



# Chemical composition, antimicrobial activity and cytotoxicity of *Murraya paniculata* (L.) Jack leaf essential oil from Assam, India: the effect of oil on cellular morphology of micro-organisms

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## Abstract

*Murraya paniculata* (L.) Jack is commonly cultivated as ornamental plant in Assam and has been used as spice and phyto-medicine traditionally for many healthcare purposes. The therapeutic potential and chemical constituents of the essential oil of *M. paniculata* leaf was investigated against several pathogenic microbial species and human cancer cell lines. 29 chemical compounds were identified by GC–MS analysis from the essential oil representing 97.62% of the oil. The major compound identified was caryophyllene (20.93%). Leaf essential oil exhibited promising antibacterial activity against *Mycobacterium smegmatis* (MIC = 4 µg/mL) and *Pseudomonas aeruginosa* (MIC = 4 µg/mL). Best anticancer activity of the oil was observed for HeLa cells (IC<sub>50</sub> = 6.28 µg/mL). Further, scanning electron microscopic studies revealed that the oil kills micro-organisms with the deformation of cellular morphology on treatment of the oil. Thus, the essential oil of *M. paniculata* leaf can be an excellent alternative for development of new antimicrobials and anticancer chemotherapeutic agents for the pharmaceutical industries.

**Keywords** Antimicrobial · Anticancer · Sesquiterpenes · Essential oil · *Murraya paniculata*

## Introduction

Antimicrobial resistance (AMR) as well as cancer is a life-threatening disease in today's world. Microorganisms develop resistance mechanism so fast and spread globally which bring complexity to fight against common infectious diseases. In 2019, Centers for Disease Control and Prevention (CDC) reported that every year above 2.8 million

microbial infections occur in United States due to antibiotic resistance, as a result almost 35,000 people die (CDC 2019). World Health Organization (WHO) identified and listed the names of such antibiotic resistant bacteria to prioritize research for the development of new and effective alternative drugs (WHO 2017). *Mycobacterium tuberculosis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* occupied top categories in the list as they become resistant to existing antibiotics like rifampicin, vancomycin, methicillin, carbapenem, etc. bringing threat to the human population. Moreover, cancer is the 2nd deadliest disease all over the world which is responsible for almost 9.6 million deaths in 2018. It was expected to double the number by 2040, mostly in low- and middle-income countries (WHO 2020).

It has been a challenging task to find out new antimicrobials for the microbes that become resistant to the established drugs. As plants are alternative source of treatment to the conventional antibiotics which can fight as resistant modifying agents (RMAs) function through different mechanisms against pathogenic micro-organisms (Abreu et al. 2012; Chassagne et al. 2021). Moreover, plant kingdom is the richest reservoir of medicines in the form of natural compounds that can be easily obtained to replace the chemotherapeutic

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drugs (Russo et al. 2015). Among the plant secondary metabolites essential oils have rich medicinal values due to the presence of terpenes, flavonoids, phenolics, etc. compounds as chemical entity (Bouyahya et al. 2017). In spite of their use as flavoring agents, plant essential oils have been utilized in the pharmaceutical industry. *Murraya paniculata* (L.) Jack is a potential medicinal plant, flowers of which have a demand in India as a source of flavor and perfumery (Semarayani et al. 2018). The plant belongs to *Rutaceae* family and is commonly known as orange jasmine in Asia and kamini kusum in Assam, India (Choudhury 2015). Although the plant was endemic to South-West Asia, now it is widely distributed from tropical to subtropical parts of the world including China, Nepal, India, Taiwan, Sri Lanka, North eastern Pakistan, Northern Australia and South eastern Asia (Dosoky et al. 2016). The leaves of *M. paniculata* are used as spice by the people of India, Pakistan and Malaysia in a variety of food preparation (Saqib et al. 2015; Ng et al. 2012). Moreover, in ancient India, China and Indonesia plant is used as phytomedicine for many healthcare purposes. In Chinese pharmacopeia, the plant is reported to possess analgesic property with potentiality to cure microbial infection and inflammatory diseases (Zhang et al. 2011). Studies revealed that *M. paniculata* is a natural source of antioxidant that may be useful for pharmaceutical industry or as a possible food supplement (Gautam et al. 2012).

Although, the chemical components of *M. paniculata* leaf essential oil were reported from Nepal (Dosoky et al. 2016), Brazil (Selestino Neta et al. 2016), China (Li et al. 1988), Nigeria (Olawore et al. 2004) and Bangladesh (Chowdhury et al. 2008), there is no report so far on the chemical composition or biological activities of the essential oil of *M. paniculata* leaf found in Assam, India. In continuation of our search for bioactive substances and molecules from the Indo-Burma Biodiversity mega center (Saikia et al. 2020; Neipihoi et al. 2020), we have studied the chemical composition of the essential oil of *M. paniculata* leaf from Assam, India and also detailed biological activities of the oil against a number of pathogenic micro-organisms and cancer cell lines. The chemical composition of the oil was found to be different from those reported and biological activities were found to be promising and are presented in this communication.

## Material and methods

### Plant materials

The leaves of *Murraya paniculata* (L.) Jack (*Rutaceae*) were collected in the month of March (2016) from the experimental farm of CSIR-NEIST, Jorhat, Assam, India. The plant samples were identified at CSIR-NEIST, Jorhat and voucher specimens [CSIR-NEIST Accession number 1830

(NPC/289)] were deposited at CSIR-NEIST, Jorhat 785006, Assam.

### Antibiotics and chemicals

Isoniazid, rifampicin, amikacin, fluconazole, resazurin sodium, gentamycin, dimethyl sulfoxide (DMSO), brain heart infusion agar (BHIA) and broth (BHIB), Mueller Hinton agar (MHA) and broth (MHB), yeast malt agar (YMA) and potato dextrose broth (PDB) were obtained from HIMEDIA Laboratories, Mumbai, India. Anhydrous sodium sulfate, isopropanol, hydrochloric acid and acetone were procured from Sisco Research Laboratories (SRL) Pvt. Ltd. Maharashtra, India. Media for culture of cell lines viz., DMEM (Dulbecco's Modified Eagle's Medium), MEM (Minimum Essential Medium Eagle), FBS (Fetus Bovine Serum) were obtained from MERCK, Sigma-Aldrich Chemicals Private Limited, Bangalore, India. Distilled water was used for the preparation of agar and broth media.

### Extraction of essential oil

The leaves of *M. paniculata* were extracted for essential oil by hydrodistillation method with the help of a Clevenger-type apparatus (Bouyahya et al. 2017) in which the condenser was connected with Buchi Recirculating Chiller F-305, circulating the mixer of ethylene glycol and water (1:1) at  $-10\text{ }^{\circ}\text{C}$ . The fresh leaves (300 g) of *M. paniculata* were taken in a round bottom flask separately and then distilled water (1 L) added to it. Then, the hydro distillation is continued for 7 h at  $100\text{ }^{\circ}\text{C}$ . The whole procedure was repeated for two times. After completion of the experiment, the essential oil was collected in glass bottles, dried over anhydrous sodium sulfate and then it was filtered to give 1.25 g (0.41% yield) of essential oil, which was stored at  $4\text{ }^{\circ}\text{C}$  for further experiments. The essential oil yield was calculated by using the following formula:

$$\text{Yield (\%)} = \frac{\text{Dry weight of crude oil (g)}}{\text{Raw leaves taken (g)}} \times 100\%.$$

### GC/MS analysis of essential oil

The investigation of chemical components present in the essential oil was conducted by Gas Chromatography–Mass Spectrometer (Clarus 680 GC & Clarus 600C MS, Perkin Elmer, USA, Liquid Autosampler, Library Software: Turbomass NIST 2008) coupled with FID detector for quantification. The GC settings were as follows: the initial oven temperature was maintained at  $50\text{ }^{\circ}\text{C}$  for 2 min and raised to maximum  $260\text{ }^{\circ}\text{C}$ , ramping temperature  $5\text{ }^{\circ}\text{C}/\text{min}$  hold time is 6 min. The injector port temperature was  $280\text{ }^{\circ}\text{C}$ ,

transfer and source temperature was maintained at 200 °C and 180 °C, respectively. The samples (1 µL) were injected with a split ratio of 50:1. The flow rate of Helium as a carrier gas was 1 mL/min. Oil sample was prepared in methanol and compounds were separated in a capillary column of 60.0 m × 250 µm thickness. The oil was analyzed by gas chromatography and gas chromatography–mass spectroscopy technique and quantification was done by external standard method along with the mass spectra of authentic samples available in NIST library data of the GC–MS system (Kovats et al. 1965; Adams et al. 1995). The retention indices (RI) of the identified compounds were calculated by Kovat's method using n-alkanes as standard using the formula,

$$\text{Retention indices (RI)} = 100 \left[ \frac{n + (N - n)}{\log \text{RT (unknown)} - \log \text{RT (n)}} \right] \times \frac{\log \text{RT (N)} - \log \text{RT (n)}}{\log \text{RT (N)} - \log \text{RT (n)}}$$

## Antimicrobial activity

### Bacterial and fungal strains

Bacterial strains used in this study were *Mycobacterium smegmatis* (ATCC<sup>®</sup>607<sup>TM</sup>), *Staphylococcus aureus* (ATCC<sup>®</sup>11632<sup>TM</sup>), *Pseudomonas aeruginosa* (ATCC<sup>®</sup>25619<sup>TM</sup>) and fungal strain is *Candida albicans* (ATCC<sup>®</sup>90028<sup>TM</sup>) procured from HIMEDIA. *M. smegmatis* was cultured on sterile Brain heart infusion (BHI) agar media. Sterile Nutrient agar (NA) was used for the culture of *S. aureus* and *P. aeruginosa*, and Yeast malt agar (YMA) media were used for *C. albicans*. Incubation temperature used for bacterial strains is 37 °C and for fungal strain is 30 °C. All the pathogens were incubated from 18 to 48 h. Stock cultures of the respected bacteria were maintained on Nutrient broth and Brain heart infusion broth, *C. albicans* was maintained on Potato dextrose broth. All the strains were stored at 4 °C for further use.

### Antimicrobial activity test by well diffusion assay

Isolated leaf essential oil was dissolved in DMSO to prepare stock solution for assay. Antimicrobial activity of the leaf oil was evaluated by well diffusion method (CLSI 2012). Bacterial and fungal suspensions were homogeneously spread over MHA and YMA medium, respectively. Wells of 6 mm diameter were bored in petriplates containing previously inoculated strains. The inoculum size used has a final inoculum density of approximately  $1 \times 10^8$  CFU/mL. 100 µL of stock solution was added to the wells and the plate containing fungal strain was incubated at 30 °C and plates containing bacterial strains were incubated at 37 °C for 18–48 h as per growth conditions of the selected pathogens. The

experiments were carried out in triplicates. Antimicrobial activity was determined by measuring the inhibition zones in the test plates and results were presented as mean ± SD.

### Determination of minimum inhibitory concentration

Resazurin assay was performed in 96-well microtiter plates (Nunc<sup>TM</sup>, Thermo Fisher Scientific Inc) to determine minimum inhibitory concentration of the isolated oil using broth microdilution method with slight modification of Wiegand et al. 2008 (Wiegand et al. 2008; NCCLS 1999). Resazurin is an indicator to determine live and dead microbial cells. It is a non-toxic, blue to purple colored dye and converted to pink color in presence of live bacterial cells and become colorless in case of live *Candida* cells (Sarker et al. 2007). For bacteria 100 µL of MHB and for fungus 100 µL of PDB were added to the each well of microtiter plates. Stock concentration of antibiotics used is 24, 50 and 60 µg/mL for Rifampicin, Amikacin and Fluconazole, respectively. 100 µL from each stock solution of antibiotics and essential oil was added to the first well and twofold serial dilutions were carried out in MHB and PDB media (Magrys et al. 2021). Then, 20 µL of microbial suspension was added to each well to achieve a concentration of  $5 \times 10^5$  CFU/mL. Finally 20 µL of resazurin indicator was added to each well. Plates were incubated at 30 °C (fungus) and 37 °C (bacteria) for 18–48 h. At the end of incubation period change of color was evaluated visually. In each plate the column C1 is for sterility check (C1 = broth + sample + resazurin), C5 and C6 are for negative control (C5 and C6 = broth + inoculum + resazurin), C7 and C8 are for positive control (C7 and C8 = broth + antibiotic + inoculum + resazurin) and in rest of the columns, test sample was added in triplicates. The color change from purple to pink or colorless indicates growth of microbes. MIC of test sample was interpreted as the lowest concentration of the essential oil that prevents a change in color of the resazurin (Sarker et al. 2007).

### Minimum bactericidal and fungicidal (MBC and MFC) concentration

Minimum bactericidal activity was tested against all the microbial strains following NCCLS guideline (NCCLS 1999). After completion of MIC experiment, the concentration of test samples that did not show any visible growth of microbes in resazurin assay, was subcultured to determine bactericidal and fungicidal concentration. For MBC and MFC, 10 µL of the samples taken from the purple wells of the micro broth susceptibility studies and placed onto the surfaces of Mueller Hinton agar and Yeast malt agar plates. The MBC and MFC were defined as the concentration of test sample at which 99.9% of the test pathogens were killed

compared to the test pathogens present in test wells at 0 h (NCCLS 1999).

### Effect of essential oil on the cell surface of tested micro-organisms

At first, the test micro-organisms were cultured in respective broth media, i.e., nutrient broth for *S. aureus* and *P. aeruginosa*, Mueller Hinton broth for *M. smegmatis* and potato dextrose broth for *C. albicans*. After attaining mid log phase, the microbial cells were harvested by centrifugation at 1000g for 10 min. Then, cells were treated with phosphate buffered saline (PBS) (HIMEDIA, Mumbai, India) for two times. Two sets of each micro-organisms were taken, one set without treatment and another set was treated with essential oil at MIC concentration. Treated samples were incubated for 1 h from 30 to 37 °C. After removing from incubator, cells were washed thrice in PBS at 6000g for 5 min. Bacterial and fungal pellets were then fixed in 2.5% (v/v) glutaraldehyde (MERCK, Bangalore, India) at 4 °C overnight. Thereafter, the cell pellets were washed two times in PBS and dehydrated using a graded acetone (SRL, Maharashtra, India) series (30, 50, 70, 80, 90, 95 and 100%) for 15 min in each. At last, the cell pellets were immersed in TMS (MERCK, Bangalore, India) for 10 min at 4 °C and then brought to room temperature (25–26 °C) for drying. The samples prepared then subjected to SEM analysis (FESEM, Carl Zeiss Microscopy Ltd., Cambridge CB1 3JS, UK) (Saikia et al. 2020).

### Culture of cell lines and cytotoxicity (MTT) assay

To study the cell toxicity activity, stock solution of the oil sample was prepared in DMSO at a concentration of 1 mg/mL. L6 (Normal Rat muscle), HeLa (Human cervix), MIA-PaCa2 (Human Pancreatic) and PA1 (Human Ovary) cell lines used in this experiment were collected from National Centre for Cell Sciences (NCCS), Pune, India. The cell lines were cultured in respective complete media (DMEM for L6 and MIA-PaCa2, MEM for PA1 and HeLa) supplemented with 10% FBS (Fetus Bovine Serum), 1% Gentamycin, 10% Penstrep and incubated at an optimum temperature of 37 °C with humidified 5% CO<sub>2</sub> atmosphere. For MTT assay confluent cells ( $1 \times 10^6$  mL<sup>-1</sup>) were added to the complete medium in tissue culture grade 96-well plates (Nunc™, Thermo Fisher Scientific Inc) and incubated for 24 h. After incubation, the complete medium was removed and FBS free medium was poured on the plates, and then it was incubated overnight. Then, the cell lines were treated with different concentrations (0.5, 1, 2, 5, 10, 20, 50, 100, 150 µg/mL) of essential oil and incubated for 24 h. All the treatments were applied in triplicates. Cells maintained in culture medium without test sample served as control.

MTT (MERCK, Bangalore, India) assay was conducted as per guideline of manufacturer's (Merck Millipore Corporation, Germany) for cytotoxicity evaluation of essential oil. After 24 h of incubation, 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL) was added to each well, mixed gently and incubated for 4 h. After completion of incubation time, 96-well plates were examined under an inverted microscope and dark purple formazan crystals were found at the bottom of the wells. 100 µL of isopropanol and 0.04 N HCl mixture was added to the wells and thoroughly mixed by repeated pipetting. HCl can convert the phenol red of the culture media into yellow color which does not disturb MTT formazan measurement. The formazan is dissolved by isopropanol and we get a homogeneous blue solution suitable for absorbance measurement. With the help of ELISA plate reader (Filter Max F3 Multi Mode Microplate Readers, Molecular Devices) absorbance was measured with a test wavelength of 570 nm and a reference wavelength of 630 nm. The effect of oil on cell proliferation was expressed in percent of viable cells compared with the control, and it was calculated as absorbance of treated cells/absorbance of control cells × 100. Cytotoxic concentration (IC<sub>50</sub>) of the sample was determined by analysis of dose response curves (Saikia et al. 2020).

### Statistical analysis

Zone of inhibition and IC<sub>50</sub> values were determined in triplicates and data are presented as mean ± standard deviation (SD) (MS Excel 2007).

## Results

### Chemical composition of the essential oil

From leaf essential oil of *M. paniculata*, 29 compounds were identified through GC–MS analysis which represents about 97.62% of chemical components present in the oil (Table 1). Figure 1 shows the gas chromatogram of the essential oil. The oil is light yellowish in color with strong aroma; yield percentage after dehydration is about 0.41% (w/w on dry weight basis). Most of the identified compounds of *M. paniculata* leaf essential oil were sesquiterpene hydrocarbons (80%) and rest were belonged to oxygenated sesquiterpenes (12.26%), diterpenoids (1.5%), fatty acids (1.80%) and nitrogenous heterocyclic compounds (2.06%). Caryophyllene **5** (20.93%), a bicyclic sesquiterpene compound, is the most abundant component present in the oil. Apart from Caryophyllene, other major compounds of the oil are δ-Elementene **2**, β-Elementene **4**, α-Caryophyllene **7**, β-Cubebene **9**, Elixene **11** and δ-Cadinene **12**. A few compounds were identified in trace amounts (< 1%) from the leaf oil viz., Isophytol **26**,

**Table 1** Chemical constituents of *Murraya paniculata* leaf essential oil

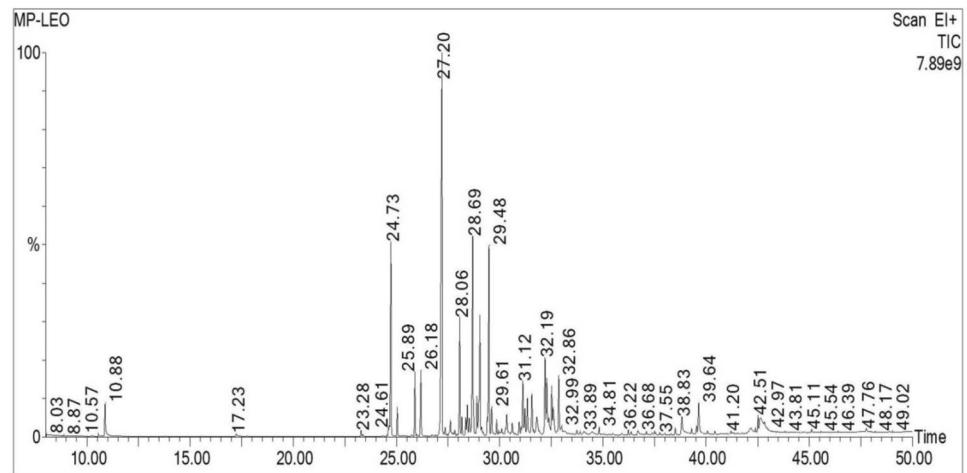
Constituents	<sup>a</sup> RI	<sup>b</sup> RT	Area%	<sup>c</sup> Identification	Mol. formula	Mol. Weight	M <sup>+</sup> m/z
5-Methyl thiazole	820	10.880	2.06	RI, MS	C <sub>4</sub> H <sub>5</sub> NS	99.15	99
δ-Elemene	1338	24.725	7.24	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
α-Cubebene	1351	25.891	2.45	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
β-Elemene	1388	26.176	3.09	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
Caryophyllene	1450	27.201	20.93	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
Alloaromadendrene	1461	27.611	1.19	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
α-Caryophyllene	1454	28.061	4.37	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
β-Cadinene	1474	28.352	2.04	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
β-Cubebene	1389	28.692	9.26	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
Viridiflorene	1486	28.892	1.61	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
Elixene	1430	29.042	4.68	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
δ-Cadinene	1530	29.477	8.31	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
4,4-Dimethyl-3-(3-methylbut-3-enylidene)- 2-methylenebicyclo [4.1.0] heptanes	1413	29.607	1.62	RI, MS	C <sub>15</sub> H <sub>22</sub>	202.33	202
Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-di- methyl-4-(1-methylethyl)	1338	29.847	0.96	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
Nerolidol	1565	30.337	1.51	RI, MS	C <sub>15</sub> H <sub>26</sub> O	222.36	222
Spathulenol	1576	31.118	2.31	RI, MS	C <sub>15</sub> H <sub>24</sub> O	220.35	220
Patchoulane	1890	31.223	1.11	RI, MS	C <sub>15</sub> H <sub>26</sub>	206.36	206
Longifolene	1416	31.338	2.37	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
Guaiol	1595	31.558	2.17	RI, MS	C <sub>15</sub> H <sub>26</sub> O	222.36	222
Rosifoliol	1601	31.793	1.83	RI, MS	C <sub>15</sub> H <sub>26</sub> O	222.36	222
γ-Gurjunene	1474	32.193	2.73	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
Dehydro-aromadendrene	1466	32.278	2.18	RI, MS	C <sub>15</sub> H <sub>22</sub>	202.33	202
γ-Muurolene	1477	32.518	1.95	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
τ-Cadinol	1624	32.588	1.74	RI, MS	C <sub>15</sub> H <sub>26</sub> O	222.36	222
α-Cadinol	1654	32.858	2.70	RI, MS	C <sub>15</sub> H <sub>26</sub> O	222.36	222
Isophytol	1943	38.501	0.77	RI, MS	C <sub>20</sub> H <sub>40</sub> O	296.53	296
N-hexadecanoic acid	1984	38.826	1.80	RI, MS	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.42	256
3,3'-Bi-p-menthane	1856	39.536	0.73	RI, MS	C <sub>20</sub> H <sub>38</sub>	278.52	278
Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl	2131	39.636	1.91	RI, MS	C <sub>15</sub> H <sub>24</sub>	204.35	204
Sesquiterpene hydrocarbons			80				
Oxygenated sesquiterpenes			12.26				
Fatty acids			1.80				
Diterpenoids			1.5				
Nitrogenous heterocyclic compound			2.06				
			<b>97.62</b>				

<sup>a</sup>RI: retention indices, <sup>b</sup>RT: retention time determined on a Thermo Fisher Scientific TR-5MS 30 m×0.25 mm ID×0.25 μm with reference to n-alkanes, <sup>c</sup>Identification (RI, MS): identification by GC–mass spectrometry analysis and RI, using NIST and Wiley library spectra and literature studies

3,3'-Bi-p-menthane **28** and Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl) **14**. The leaf essential oil was analyzed through NMR spectroscopy (Fig. 2a, b) which validates the presence of all the identified compounds. Here, all the chemical shifts (δ) are presented in ppm. The signals appearing from δ 0.82 to δ 2.41 in <sup>1</sup>H NMR indicates the higher percentage of compounds containing hydrocarbon

or terpene types of analogs. Moreover, signals δ 3.91–δ 5.76 assign as proton under hydroxyl groups and C=C bonded moieties of the components of the essential oil (Fig. 2a). The <sup>13</sup>C NMR spectrum also, indicated the same inferences (Fig. 2b). Since, essential oil contains many chemical constituents, so overlapping signals of chemical shifts were observed in the NMR spectrum.

**Fig. 1** Gas chromatogram of *M. paniculata* leaf essential oil



### Antimicrobial activity (MIC, MBC, MFC)

Agar well diffusion assay displayed that leaf essential oil of *M. paniculata* is active against all the test pathogens (Table 2; Fig. 3a). Minimum inhibitory concentration (MIC), bactericidal concentration (MBC) and fungicidal concentration (MFC) of the leaf essential oil were determined and presented in Table 2 (Fig. 3b, c). Antibacterial activity of the oil is first time reported against *P. aeruginosa* and *M. smegmatis* where MIC of the oil is close to standard drug rifampicin against *M. smegmatis* (Table 2). Here, the antimicrobial activity of the *M. paniculata* leaf essential oil is considered as susceptible for *P. aeruginosa* and *M. smegmatis* whereas, intermediate for *C. albicans* and *S. aureus* according to CLSI guideline (CLSI 2012).

Antimicrobial efficacy of the oil was further analyzed by scanning electron microscopy (SEM). The differences in cellular morphology were distinctly observed on the tested microbial samples after application of *M. paniculata* leaf essential oil (Fig. 4).

### Cytotoxicity assay

MTT assay of L6, HeLa, MIAPaCa2 and PA1 displayed that increase in the dose of leaf oil led to a decrease in the percentage viability of the cell lines (Fig. S1a). L6 cells showed 50% viability ( $IC_{50}$ ) at  $13.62 \pm 4.02 \mu\text{g/mL}$  which was close to  $13.14 \pm 1.56 \mu\text{g/mL}$  for essential oil-treated PA1 (Human Ovary) cells.  $IC_{50}$  value of  $6.28 \pm 1.82 \mu\text{g/mL}$  was seen for HeLa (Human cervix) cells implying that the oil is considerably toxic to it. However, oil is least toxic to MIAPaCa2 (human pancreatic) cell lines with  $IC_{50}$  value of  $55.12 \pm 0.77 \mu\text{g/mL}$ . The photomicrograph also supports the result where change in cell morphology of the treated cells is visible in comparison to control cells (Fig. S1b). The oil was previously reported with  $IC_{50}$  value  $63.73 \mu\text{g/mL}$  against

Hepa1c1c7 cell lines (Selestino Neta et al. 2016). We have first time reported the anticancer activity of *M. paniculata* leaf essential oil against human cervical (HeLa), pancreatic (MIAPaCa2) and ovary (PA1) cell lines with distinguished activity for cervical cancer cells. The compound Caryophyllene **5** is reported to have cytotoxicity against breast cancer cell lines (MDA-MB-231 and Hs 578 T), which may definitely impart toxic effect along with other compounds on three cancer cell lines, HeLa, MIAPaCa2 and PA1 in our experiment (Palazzo et al. 2009).

### Discussion

Chemical constituents of medicinal plant *M. paniculata* leaf essential oil are first time reported from India, though a few countries have reported earlier (Selestino Neta et al. 2016; Li et al. 1988; Olawore et al. 2004). Due to the variation in geographical location, climatic factors and edaphic factors chemical composition and biological activity of the essential oils vary from country to country (Boira and Blanquer 1998). Our group at Jorhat, India and other groups reporting are in different geographical regions in different countries with the distance between the two collections sites is approximately 1000–14,000 km. This factor may be responsible for the variation in the phytochemical constituents (Akula et al. 2011; Verma et al. 2015). Here, we have found promising anticancer and antimicrobial activity of the oil for some of the tested pathogens and cancer cell lines that are reported first time. Leaf oil from China was characterized by 15 compounds with highest percentage of  $\gamma$ -Elemene (31.7%) (Li et al. 1988). Moreover, study of leaf essential oil from Bangladesh and Nepal reported with total 58 and 76 compounds, indicated the presence of Caryophyllene oxide (16.63%) and Methyl palmitate (11.05%) with highest percentage, respectively (Dosoky et al. 2016; Chowdhury et al. 2008). Among

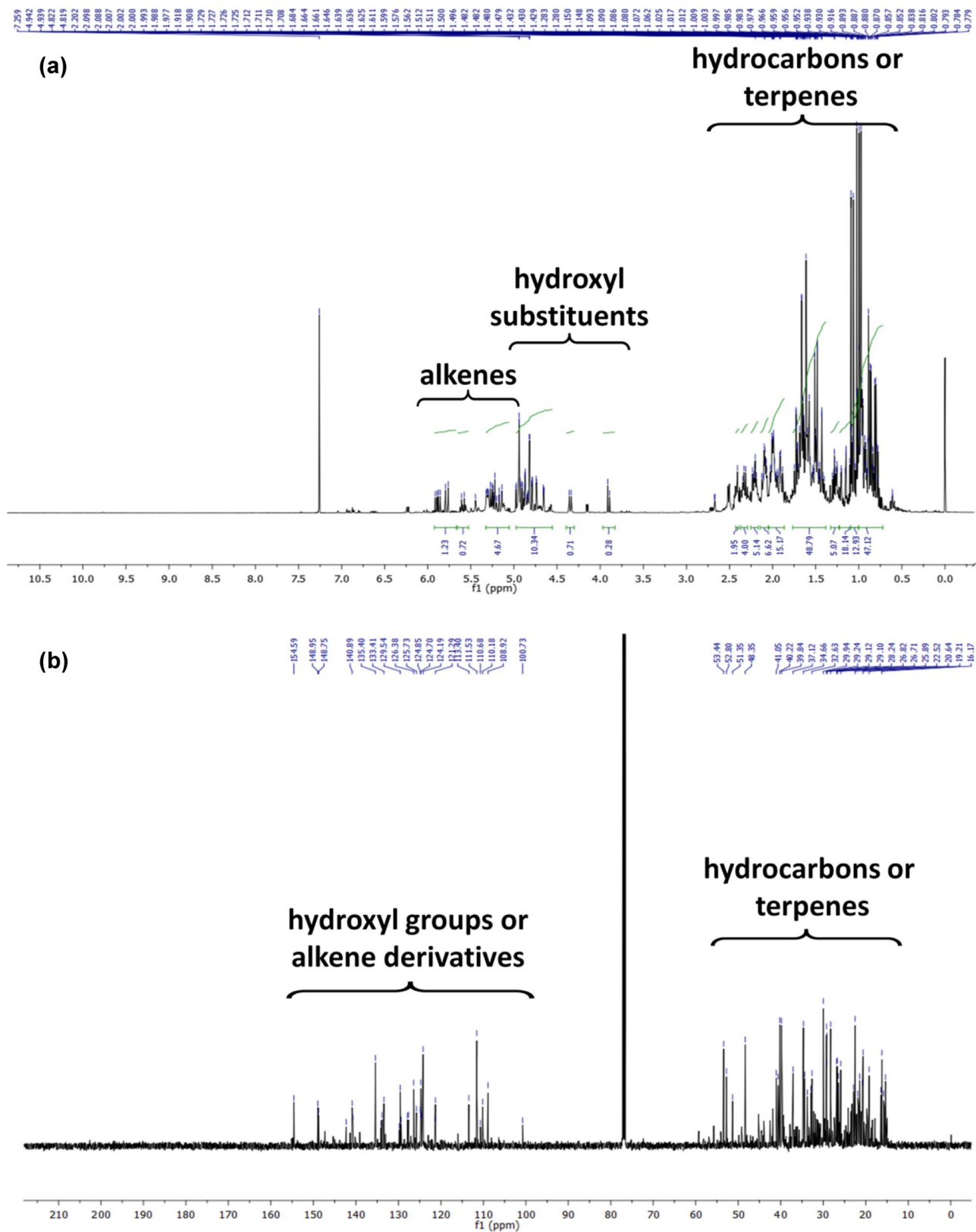
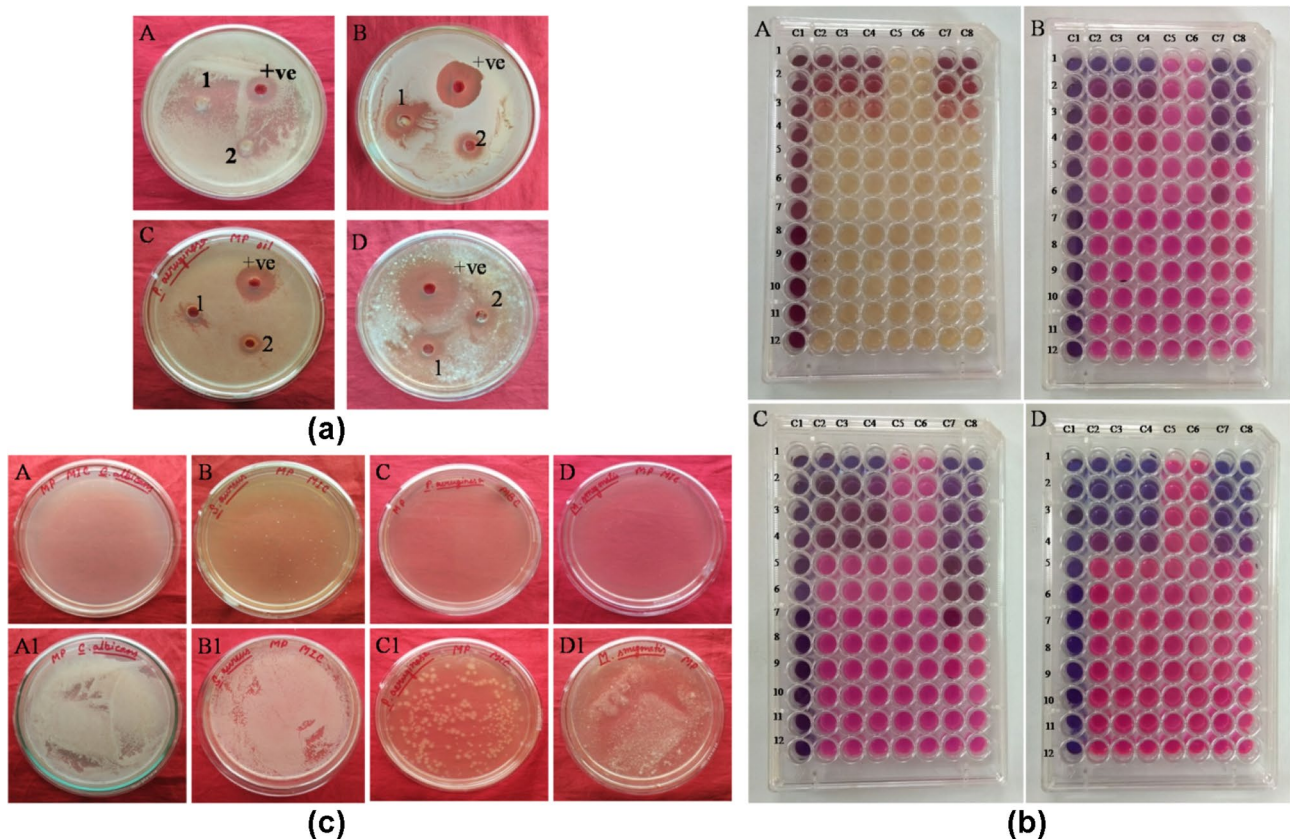


Fig. 2 a <sup>1</sup>H NMR and b <sup>13</sup>C NMR of the leaf essential oil of *M. paniculata*

**Table 2** Antimicrobial activity of *Murraya paniculata* (L.) Jack leaf essential oil

Name of the samples	Experiments	<i>S. aureus</i>	<i>M. smegmatis</i>	<i>C. albicans</i>	<i>P. aeruginosa</i>
<i>Murraya paniculata</i> leaf essential oil	ZOI <sup>a</sup>	11.8 ± 0.16	13.8 ± 0.17	12.8 ± 0.14	10.6 ± 0.57
	MIC <sup>b</sup>	16	4	8	4
	MBC <sup>c</sup>	32	8	NA	16
	MFC <sup>d</sup>	NA	NA	8	NA
Rifampicin	ZOI	35.8 ± 0.1	24.5 ± 0.5	NA	NA
	MIC	1	3	NA	NA
	MBC	0.01	3	NA	NA
Isoniazid	ZOI	NA	21.9 ± 0.06	NA	NA
Fluconazole	ZOI	NA	NA	15.9 ± 0.11	NA
	MIC	NA	NA	7.5	NA
	MFC	NA	NA	7.5	NA
Amikacin	ZOI	NA	NA	NA	19.1 ± 0.76
	MIC	NA	NA	NA	0.5
	MBC	NA	NA	NA	1

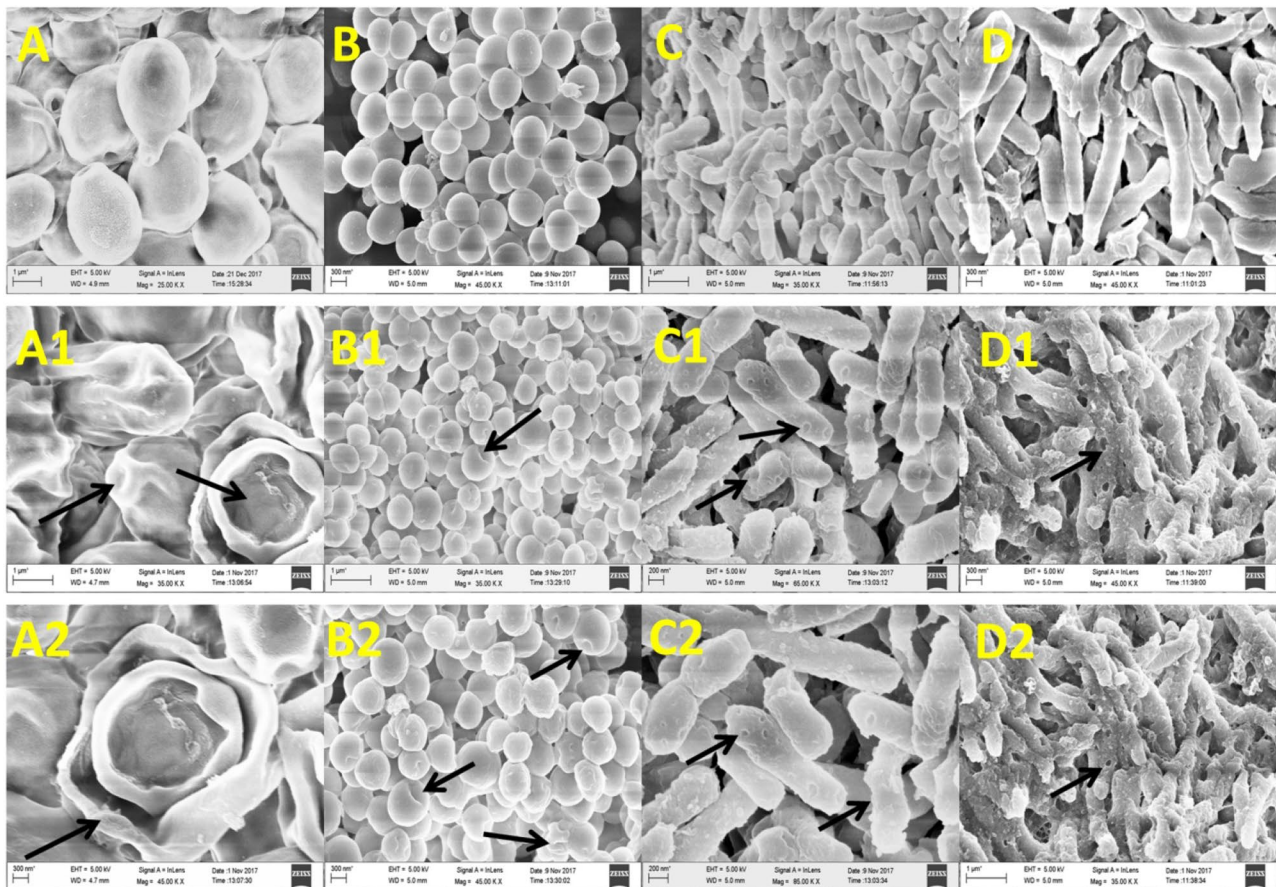
ZOI<sup>a</sup> zone of inhibition in mm (means of triplicate ± standard deviation), MIC<sup>b</sup> minimum inhibitory concentration in µg/mL, MBC<sup>c</sup> minimum bactericidal concentration in µg/mL, MFC<sup>d</sup> minimum fungicidal concentration in µg/mL, NA not applicable/not applied



**Fig. 3** Antimicrobial activity of *M. paniculata* leaf essential oil. Photograph of **a** Zone of inhibition by leaf essential oil of *M. paniculata* against (A) *C. albicans*; (B) *S. aureus*; (C) *P. aeruginosa* and (D) *M. smegmatis*. **b** Inhibition of (A) *C. albicans*, (B) *S. aureus*, (C) *P. aeruginosa* and (D) *M. smegmatis* by *M. paniculata* leaf essential oil with minimum inhibitory concentrations (MIC). **c** A. Growth inhibition of *C. albicans* at MFC 8 µg/mL, A1. visible growth of

*C. albicans* at less than MFC; B. growth inhibition of *S. aureus* at MBC 32 µg/mL, B1. visible growth of *S. aureus* at less than MBC; C. growth inhibition of *P. aeruginosa* at MBC 16 µg/mL, C1. visible growth of *P. aeruginosa* at less than MBC; D. growth inhibition of *M. smegmatis* at MBC 8 µg/mL, D1. visible growth of *M. smegmatis* at less than MBC





**Fig. 4** Photomicrograph of *C. albicans* (A—control cells, A1 and A2—treated cells), *S. aureus* (B—control cells, B1 and B2—treated cells), *P. aeruginosa* (C—control cells, C1 and C2—treated cells) and *M. smegmatis* (A—control cells, A1 and A2—treated cells) under

scanning electron microscope. *M. paniculata* leaf essential oil at MIC concentration were applied on the micro-organisms and some of the damaged cells were marked with arrows

the similar compounds, the relative percentage of  $\delta$ -Elemene **2** and Caryophyllene **5** is higher in the leaf oil representing India than the *M. paniculata* leaf oils of other countries (Dosoky et al. 2016; Chowdhury et al. 2008). A few compounds are first time reported from *M. paniculata* leaf essential oil are, 5-methyl thiazole **1**,  $\beta$ -cadinene **8**, viridiflorene **10**, elixene **11**, patchoulane **17**, longifolene **18**, guaialol **19**, rosifoliol **20**, dehydro-aromadendrene **22**,  $\gamma$ -muurolene **23**, isophytol **26**, 4,4-dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptanes **13**, naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl) **14**, *N*-hexadecanoic acid **27**, 3,3'-Bi-p-menthane **28** and Bicyclo [5.3.0] decane, 2-methylene-5-(1-methylvinyl)-8-methyl **29** (Fig. 5).

The leaf essential oil of *M. paniculata* showed significant anticandidal activity in our experiment with almost similar MIC and MFC value to standard drug Fluconazole (Table 2) whereas oil was inactive against *C. albicans* reported from Nepal (Dosoky et al. 2016). Literature depicted that, Gram-negative bacteria restricts accumulation of oil on cell wall

and become resistant to the antimicrobial drugs (Oussalah et al. 2007). For this reason, in our experiment MBC is four times greater than the MIC of leaf essential oil against *P. aeruginosa*. Based on earlier studies, the anticancer activity and antimicrobial activity of the leaf essential oil can be attributed to the presence of various groups of chemical entities in the oil. Sesquiterpene hydrocarbons are abundantly present in the leaf essential oil of *M. paniculata* that may be the cause of potential bioactivity of the oil. *M. paniculata* leaf essential oil was active against *Escherichia coli*, *S. aureus*, *Enterococcus faecalis* and *Salmonella typhimurium* with MIC values 0.5–1 mg/mL (Selestino Neta et al. 2016). We have found better antibacterial activity against Gram-positive bacteria *S. aureus* (Table 2) than earlier report (MIC 1 mg/mL) by Selestino Neta et al. 2016. Previous studies also revealed that, the oil had antifungal activity against *Aspergillus niger*, *A. fumigatus*, *A. parasiticum* and *Fusarium solani* with MIC 0.1–0.2 mg/mL and Dosoky et al. (2016) reported that *M. paniculata* leaf oil is inactive against *C. albicans* and *Bacillus cereus* (Dosoky et al.

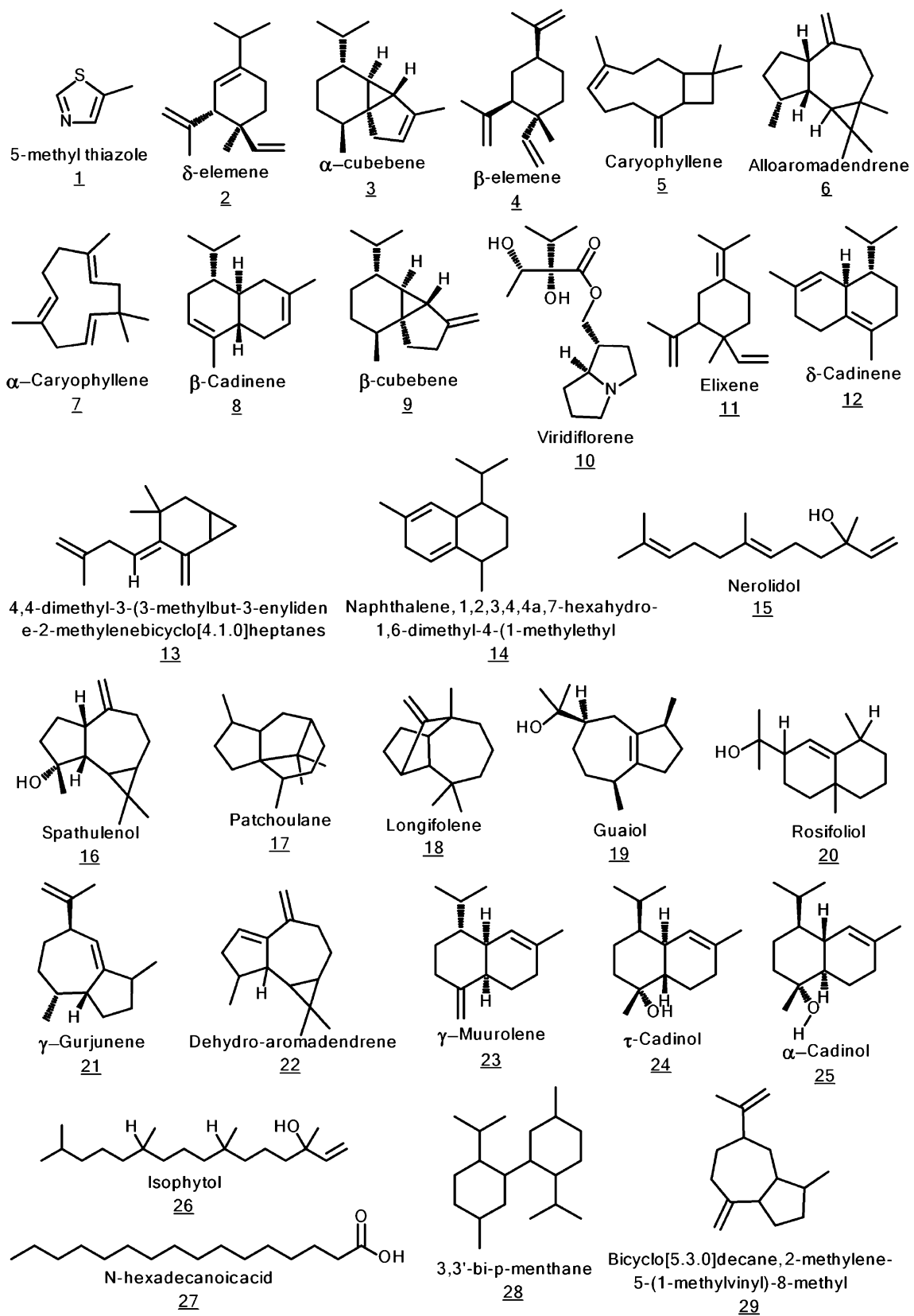


Fig. 5 Structures of the identified compounds from leaf essential oil of *M. paniculata*

2016; Selestino Neta et al. 2016). In our experiment, *M. paniculata* leaf essential oil showed better antifungal activity (Table 2) than previous reports. Reports suggested that  $\beta$ -caryophyllene possesses bactericidal activity against Gram-positive bacteria *Bacillus cereus*, *S. aureus*, *E. faecalis* and Gram-negative bacteria *E. coli*, *S. typhimurium* (Moo et al. 2020; Selestino Neta et al. 2016). In another report, the compound was significantly able to inhibit the growth of bacterium *P. aeruginosa* and fungal cells *Trichoderma reesei*, *Aspergillus* spp. and *F. solani* (Dahham et al. 2015; Selestino Neta et al. 2016).  $\beta$ -Caryophyllene has the ability to damage the cell permeability of bacterial cells that was proved by various experiments on *B. cereus* cells. As a result of interaction between compound and cell membrane some non-selective pores were formed that results into leakage of intracellular materials of microbial cells (Moo et al. 2020). Thus, the cell integrity is disturbed and finally cells died. This mechanism is possible for other microbial cells also. Perez-Lopez reported that  $\delta$ -cadinene is responsible for antibacterial activity against *Streptococcus pneumoniae* (Perez-Lopez et al. 2011). Cis and trans-nerolidol was also reported to possess antimicrobial activity against *C. albicans*, *Salmonella enterica* and *S. aureus* (Chan et al. 2016). According to Togashi et al., long-chain sesquiterpene alcohols possess bactericidal activity (Togashi et al. 2007). Nerolidol has potential activity against MSSA (methicillin-susceptible *Staphylococcus aureus*) and MRSA (Methicillin-resistant *Staphylococcus aureus*) strains that can interact with cell membrane and destroy the cell integrity by releasing  $K^+$  ions (Chan et al. 2016). Moreover,  $\beta$ -caryophyllene,  $\delta$ -cadinene and nerolidol were reported to have anticancer activity. Nerolidol was effective against human cervical (HeLa) and breast cancer (BT 20) cell lines with  $IC_{50}$  values 3.02 and 2.96  $\mu\text{g/mL}$ , respectively (Chan et al. 2016). Another terpenoid  $\delta$ -cadinene showed anticancer activity against human ovarian cancer cell line OVCAR-3. The ovarian cancer cells after treatment with  $\delta$ -cadinene became shrink, detached from the substratum and nuclear material get condensed (Hui et al. 2015). With increasing concentration of  $\delta$ -cadinene cellular blebbing took place and finally apoptosis occurred to the cancerous cells.  $\beta$ -caryophyllene exhibited antiproliferative activity against human colon cancer (HCT 116, HT 29) and pancreatic cancer (PANC-1) cell lines. The HCT 116 cell lines treated with  $\beta$ -caryophyllene showed fragmented DNA and cause condensation of nuclear material (Dahham et al. 2015). So, in our experiment there are two possibilities related to bioactivity of the essential oil. The major compound caryophyllene alone is responsible or synergistic effect of active compounds like  $\delta$ -cadinene, nerolidol, caryophyllene, etc. play major role to inhibit the growth of microbial cells and cancer cells.

Mechanism of antimicrobial effect of the *M. paniculata* leaf essential oil on microbial cells was analyzed by

scanning electron microscopic (SEM) study. The cellular morphology of the tested micro-organisms observed under SEM, depicted that cells lost their normal structure after the application of leaf essential oil at minimum inhibitory concentrations (Fig. 4). *P. aeruginosa* is a Gram-negative bacterium consists of thick cell wall with outer lipopolysaccharide and inner peptidoglycan layer whereas, Gram-positive bacteria *S. aureus* consist of outer thick layer of only peptidoglycan (Selvamani et al. 2020). Oil-treated cells of *P. aeruginosa* and *M. smegmatis* showed porous structure through which intracellular materials are moved outside (Fig. 4). Untreated cells of acid-fast bacteria *M. smegmatis* showed distinct rod-shaped structure under SEM, whereas oil-treated *M. smegmatis* cells undergone structural deformation. Cell wall is composed of mycolic acid that provide waxy texture to *M. smegmatis*, after oil treatment we noticed that cells are attached with each other losing their original structure and finally got lysed (Fig. 4). On the other hand, oil-treated *S. aureus* and *C. albicans* cells noticeably showed rough surface and cell shrinkage (Fig. 4). Whereas normal cells were observed with smooth surface. The structural rigidity of *C. albicans* cells is provided by its cell wall which is formed of  $\beta$ -glucan–chitin skeleton (Garcia Rubio et al. 2020). Oil-treated *C. albicans* cells became flattened with ridges like appearance. Generally, bacterial cells are surrounded by a layer of polysaccharide molecule called peptidoglycan that provides cell shape and structural rigidity by maintaining the turgor pressure. When antimicrobial agents are applied this peptidoglycan biosynthesis is blocked that results into cell wall disorganization and finally the cells die (Abrahams and Besra 2018; Neu and Gootz 1996). *M. paniculata* leaf essential oil mostly contains sesquiterpene compounds which are lipophilic in nature (Chadwick et al. 2013) and act as antimicrobials on tested pathogens. Due to the presence of sesquiterpene compounds, oil can easily interact with the phospholipid bilayer and damage the outer cover of bacterial cells. As a result, physical integrity of the cells is lost and affects the cellular mechanism (Sikkema et al. 1995). One-third of the cellular proteins of bacteria are embedded in cell membrane that is essential site for cell functions. When lipophilic compounds are accumulated on cell membrane they disturb the function of proteins as well as enzymes attached on the cell membrane (Sikkema et al. 1995). Therefore, it can be suggested that damage of bacterial cells by *M. paniculata* leaf essential oil occur mainly due to sesquiterpene-lipid interaction as well as sesquiterpene-protein interaction. In case of yeast cells, the mechanical strength is achieved through a network of carbohydrate molecules viz., chitin, mannan and  $\beta$ -glucan present as cell wall components (Torey et al. 2016). Cell wall is the first target of any antimicrobials,

when sesquiterpene rich essential oil interacts with the yeast cell wall components normal function of the cells disrupted.

## Conclusions

The chemical composition of the leaf essential oil of *M. paniculata* of Assam, India was found to be different from those reported from Nepal, Bangladesh, China, Brazil and Nigeria. Most of the compounds identified in the oil belonged to the sesquiterpenes class. A few compounds of this oil viz., 5-methyl thiazole,  $\beta$ -cadinene, viridiflorene, elixene, nerolidol, patchoulane, longifolene, guaiol, rosi-foliol, dehydro-aromadendrene,  $\gamma$ -muurolene, 3,3'-bi-p-menthane, isophytol and N-hexadecanoic acid, etc. were reported for the first time from this region. It was found for the first time that the leaf essential oil possesses very good antibacterial activity against *Mycobacterium smegmatis* (MIC 4  $\mu\text{g/mL}$ ) and *Pseudomonas aeruginosa* (MIC 4  $\mu\text{g/mL}$ ). The oil also showed excellent activities against *S. aureus* and *C. albicans* as compared to the earlier report. Moreover, anticancer activity of the oil was also studied for the first time against human carcinoma cell lines, HeLa (cervix), MIA PaCa2 (pancreatic) and PA1 (ovarian) using MTT assay. The best anticancer activity was observed for HeLa cells (IC<sub>50</sub> = 6.28  $\mu\text{g/mL}$ ). The antimicrobial and anticancer activity of the oil can be attributed to the most abundant compound caryophyllene **5** (20.93%) and synergistic effect of bioactive compounds identified in the leaf essential oil. Further, scanning electron microscopic studies revealed that the oil kills micro-organisms with the deformation of cellular morphology of the microbial cells. Drug resistance in microbial pathogens and cancer causes severe health issues in human being all over the world. Thus, the essential oil of *M. paniculata* leaf from Assam, India can be an excellent alternative source of new antimicrobials and anticancer agents for the pharmaceutical industries.

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## Declarations

**Conflict of interest** The authors have no conflict of interests to declare.

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