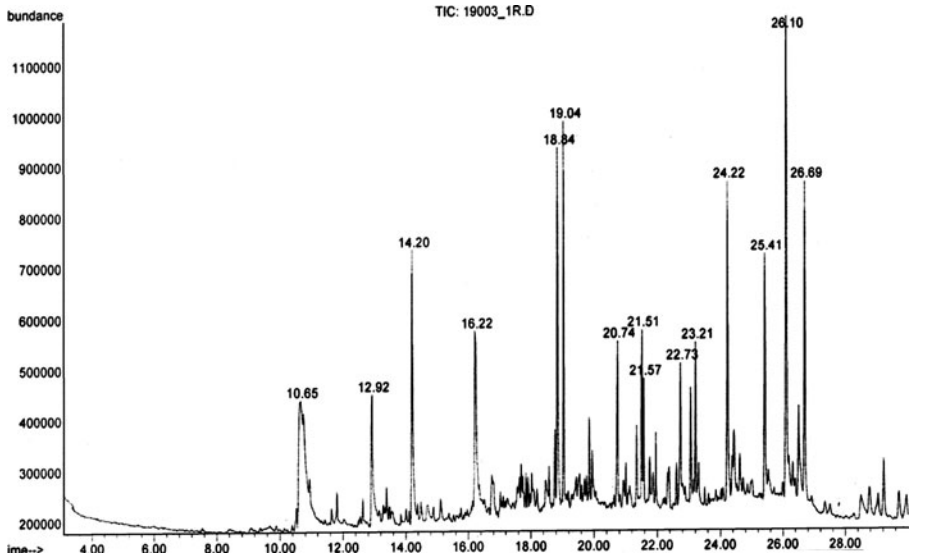

Fig. 1 Chromatogram of methanol leaf extract of *A. galanga*

were similar to the standard. Whereas the values for *B. subtilis*, *S. aureus* and *S. typhimurium* were lower than the standard, making it an excellent choice for the development of new drugs. *Alpinia* methanol root extract has the advantage of being completely microbiocidal at lower concentrations, i.e as low as 0.080 mg/ml for *S. typhimurium*. This is quite contrary to the report given by Voravuthikunchai et al. in 2006, that methanol extract of *A. galanga* did not show any activity. Also the MIC and MBC values of *A. galanga* in $CHCl_3$ against most of the clinical isolates were 0.19 and 1.57 mg/ml,

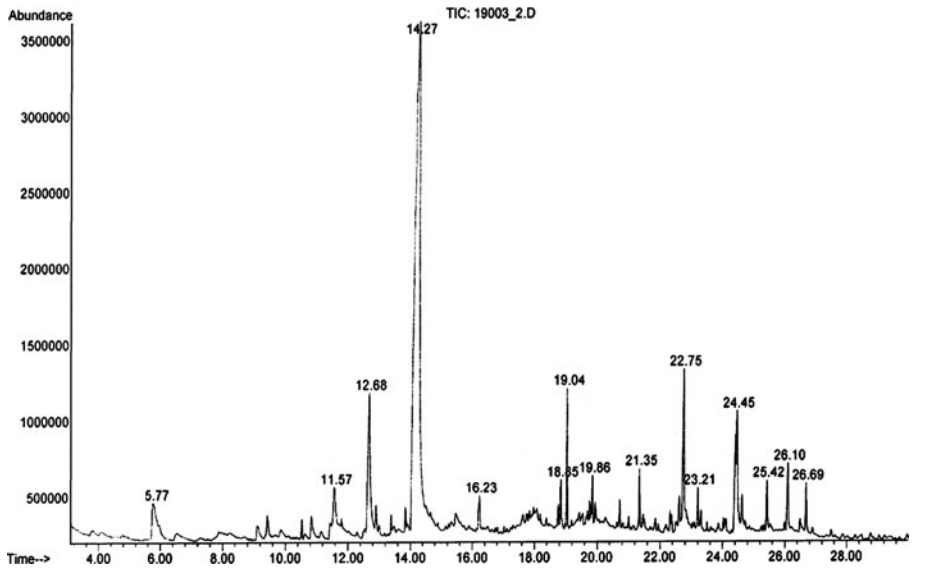

Fig. 2 Chromatogram of methanol rhizome extract of *A. galanga*

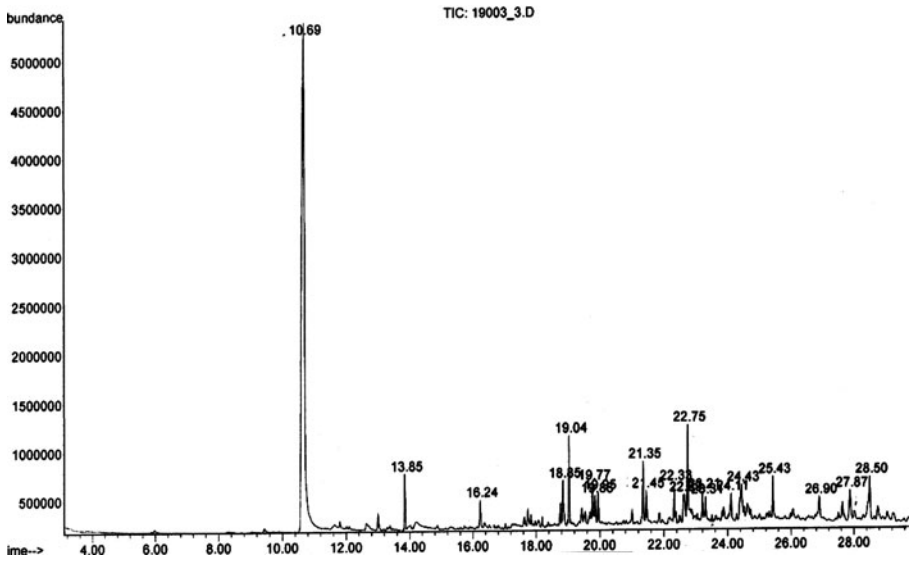

Fig. 3 Chromatogram of methanol root extract of *A. galanga*

respectively; which is less when compared to the present value of 0.08 and 0.64 mg/ml [30]. Even though the acetone and diethyl ether extracts have shown considerable activity, in few cases the MBC values were >5.12 mg/ml. In an earlier report, galangal rhizome oil could not inhibit the growth of *P. aeruginosa* due to a failure of outer membrane penetration [31]. But in the present study, the leaf extracts were successful in inhibiting *K. pneumoniae* and *P. aeruginosa*. A related possibility is that terpenes, synergise the effects of other compounds by acting as solvents to facilitate their passage through membranes. The terpenoids and phenolic compounds in pure form demonstrate high antibacterial activity [32]. The lipophilic nature of terpenes, suggests that their principle targets are the cell membranes and their toxicity is caused by loss of chemiosmotic control [33, 34]. According to the earlier study by Jirawan Oonmetta-aree in 2006, the MBC value of *Alpinia* ethanol extract against *S. aureus* was 1.3 mg/ml and chloroform extract was 0.256 mg/ml as reported by Voravuthikunchai et al in 2005, in contrast to the present report of 0.16 mg/ml for methanol root extract, which is 12.5 folds and 1.5 folds higher respectively [16, 35]. This clearly indicates the potential of *A. galanga* extract as a good source of new age antimicrobials.

The GC–MS results of the methanol extract have furnished an array of compounds in leaf, rhizome and root. The major constituents identified in *Alpinia* leaf are 1,8-cineole, phenylpropionaldehyde and methylcinnamate among many others which could be responsible for the antimicrobial activity. The activities of 1,8-cineole, phenylpropionaldehyde and methylcinnamate have already been analyzed in other plant species [36–38]. In case of rhizome, 5-hydroxymethyl furfural is the major constituent (59.9%), which could be responsible for its activity. The activity was confirmed by Fausto Rivero et al. in *Vitis vinifera* against selected oral pathogens [39]. Whereas in root, the major phytochemical identified was benzyl alcohol (57.6), whose antimicrobial activity has been confirmed by Zheng-Zhu. et al. in 2006 [40].The Directorate General of the European commission for health and consumer protection has recommended the use of benzyl alcohol in food preservation and also as flavouring agent.

Table 3 GC-MS results of *A. galanga* in methanol.

S.No	Rt (min)	Name of the Compound	Percent of the Total		
			Leaf	Rhizome	Root
1	5.77	Furfuraldehyde	–	4.595	–
2	10.65	Benzenemethanol	4.674	–	–
3	10.69	BenzylAlcohol	–	–	57.665
4	11.57	Unknown	–	2.982	–
5	12.68	2,3-Dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one	–	7.441	–
6	12.92	3-Phenylpropionaldehyde	5.237	–	–
7	13.85	β-Fenchyl Acetate	–	–	1.704
8	14.20	3-Phenyl-2-Butanone	8.517	–	–
9	14.27	5-Hydroxymethyl furfural	–	59.975	–
10	16.22	Methyl Cinnamate	9.423	1.235	–
11	16.24	5-Tetradecene	–	–	1.154
12	18.84	1,2-Benzene dicarboxylic acid	8.932	1.443	2.361
13	19.04	γ-Cadinene	6.604	–	–
14	19.04	Carotol	–	2.503	2.825
15	19.77	Unknown	–	–	1.830
16	19.86	Unknown	–	1.213	1.258
17	19.95	Unknown	–	–	1.168
18	20.74	Unknown	4.609	–	–
19	21.35	Unknown	–	1.169	2.362
20	21.45	Unknown	–	–	1.595
21	21.51	Neophytadiene	3.753	–	–
22	21.57	Hexahydrofarnesyl acetone	2.235	–	–
23	22.33	Unknown	–	–	1.831
24	22.63	Unknown	–	–	2.230
25	22.74	Hexadecanoic acid	3.693	5.500	4.785
26	23.21	Unknown	3.252	0.849	1.576
27	23.34	Unknown	–	–	1.474
28	24.11	Unknown	–	–	1.926
29	24.22	2,6,10-Trimethyl, 14-Ethylene-14-Pentadecene	8.637	–	–
30	24.44	9-Octadecenoic acid	–	6.454	3.766
31	25.41	Unknown	6.234	1.301	–
32	25.43	Unknown	–	–	1.537
33	26.10	1,8-Cineole	15.650	1.963	–
34	26.69	Unknown	8.550	1.376	–
35	26.90	Unknown	–	–	1.383
36	27.87	Unknown	–	–	2.042
37	28.50	Unknown	–	–	3.538

Rt Retention time in minutes

Developing countries are paying increased attention to food safety, because of growing recognition of its potential impact on public health, food security, and trade competitiveness. Increasing scientific understanding of the public health consequences of unsafe food, amplified by the rapid global transmission of information regarding the public health threats associated with food-borne and zoonotic diseases (e.g. *E. coli* and *Salmonella*) [41, 42]. In India, it is estimated that 20% of deaths among children under five are caused by diarrheal disease [43]. The presence of high amount of benzyl alcohol has been reported for the first time in this particular Indian variety, which can be used as food preservative in view of increasing the shelf life of foods as well as in the control of food borne diseases. Previously, the rhizome and root parts were studied, as they were used since long in ayurveda and unani medicines. In the present study, in addition to the root and rhizome, antimicrobial activity of the leaf towards various pathogens has been reported for the first time which could be a source for alternative antimicrobials.

Conclusion

Even though, pharmaceutical industries have produced a number of new antimicrobial drugs in the last few years, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents [44]. The use of phytochemicals as natural antimicrobial agents commonly called 'biocides' is gaining popularity. Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth [45, 46]. The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs. Many plant extracts owe their potency to the presence of substances like tannins, phenolic compounds and so on. *A. galanga* represents a potent antimicrobial system for the development of natural drugs, as the whole plant can be used. Though in a crude form, the plant extracts have displayed broad spectrum activity towards the microorganisms. It contains a mixture of compounds like phenylpropanoids, monoterpenes, sesquiterpenes and essential oils that help in checking the antibiotic resistance. *A. galanga* can be used for preservation of foods, as it possesses characteristic flavour as well as antimicrobial activity.

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