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EVALUATION OF ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF THE LEAF EXTRACTS OF *ALPINIA GALANGA* (L.) WILLD.

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The antibacterial activity of *Alpinia galanga* (L.) Willd. leaf extracts prepared from methanol,acetone, chloroform, ethanol, petroleum ether extracts of *Alpinia galanga* were checked against pathogens isolated from wound isolates like burn, accident, skin, abscess, trauma etc *viz. Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus* and *Streptococcus* sps, *Proteus* sps, *Staph aureus,* using Agar well diffusion method. Acetone extracts have shown excellent anti bacterial activity towards all the pathogens with plant extract ranging from 5-25mg/ml and size of zone (mm) ranged 8-26 mm (12.5 mg) concentration and 8-35 mm (25 mg), respectively. The ethanolic extract contained the highest concentrations of total phenolic compounds (92.40 ± 0.83(mg TAE/g) and flavonoids (13.78 mg TAE/g) and Tannins 48.80 ± 0.83(mg TAE/g). The LC-MS analysis of methanol extracts have yielded compound like 1-acetoxy chavicol Acetate which could be responsible for its broad spectrum activity. So, *A. galanga* can be quite resourceful for the development of new generation drugs.

Keywords : 1´-acetoxycavichol acetate; *Alpinia galanga;* Antibacterial activity; Disc diffusion; Galangal extract; LC - MS, Phenolic compounds.

Introduction

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 south east Asia. Most of the south Indian physicians of traditional Ayurveda and Siddha medicine system use *Alpinia galanga* to treat various kinds of disease including diabetes mellitus. *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, 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[1]. The essential oil of *A. galanga* rhizome has been found to have inhibitory activity against certain dermatophytes, filamentous fungi and yeast[2]. Vudhakul *et al.*[3] have reported that the 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.

The essential oils of the leaves, stems, rhizomes and roots of the medicinal plant *A. galanga* from south India were investigated by LC-MS. The major constituents of the leaf oils from the same locations were: alpha-pinene (6.6% and 6.3%, respectively), camphene (5.0% and 5.1%, respectively), 3-pinene (21.5% and 23.5%, respectively), 1, 8-cineole (34.4% and 30.7%, respectively) and camphor (7.8% and 12.8%, respectively). Natural antioxidants from plants have attracted significant interest because of their safety and potential nutritional and therapeutic effects.

Several plant materials have been investigated as a potent source of antioxidants. Antioxidants in herbs and spices include: vitamins; phenolic compounds including flavonoids and phenolic acids; tannins and volatile compounds. In the present study antibacterial activities of the crude leaf extracts of *Alpinia galanga* were investigated for the aim of discovering the medicinal potential of the plant against various microorganisms.

Material and Method

Plant Material: The fresh plants of *Alpinia galanga* were collected and taxonomical identification of the plant was

Table 1. Antibacterial activity of *Alpinia galanga* leaf extract.

Extract/Organism	Zone of inhibition (mm in diameter) 25 mg/ml					
	E. coli	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*	*Proteus* sps	*Strept pyogenes*
Acetone	35	15	30	15	11	12
Chloroform	11	12	11	17	15	12
Ethanol	-	26	-	-	-	-
Methanol	-	-	-	-	-	-
Petroleum ether	-	-	-	11	-	12
Ampicillin	28	25	25	17	19	15

Table 2. Preliminary phytochemical screening.

Phytoconstituent	Name of test	Presence/ Absence
Alkaloids	Wagner's test	+
Flavonoids	Lead acetate test	+
	Shinoda test	+
Saponins	Honey comb test	+
	Foam test	+
	Lead acetate test	+
Phenols & Tannins	Ferric chloride test	-
	Sodium hydroxide test	-
Carbohydrates	Fehling's test	+
	Benedict's test	+
Protein & Aminoacids	Biuret test	+
	Ninhydrin test	-
Steroids & Sterols	Salkowshi's test	+
Glycosides	Glycoside test	+

Note: "+"-Presence of compounds "-" - Absence of compounds

Table 3. Total phenolics, tannin and flavonoid content.

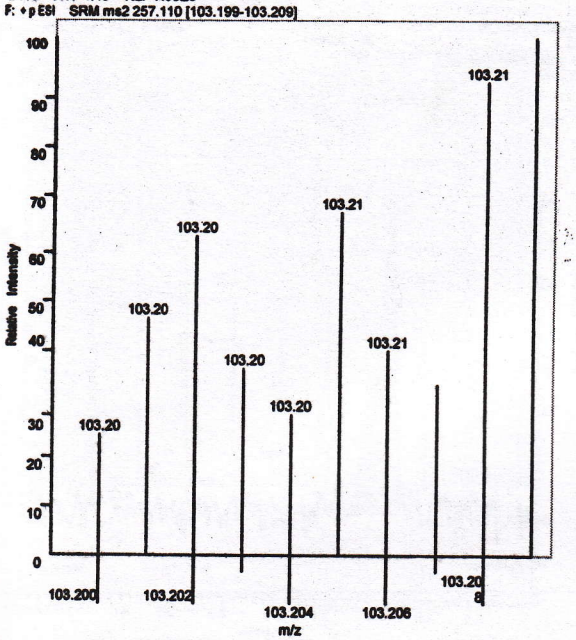
Constituents	Content
Total Phenolics (mg TAE/g extract)	92.40 ± 0.83
Flavonoid (mg RE/g extract)	1.17 ± 0.03
Total Tannin (mg TAE/g extract)	48.80 ± 0.83

Values are means of three independent analyses of the extract ± standard deviation (n = 3).

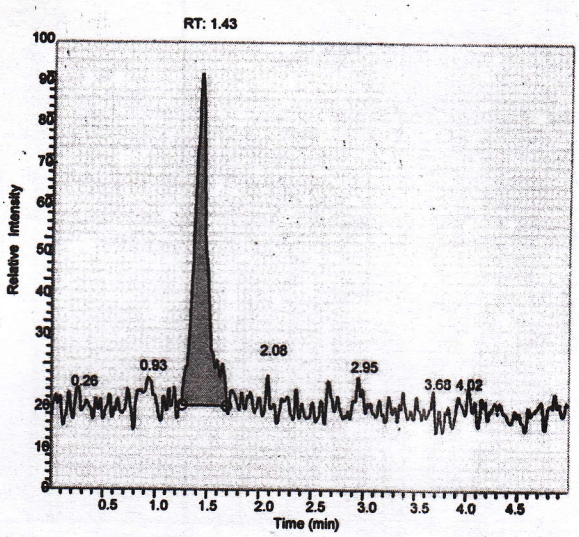
TAE - Tannic acid equivalent; RE - Rutin equivalent

Component Name: 1-acetoxy chavicol acetate

#410 RT: 1.43 NL: 7.53E3
F: + p ESI SRM ms2 257.110 [103.199-103.209]



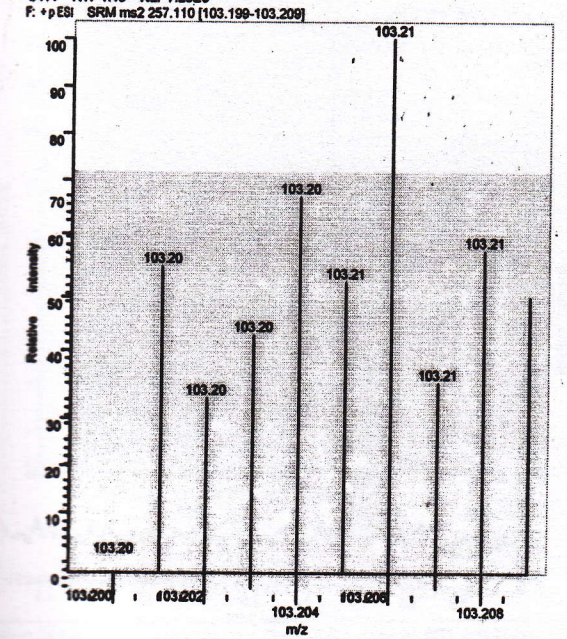
329ppb-REP-1 - TIC RT: 0.00 - 5.00 NL: 3.37E4
F: + p ESI SRM ms2 257.110 [103.199-103.209]



File Name	Sample Type	Acq Date	Sample ID	Component Name	Area	Specified Conc	Calculated Conc	% Diff
329ppb-REP-1	Unknown	Tuesday, December 06, 2011 8:45:56 PM	1-acetoxy	1-acetoxy chavicol	236274	N/A	150.470	N/A

Component Name: 1-acetoxy chavicol acetate

#411 RT: 1.43 NL: 7.26E3
F: + p ESI SRM ms2 257.110 [103.199-103.209]



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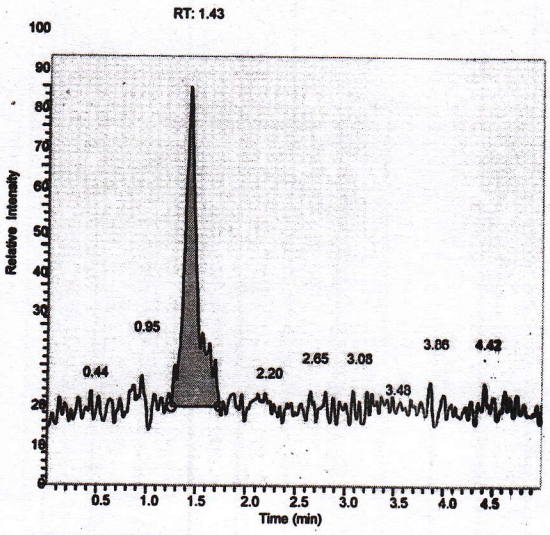
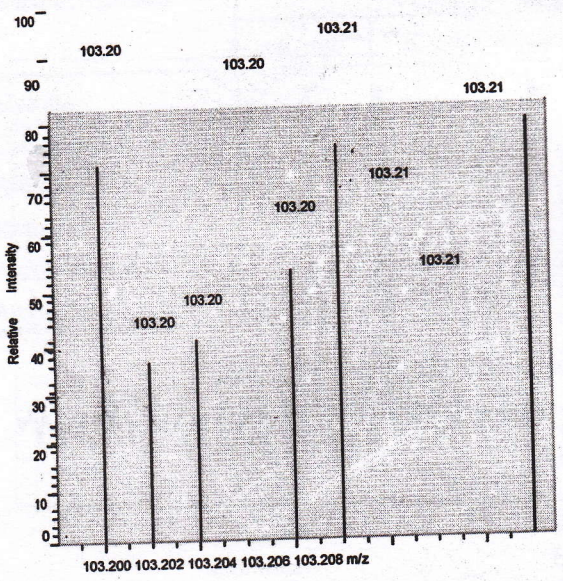


File Name	Sample Type	Acq Date	Sample ID	Component Name	Area	Specified Conc	Calculated Conc	% Diff
329ppb-REP-2	Standard	Tuesday, December 06, 2011 8:51:47 PM	1-acetoxy chavicol acetate	1-acetoxy chavicol acetate	238101	156.000	161.083	-2.63

Component Name: 1-acetoxy chavicol acetate

#411 RT: 1.43 NL: 3.91E3
F: + p ESI SRM ms2 257.110 [103.199-103.209]



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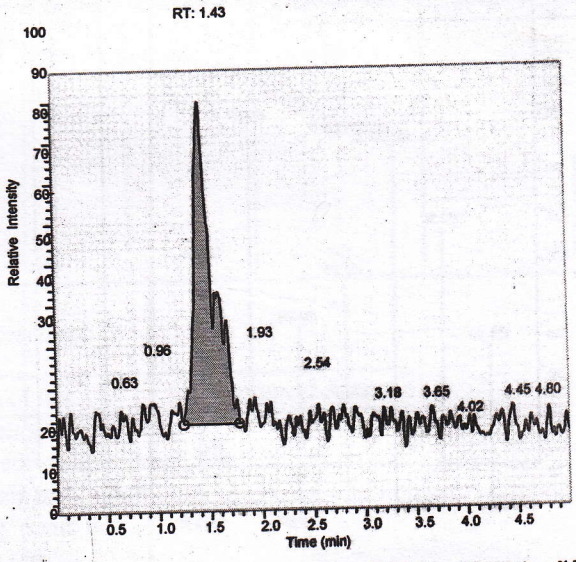
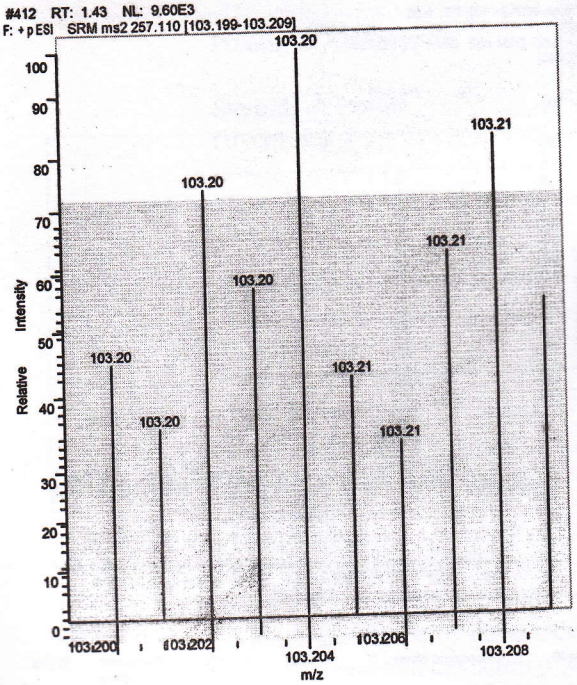
F: + p ESI SRM ms2 257.110 [103.199-103.209]



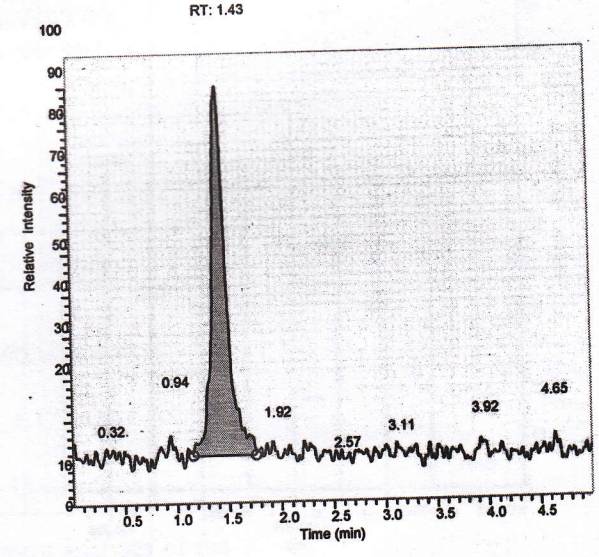
File Name	Sample Type	Acq Date	Sample ID	Component Name	Area	Specified Conc	Calculated Conc	% Diff
329ppb-REP-3	Unknown	Tuesday, December 06, 2011 8:57:37 PM	1-acetoxy chavicol acetate	1-acetoxy chavicol acetate	282233	N/A	186.260	N/A

Component Name: 1-acetoxy chavicol acetate

#412 RT: 1.43 NL: 9.60E3
F: + p ESI SRM ms2 257.110 [103.199-103.209]



658ppb-REP-1 - TIC RT: 0.00 - 5.00 NL: 5.15E4
F: + p ESI SRM ms2 257.110 [103.199-103.209]



File Name	Sample Type	Acq Date	Sample ID	Component Name	Area	Specified Conc	Calculated Conc	% Diff
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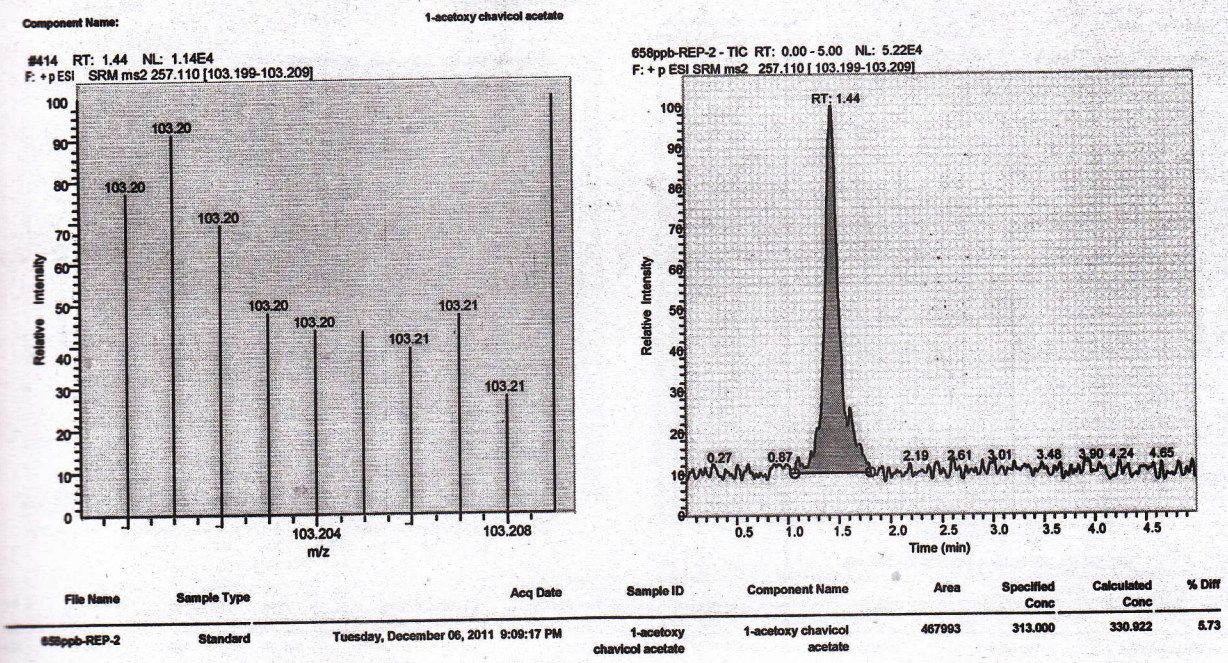
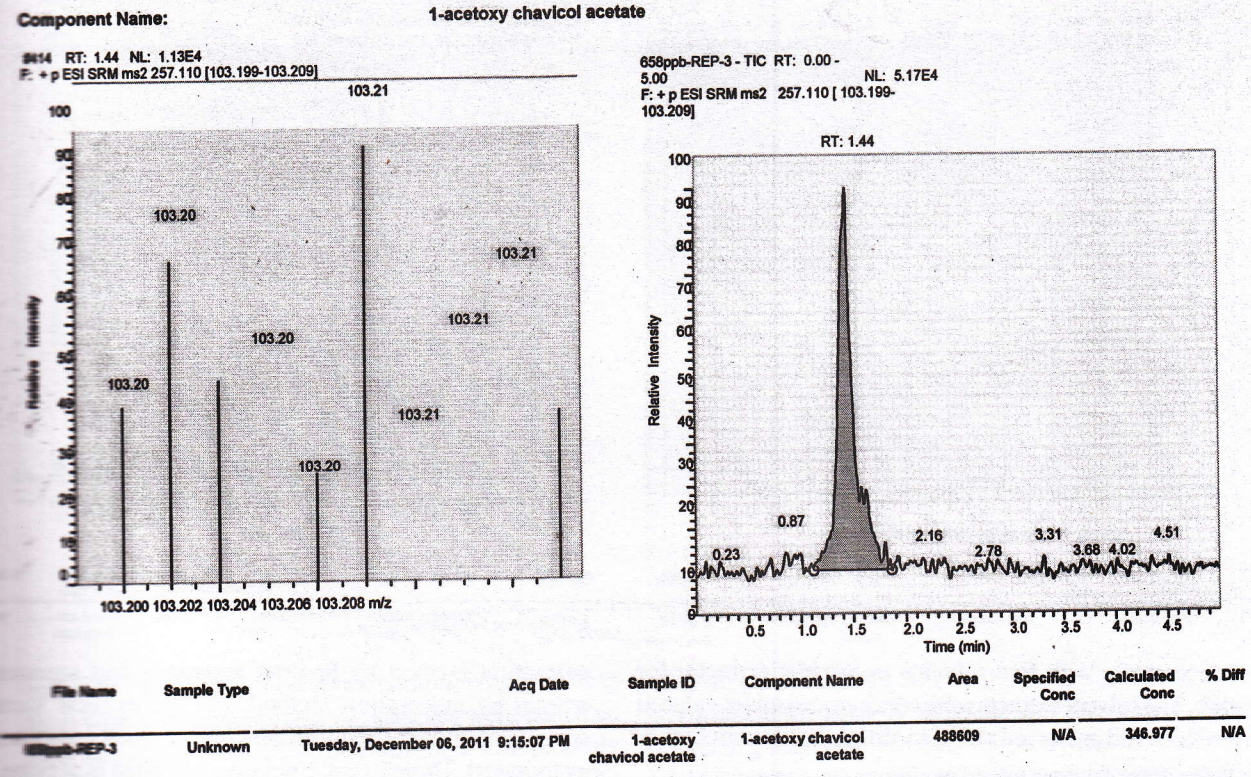
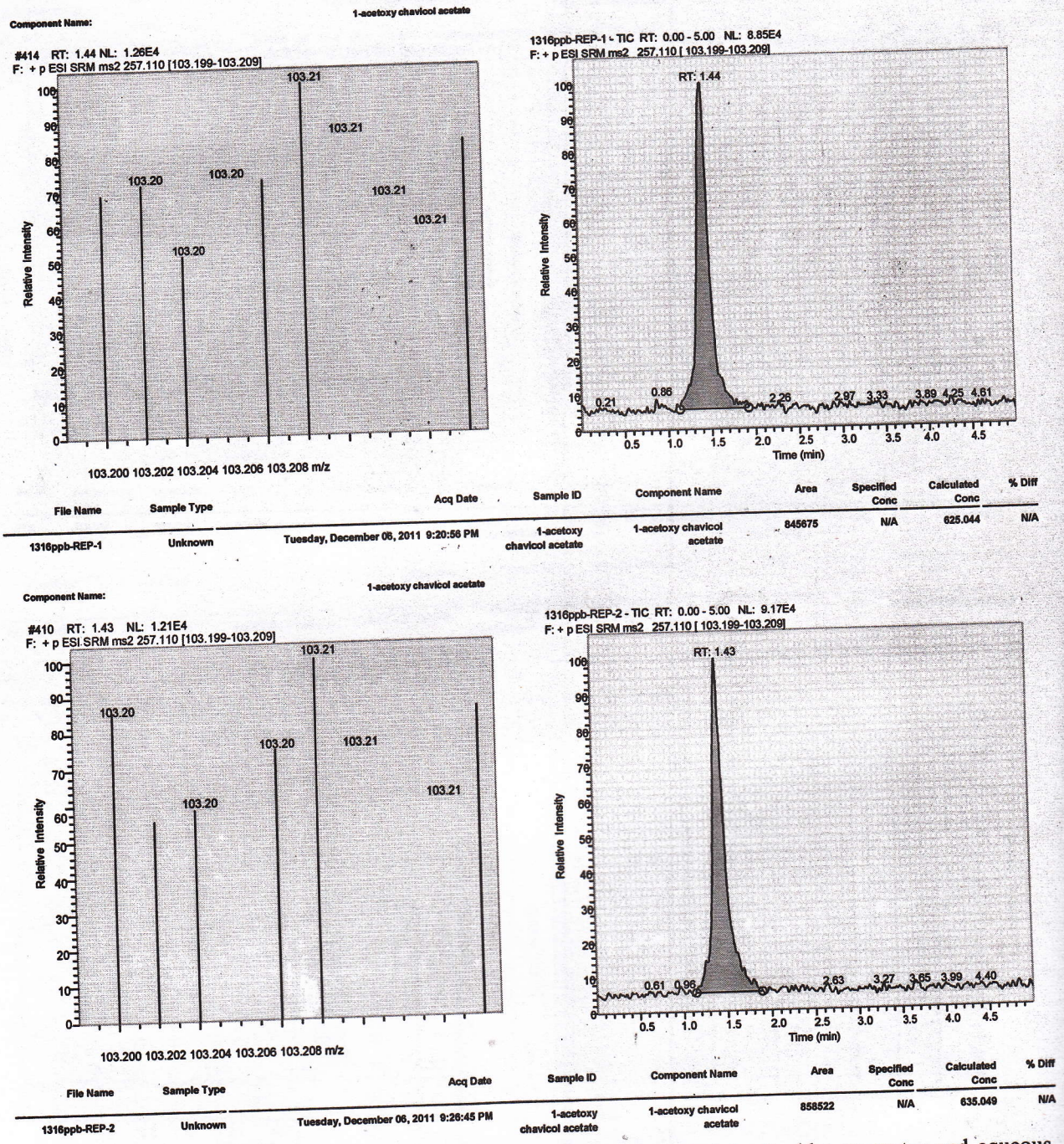




confirmed by Department of Biotechnology, G.K.V.K, Bangalore. The collected plants were washed with running water, dried, homogenised to a fine powder and stored in air tight bottles at 4°C.

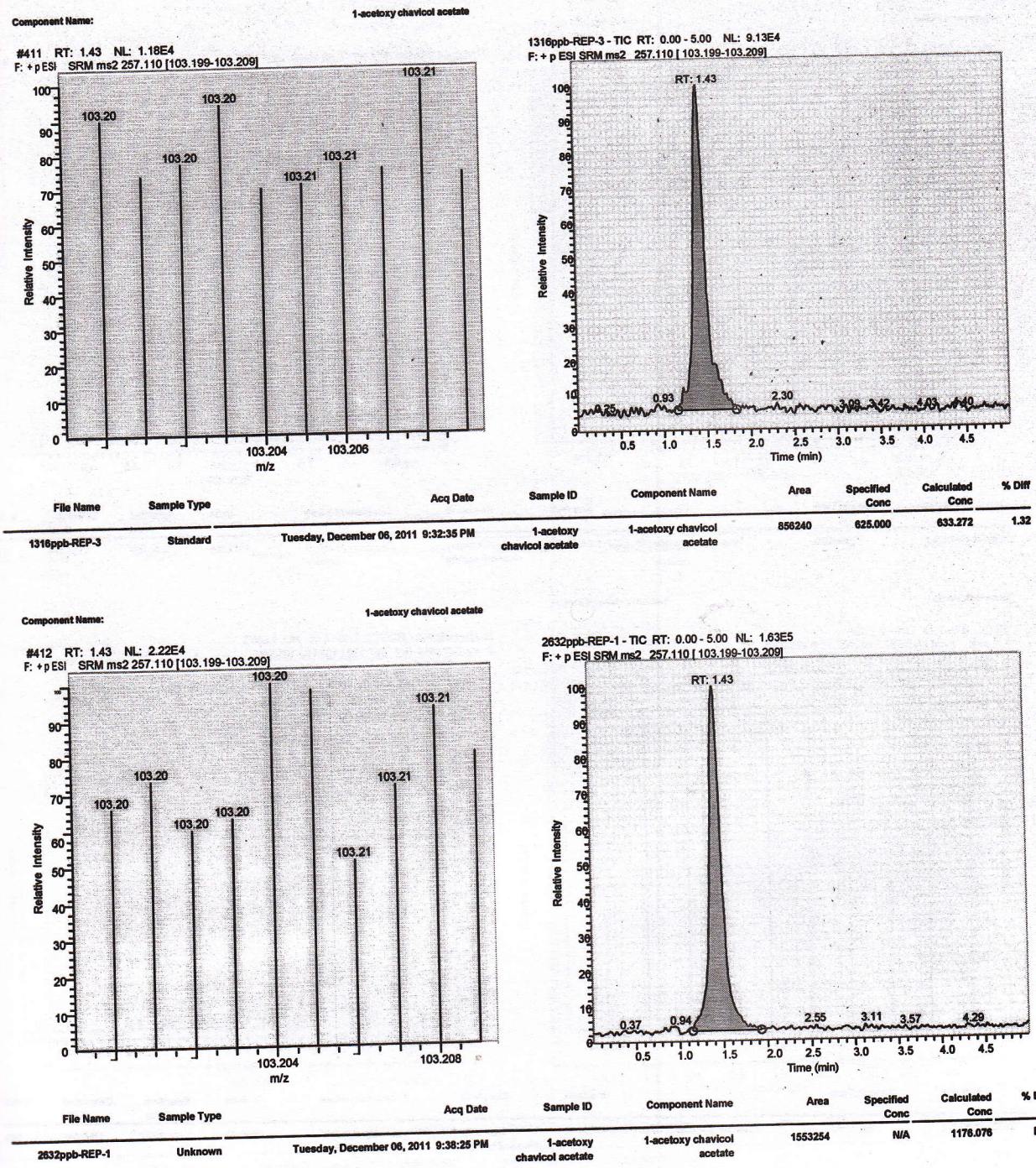
Preparation of Plant Extract: Thoroughly washed leaves of *A. galanga* were dried in shade for five days and then powdered with the help of Warring blender. 25 g of shade-dried powder was filled in the thimble and extracted



successively with five solvents in Soxhlet extractor for 48h. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use.

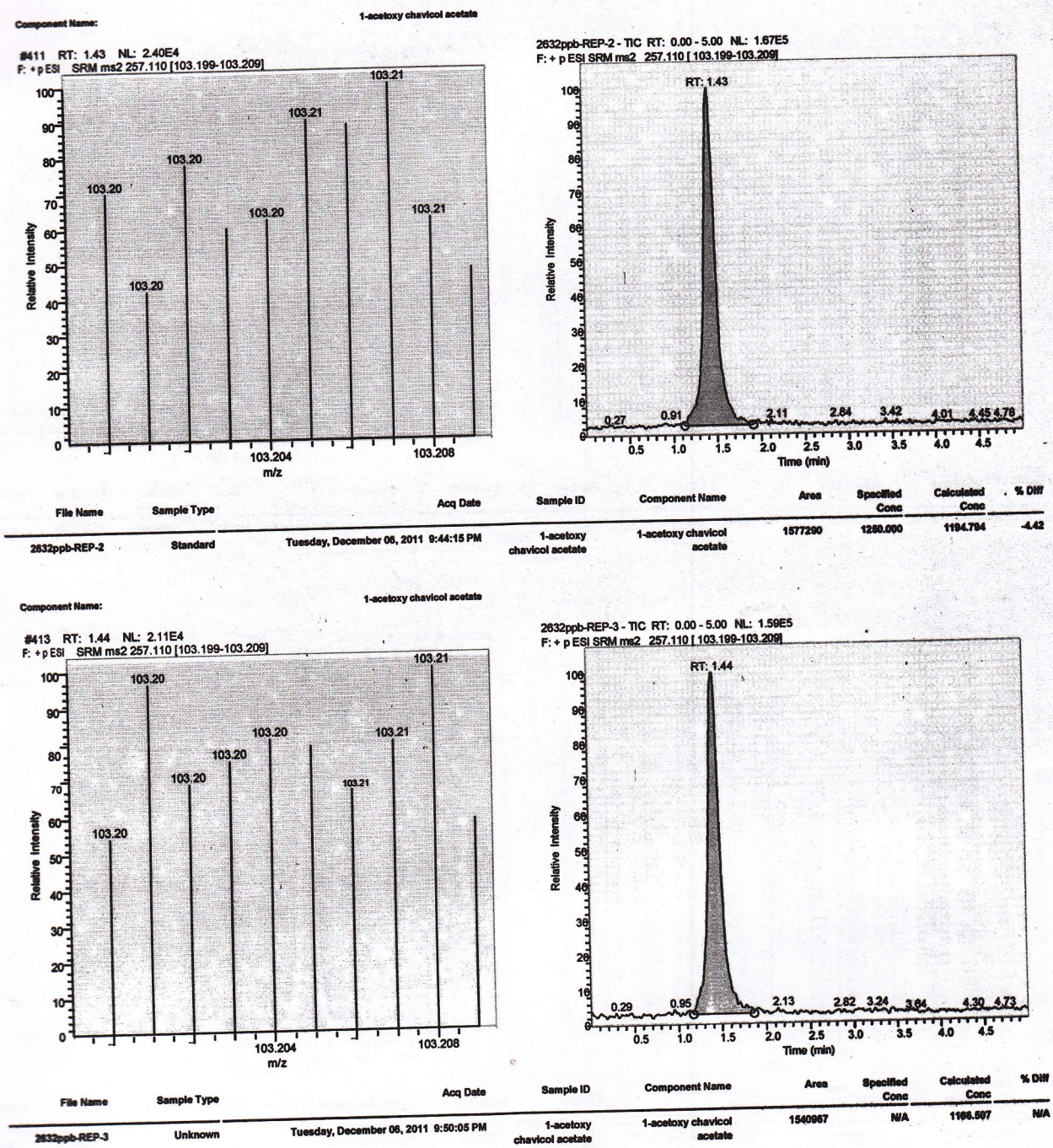
For the preparation of various extracts from the *A. galanga* were shade dried at room temperature and powdered by mixer. Petroleum ether, acetone, chloroform, ethanol and methanol extract were obtained by successive extraction method by Soxhlet apparatus and aqueous extract by maceration method. All the extracts were concentrated under reduced pressure by rotary vacuum evaporator[4]. These extracts were resuspended in acetone, chloroform, ethanol, methanol and petroleum ether to yield 100 mg residue 100 ml solvent[5].

Collection of wound pus samples: A totally 35 pus swabs were obtained from wound sites . The specimen was



collected on sterile cotton swab without contaminating them with skin commensals. All samples were collected from in around Namakkal area hospitals and properly labelled indicating the source and age of patients. The samples were transported to the laboratory soon after being obtained. In the laboratory, the specimens were registered and swabs were cultured on nutrient broth and incubated at 37°C for 24 hrs.

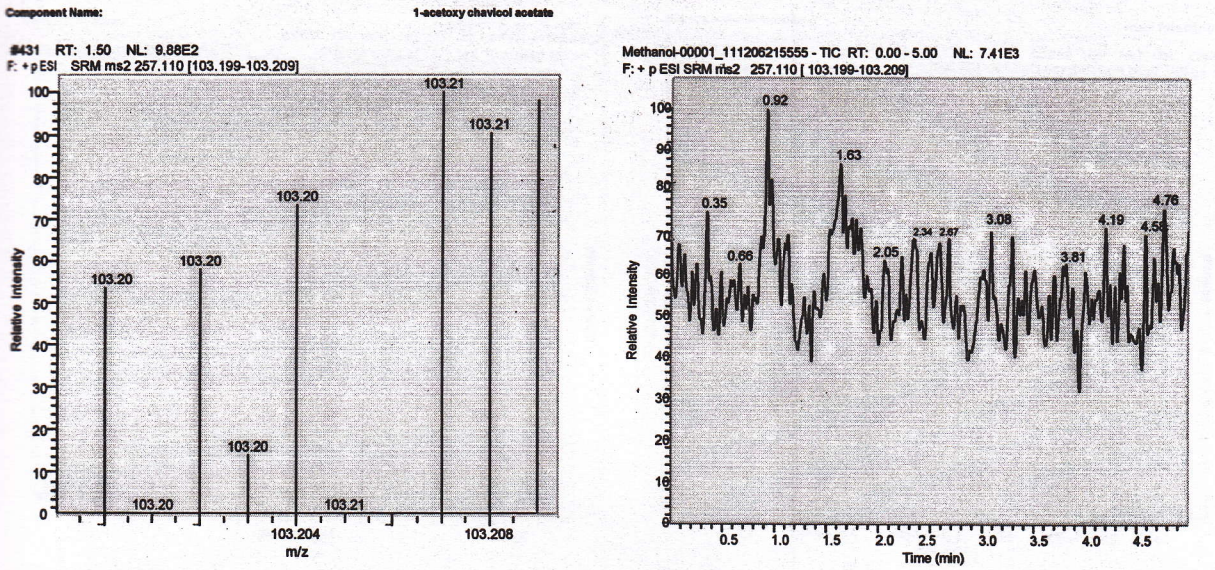
Antibacterial activity of plant extract against wound infecting bacteria : Antibacterial activities of crude plant leaf extracts were examined by the well diffusion method. Each plant extract was dissolved in respective solvents such as acetone, chloroform, ethanol, hexane, and petroleum ether, tested and they were evaluated for each

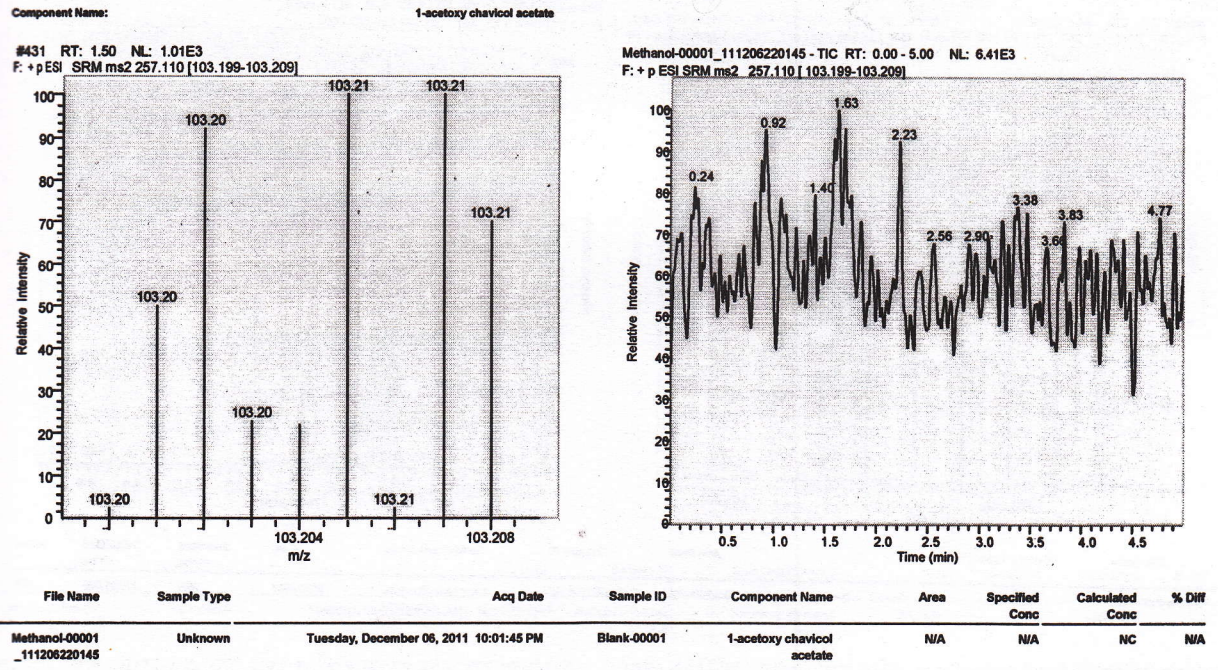


bacterium. The antibacterial activity of the plant extract was determined by measuring the diameter of the inhibition zone.

Experimental procedure

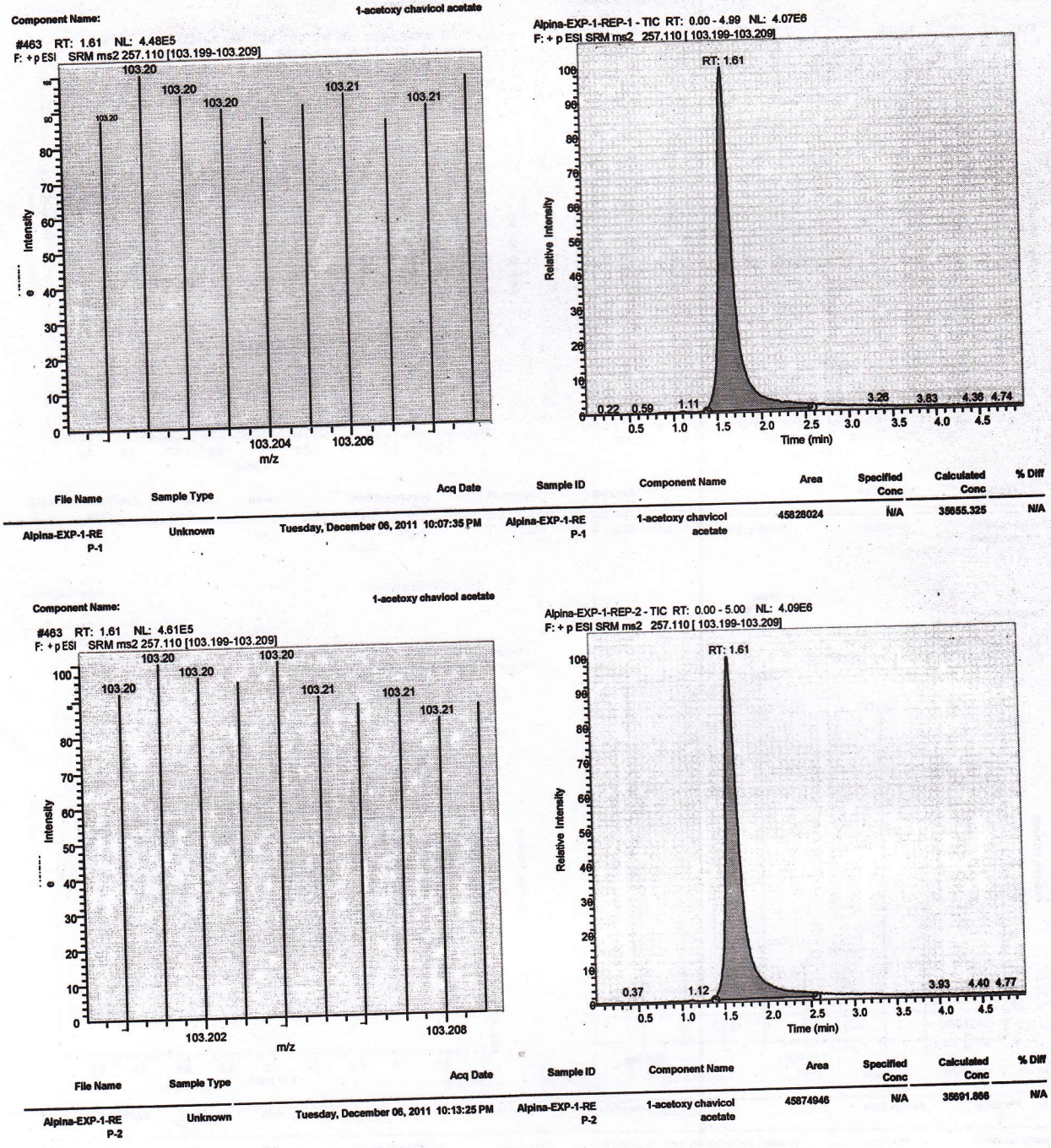
Well diffusion method (zone of inhibition)- The plant material extracts were tested for antimicrobial activity by the well diffusion method[6]. This method depends on the diffusion of the various extracts from a cavity through the solidified agar layer of Petri dish to an extract such that growth of the added microorganism is prevented entirely in circular area or zone around the cavity containing the extracts[7]. Using micropipette, 0.5 ml of each of the leaf broth containing 10^{-5}-10^{-6} cfu/ml test organisms were incubated on the four plates of solidified





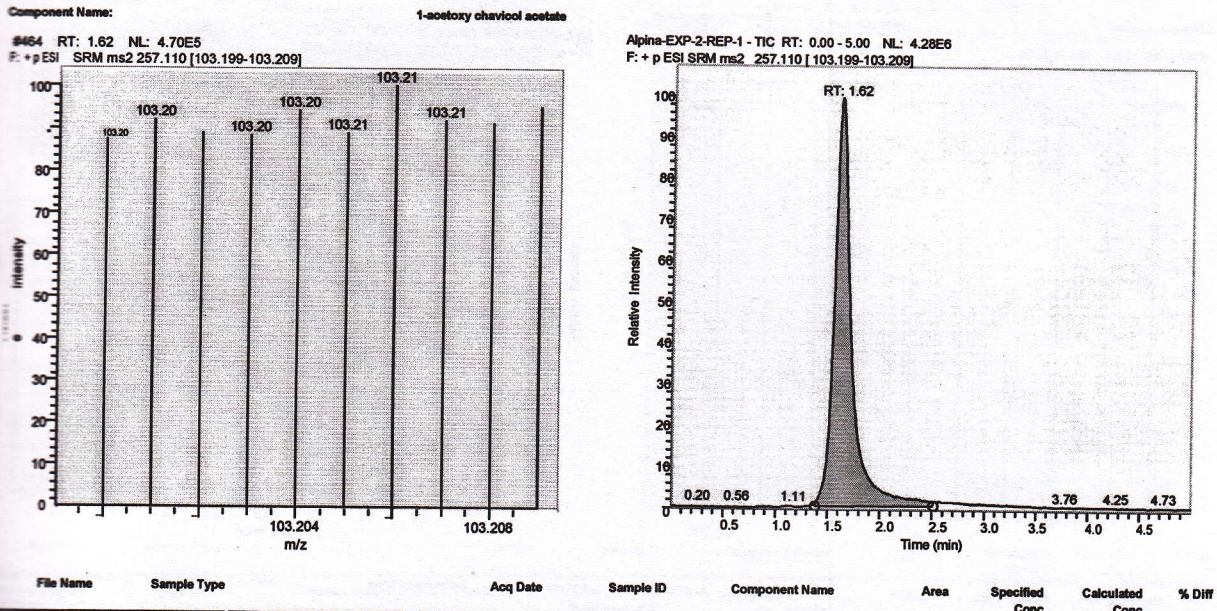
agar and spreaded uniformly with a glass spreader. Then four well were cut out in the agar layer of each plate with an aluminium bore of 5 mm diameter to contain 0.5 ml extract, standard drug. All the work was carried out in freeze for one day. After addition to allow diffusion of the solution in to the medium and then incubated for 37°C for 24 hours for antibacterial activity. After the incubation period the mean diameter of the zone of inhibition in mm obtained around the well was measured. Ampicillin was used as standard drug for antibacterial activity.

Phytochemical screening of Alpinia galanga leaf extract : The leaf extract of *A. galanga,* were analyzed for the

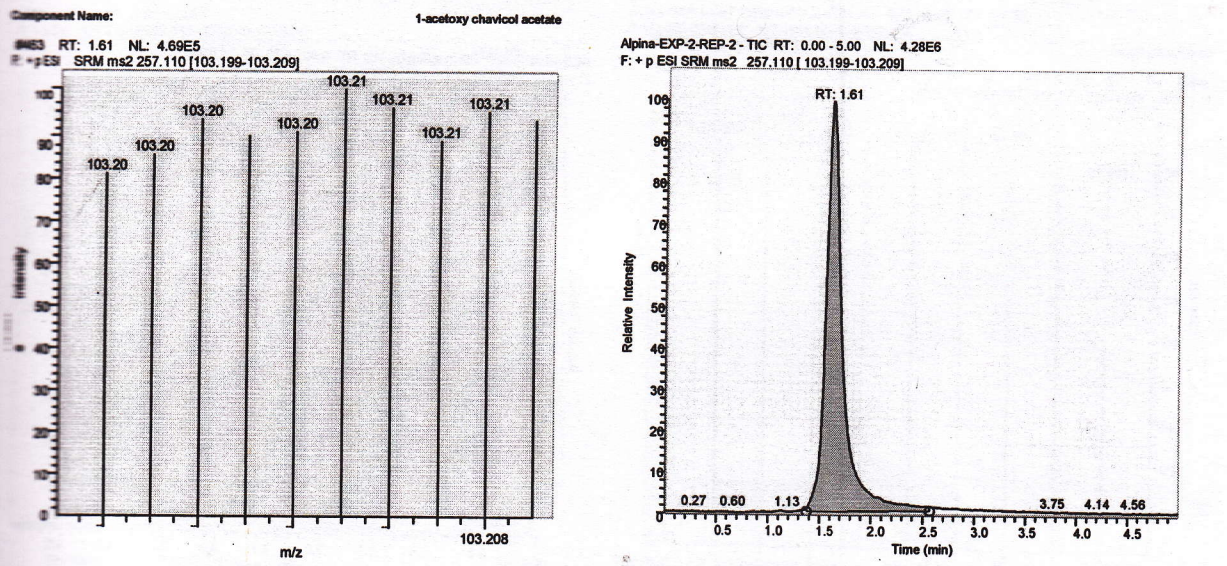


presence of saponins, phenolic compounds, alkaloids, flavonoids, glycosides, starch, phenols and tannins, carbohydrates, proteins and amino acids, steroids and sterols (Table 2).

Determination of total phenolics and tannins- The total phenolic content was determined according to the method described by Siddhuraju and Becker[8]. Ten microlitre aliquots of the extracts (10mg/2ml) were taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing

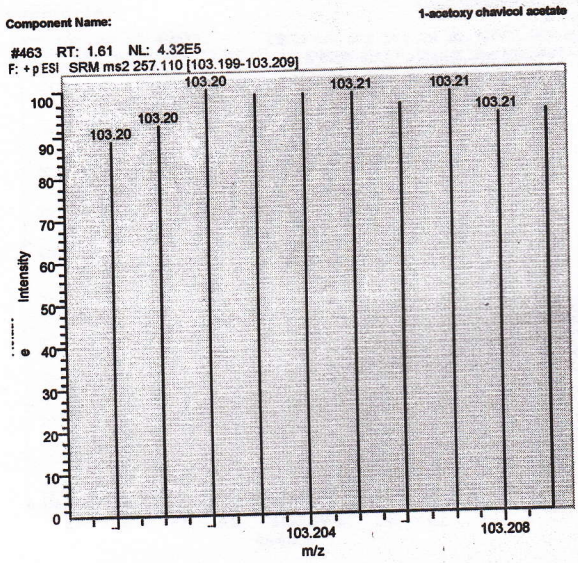


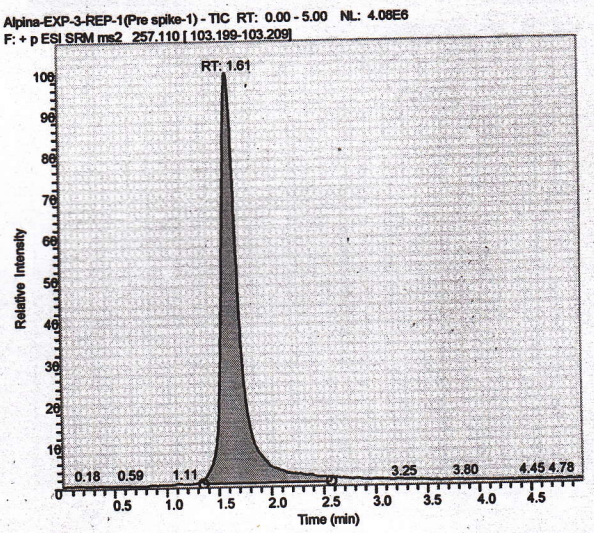
File Name	Sample Type	Acq Date	Sample ID	Component Name	Area	Specified Conc	Calculated Conc	% Diff
Alpina-EXP-2-RE P-1	Unknown	Tuesday, December 06, 2011 10:19:15 PM	Alpina-EXP-2-RE P-1	1-acetoxy chavicol acetate	48640139	N/A	37845.277	N/A



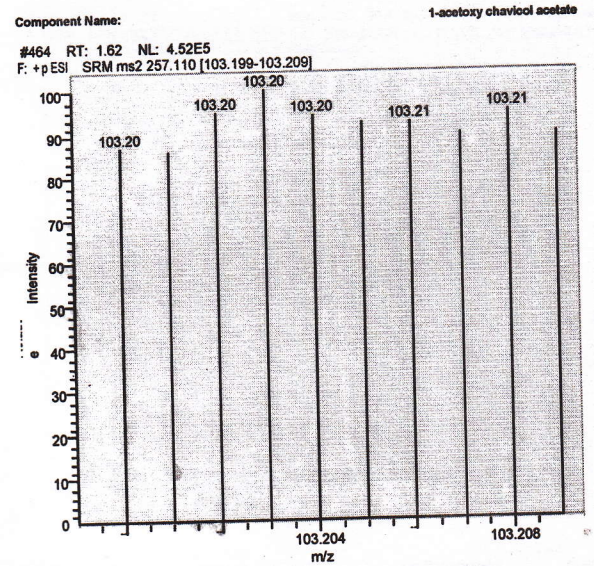
File Name	Sample Type	Acq Date	Sample ID	Component Name	Area	Specified Conc	Calculated Conc	% Diff
Alpina-EXP-2-RE P-2	Unknown	Tuesday, December 06, 2011 10:25:05 PM	Alpina-EXP-2-RE P-2	1-acetoxy chavicol acetate	49228847	N/A	38303.736	N/A

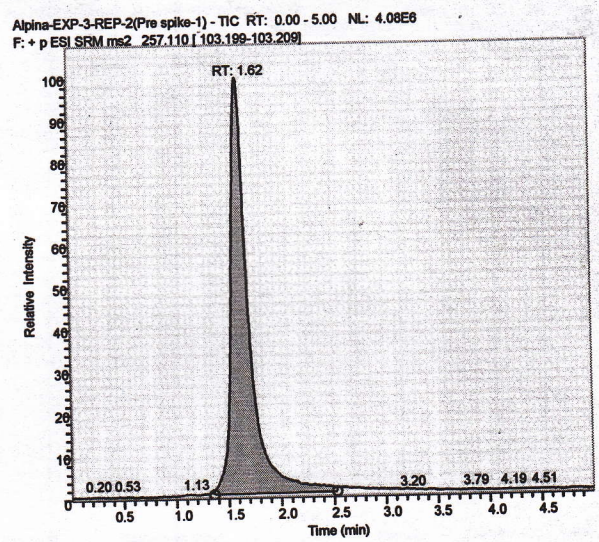
the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as gallic acid equivalents. Using the same extracts the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP)[3]. One hundred milligrams of PVPP was weighed into a 100×12 mm test tube and to this 1 ml distilled water and then 1 ml of the sample extracts were added. The content was vortexed and kept in the test tube at 4°C for 4h. Then the sample was centrifuged (3000 rpm for 10 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been





File Name	Sample Type	Acq Date	Sample ID	Component Name	Area	Specified Conc	Calculated Conc	% Diff
Alpina-EXP-3-RE P-1(Pre spike-1)	Unknown	Tuesday, December 06, 2011 10:30:55 PM	Alpina-EXP-3-RE P-1(Pre spike-1)	1-acetoxy chavicol acetate	46937197	N/A	36519.100	N/A

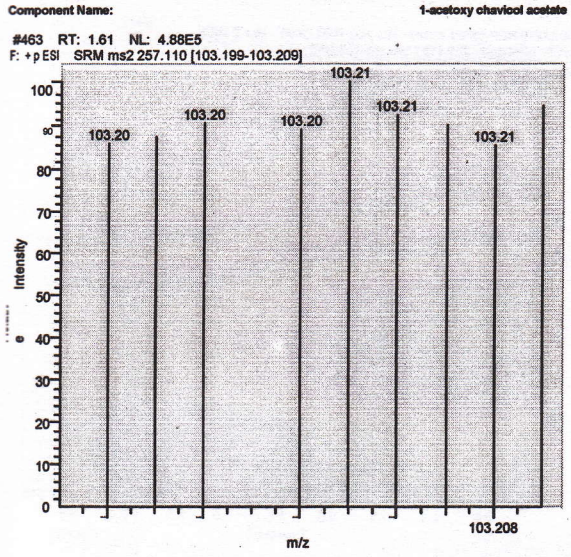


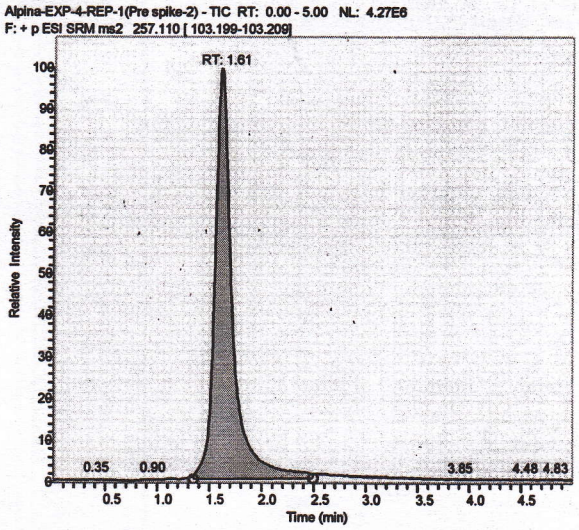


File Name	Sample Type	Acq Date	Sample ID	Component Name	Area	Specified Conc	Calculated Conc	% Diff
Alpina-EXP-3-RE P-2(Pre spike-1)	Unknown	Tuesday, December 06, 2011 10:36:45 PM	Alpina-EXP-3-RE P-2(Pre spike-1)	1-acetoxy chavicol acetate	46758424	N/A	36379.879	N/A

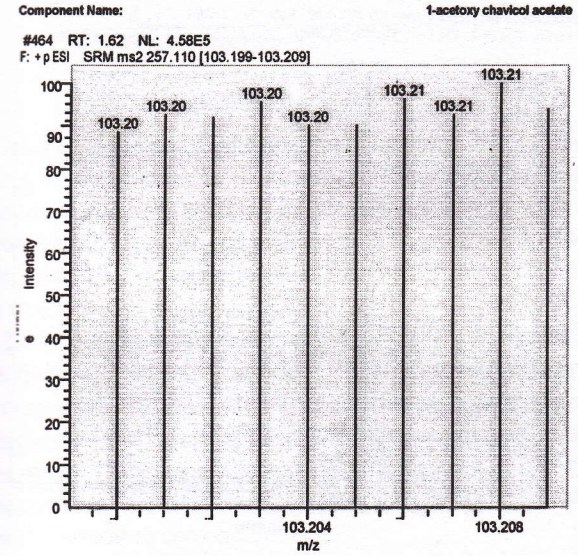
precipitated along with the PVPP). The phenolic content of the supernatant was measured as mentioned above and expressed as the content of non-tannin phenolics (tannic acid equivalents) on a dry matter basis. From the above results, the tannin content of the sample was calculated as follows: Tannin (%) = Total phenolics (%) - Non-tannin phenolics (%).

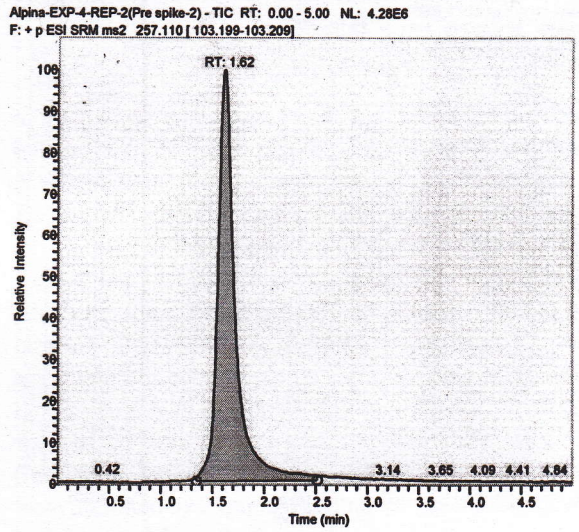
Determination of total flavonoid content- The flavonoid content was determined by the use of a slightly modified colorimetry method described previously by Zhishen *et al.*[10]. A 0.5ml aliquot of appropriately (10mg/2ml) diluted sample solution was mixed with 2ml of distilled water





File Name	Sample Type	Acq Date	Sample ID	Component Name	Area	Specified Conc	Calculated Conc	% Diff
Alpina-EXP-4-RE P-1(Pre spike-2)	Unknown	Tuesday, December 06, 2011 10:42:35 PM	Alpina-EXP-4-RE P-1(Pre spike-2)	1-acetoxy chavicol acetate	49395126	N/A	38433.227	N/A





File Name	Sample Type	Acq Date	Sample ID	Component Name	Area	Specified Conc	Calculated Conc	% Diff
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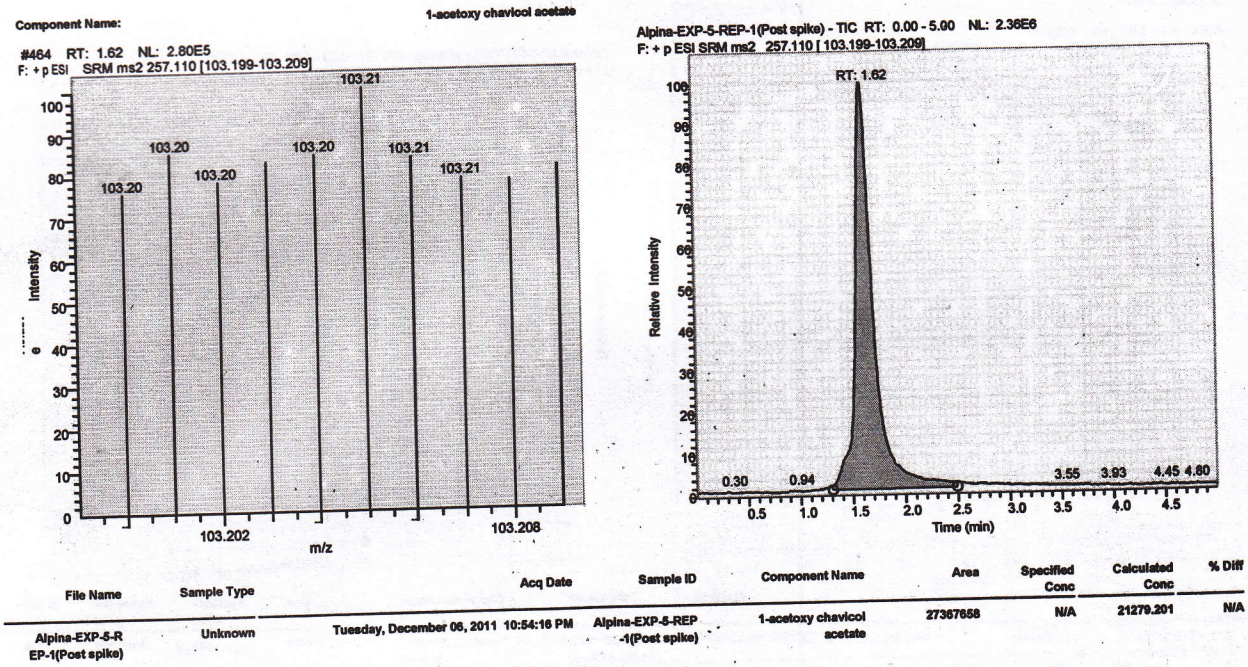
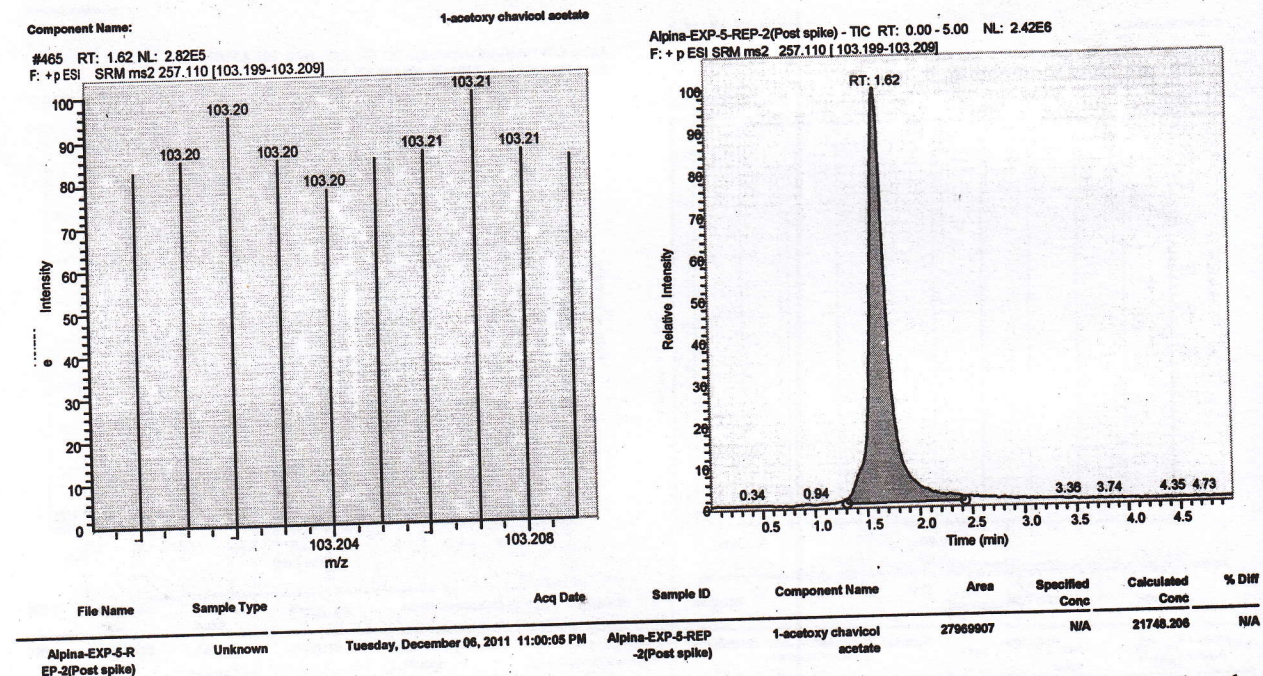
and subsequently with 0.15ml of 5%$NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5ml, and then the mixture was thoroughly mixed and allowed to stand for another 15min. Absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate and the results were expressed as rutin equivalent (Table 3).

LC-MS ANALYSIS : LC-MS Analysis were carried out with Thermo-Accela 1250, equipped with Quaternary pumps, Auto injector, Column Oven with PDA Detector supported by LC- quan Software. The instrument was set

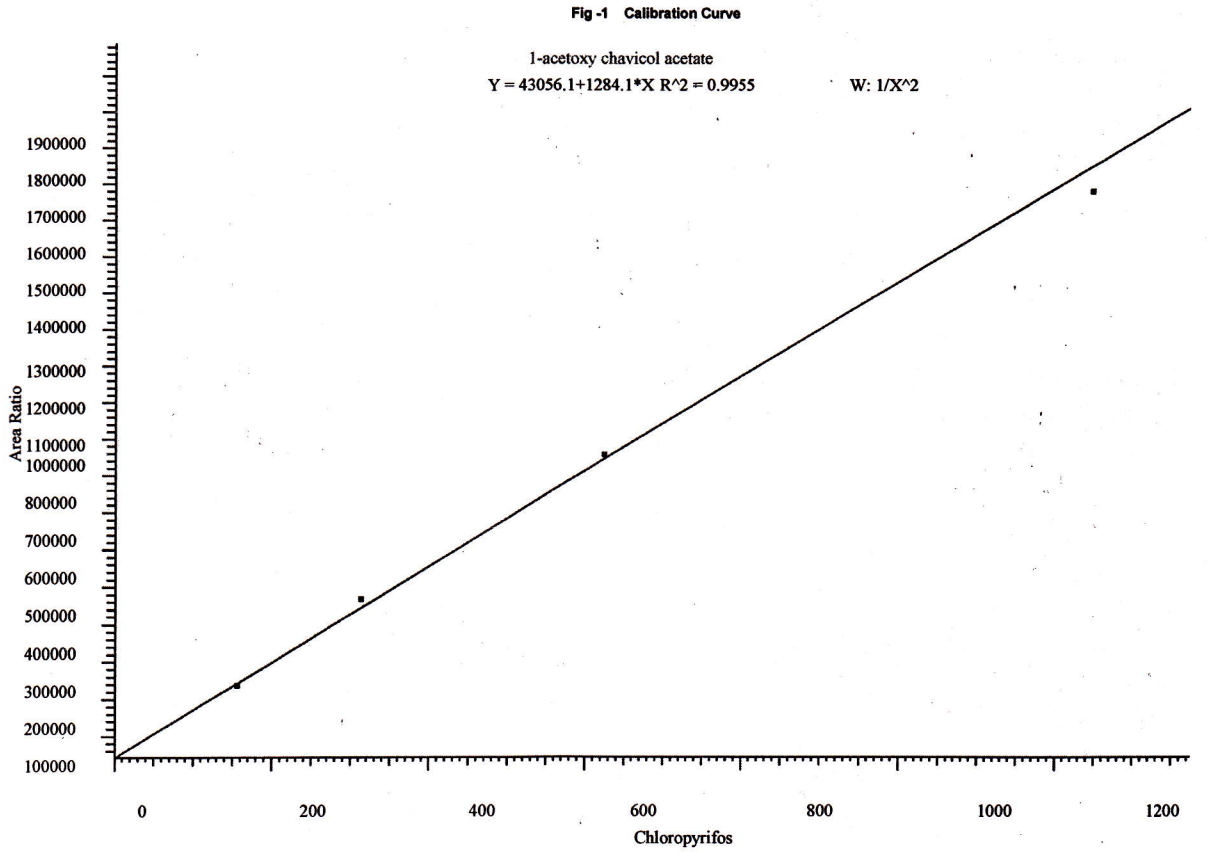




as per the chromatographic condition as prescribed and the chromatogram was recorded and calculations were carried out. The LC-MS profile showed the presence of peaks. Identification and peak assignment of the isolated compound was based on comparison of its retention time with corresponding standards. The compound 1-acetoxy chavicol acetate was obtained. This suggests that the compound identified may possibly contribute to the anti oxidative and other pharmacological activities (Table 4).

Results and Discussion

Alpinia galanga leaf extracts in solvents of different polarity were evaluated for antimicrobial activity against



Gram-positive and Gram-negative bacteria. Among the 5 types of solvents extract acetone (80%) showed highest antibacterial activity. Second most chloroform showed (64.2%) and followed by p.ether (28.5%), ethanol (8.5%). In this study no activity was observed from methanol extract.

Acetone extract- Acetone extract showed antibacterial activity against burn, accident, skin infection, abscess, trauma and post operative wound isolates. Among them highest activity was observed against accident wound isolates (85.7%), particularly *P. aeruginosa* was highly suppressed by acetone extract but at the same time *E.coli* was resistant to acetone extract.

Chloroform extract- The highest antibacterial activity of chloroform extract was found against burn and trauma wound isolates (77.7%) followed by abscess wound isolates (75%), skin infection and post operative wound isolates (50%). In percentage wise *P. vulgaris* was highly suppressed by chloroform extract but *P.aeruginosa* was resistant to chloroform extract.

Methanol extract-In this studies no antimicrobial activity were observed from methanol extract. All wound isolates were resistant to methanol extract.

Petroleum ether extract- The highest antibacterial activities of petroleum ether was found against accident wound isolates (35.7%). Among the 7 isolates, *S.aureus* and *S.pyogenes* was highly suppressed by p.ether extract.

Ethanol Extract-The highest antibacterial activities of ethanol extract was against burn wound isolates (14.8%). Among the 7 isolates *K.pneumoniae* only suppressed by ethanol extract. The inhibition zone ranged from 13-26 mm was observed. In the present investigation MIC value also observed by all 5 plants extracts. The plant extracts ranged from 5mg, 12.5mg and 25mg. The inhibition zone was started from 12.5mg concentration and size of zone (mm) ranged from 8 to 26 mm, at the same time all isolates were inhibited by 25mg concentration and inhibition zone (mm) ranged from 8-35 mm.

Conclusion- From the above experiment it can be seen that *A. galanga* leaf acetone extracts showed significant activity against Gram-positive and Gram-negative bacteria. The activity of leaf was found to be quite comparable with the standard antibiotics screened under similar conditions. So the oil can be used as an external antiseptic in the prevention and treatment of bacterial infections caused by various pathogenic bacteria. As the leaf acetone extract is found to be active against *Pseudomonas aeruginosa* and chloroform extract against

Proteus vulgaris, and their activities are comparable with the standard antibiotics they can be used for the treatment of bacterial infections. The activity of the leaf extracts with acetone on *P.aeruginosa* was higher (85.7%) . However, the activity of leaf chloroform extract against *Proteus vulgaris* is slightly greater. As the leaf extract exhibited pronounced activity, essential oil from leaves is recommended in the drug formulations.

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