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Antibacterial activity of four traditional medicinal plants from Western Ghats, India and identification of most active extract by GC-MS analysis

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Abstract
Infectious diseases caused by microbes lead to the increase in the death toll every year. Bacterial strain undergoes chromosomal mutation and thereby achieve antibiotic resistant which stands out to be an important reason for the current health problems. Medicinal plants inhibit the growth of microbes and helps in achieving better health. In the present study, *in vitro* antibacterial activity was conducted for the 4 medicinal plants *Alpinia galanga* (L.) Willd, *Blumea lacera*, *Curcuma aeruginosa* Roxb, and *Pentanema indicum* collected from the western ghats against 10 pathogens. The activity was carried out for the different extracts of all 4 plants. *Pentanema indicum* recorded highest activity by inhibiting the growth of tested microbes at a concentration of 5mg/disc, against *Vibrio cholerae with* 17mm, *Staphylococcus aureus* – 15mm, *Pseudomonas fluorescens* – 13mm, *Klebsiella pneumoniae* – 13mm, followed by the chloroform extract of *Pentanema indicum* against *Vibrio cholerae with* 15mm, *Staphylococcus aureus* – 14mm, *Klebsiella pneumoniae* – 13mm. *Alpinia galanga* (Ethyl acetate) gave a result against *Pseudomonas fluorescens* – 15mm, *Bacillus aureus* – 14mm, *Bacillus subtilis* – 13mm. Hence, from the results, it can be concluded that the ethyl acetate extract of *P. indicum* is a potent antibacterial agent.

Keywords: Medicinal plants, infectious disease, bacteria, antibiotic resistance, antibacterial activity

Introduction
Infectious diseases are risking human lives as there is a proliferation and wide spread of pathogenic bacterial strains and fungus (Danish et al., 2020) [1]. Among the many bacterial pathogens, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus mitis*, and *Pseudomonas fluorescens* are a few which are highly dangerous in causing infections (Marasini et al., 2015) [12]. Discovery of several antibiotics in the past were useful to control microbes and thereby reduce the risk factor, however, these microbes in the course of time have gained resistance to such antibiotics (Bhatia et al., 2021) [3]. Antimicrobial Resistance (AMR) is the leading cause for the current health hazards in today's millennium (Prestinaci et al., 2015) [4].
Plants are best medium in producing new antibiotic drugs. In the developing countries, more than two third of the masses rely on plant derived drugs (WHO,2020). Based on the traditional uses of plants, varieties of drugs are found. Plant drugs like Quinine, Morphine, atropine, reserpine, ephedrine are of greater importance in treating infectious diseases (Abdalla et al., 2016) [5]. Alkaloids, phenols, tannins, saponins, steroids, terpenoids, flavonoids, coumarins, organic acids, and cardiac glycosides which possess antimicrobial properties are found in plants of medical importance (Khan et al., 2013, Nduche et al., 2016) [6, 7]. According to the ethnobotanical survey, India is rich with medicinal plants and have documented 48,655 plant species, of which 9,500 species are important from the ethnobotanical point of view and 7,500 species are of medicinal value as practiced by the indigenous people (Gowthami et al., 2021) [8]. Western Ghats in India is rich in biodiversity
The present study is carried out to authenticate the *in vitro* antibacterial property of medicinal plants namely *Alpinia galanga* (L.) Willd, *Blumea lacera*, *Curcuma aeruginosa* Roxb, and *Pentanema indicum* from the western ghats against pathogens – *S. aureus, E. faecalis, S. mitis, B. subtilis, B. cereus, E. coli, K. pneumoniae, P. aeruginosa, V. cholerae* and *P. fluorescens.*

Materials and method
Collection of plant materials
For antibacterial investigation, 4 plants viz. *Alpinia galanga* (L.) Willd (Rhizome), *Blumea lacera* (Whole plant), *Curcuma aeruginosa* Roxb (rhizome) and *Pentanema indicum* (whole plant)) were field collected from Palakkad district, Kerala, India (Latitude - 10.786703^0 N Longitude – and 76.653145^0 E). The collected plants were validated and submitted to herbarium, Entomology Research Institute, Loyola college, Chennai-600 034.

Plant crude extraction
The freshly collected plants were cleansed in the clean tap water and were subjected to shade dry for 20 days. The rhizomes were also washed cleanly and allowed for drying. After 23 days of drying, they were pulverised. Hexane, chloroform, ethyl acetate and methanol solvents Successive extraction method by cold percolation was carried out to get the extraction yield (Duraipandiyan et al., 2009) [9]. One kilogram of each plant extract was soaked in hexane (1:3lit) for 72 h and periodically shaken so as to bring a complete and uniform soaking and then it was filtered. Concentration of the filtered solvent was carried out using vacuum rotary evaporator and the extract was collected. Other extracts were collected in the like manner.

Test organism
Ten types of microorganisms used for the antibacterial study from National Culture Collection Centre (NCCC), Trichy, Tamil Nadu, India. Five-gram positive bacteria namely *S. aureus* (NCT45), *E. faecalis* (NCT34), *S. mitis* (NCT12), *B. subtilis* (NCT21), *B. cereus* (NCT11) and five-gram negative bacteria namely *E. coli* (NCT01), *K. pneumoniae*

(NCT40), *P. aeruginosa* (NCT114), *V. cholerae* (NCT25), and *P. fluorescens* (NCT338), were used for the study.

Preparation of microbial Inoculum

Bacterial cultures obtained from National Culture Collection Centre (NCCC), were maintained as the stock culture from which new set of bacterial cultures were established by sub-culturing in the MH agar and was incubating at 37˚C for 24 h. Thereafter, fresh colonies which were developed were removed using sterile inoculating loop. Collected colonies were transferred into a new sterile solution. The turbidity was maintained at 0.5 McFarland's standard solution (Esimone et al., 2012) [11]. Each bacterial strain's inoculum was introduced into 3ml of Mueller Hinton Broth and incubated at 37ºC for 24 hours. Following the incubation, the culture was diluted.

Disc diffusion method

For investigating this study Disc diffusion method was adopted (Duraipandiyan et al., 2007) [12]. Sterile Muller Hinton Agar (20ml) was prepared and poured in to petri plates. After the solidification of the media, swabbing of the test culture (100µl of suspension containing 108 CFU/ml bacteria) was done and was left undisturbed for 10 minutes to bring about the drying of the culture. A concentration of 5mg per disc of the extracts were used for the test. To the sterile discs, extract dissolved in Dimethyl sulfoxide (DMSO) was loaded. Ciprofloxacin 5µg was used as positive control. The control discs and discs loaded with dilute plant extracts were placed on the surface of agar plates. The plates were incubated for 24 h at 37ºC. After incubation, the zone of inhibition was recorded in millimeters and noted.

Gas chromatography-mass spectrometry

GC-MS was carried out at the Indian Institute of Technology, Madras for Gas GC-MS study (GC-MS-5977 MSD) so as to list the number of phytochemicals present in the extract (Ganesan et al., 2018) [10]. The dimension of the column was 30 m X250 µm X 0.25µm and HP 5ms, with a temperature range between -60˚ C - 325˚ C.

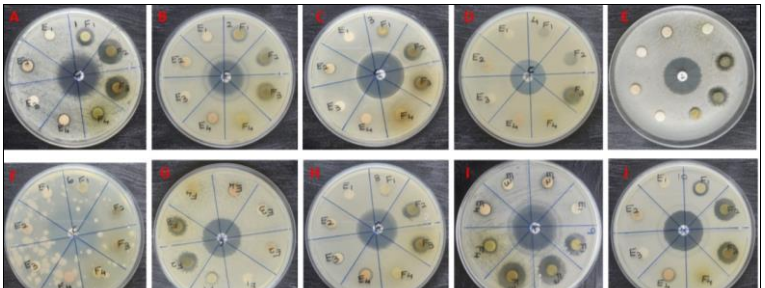
Results

Plant extraction

The plant materials soaked in different solvents were concentrated and filtered using vacuum rotary evaporator and the crude extracts were collected and preserved for biological assays. The yield of the plant extracts was measured in grams.

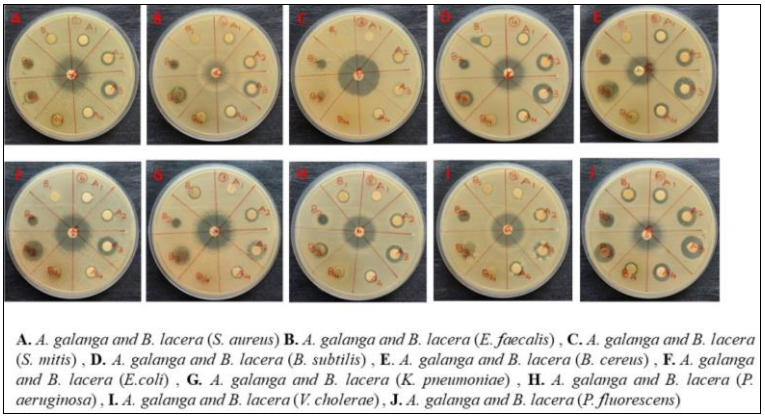
Disc diffusion method

Antibacterial activity for the 4 selected plants was done against 10 pathogens. Among 16 crude extracts obtained, ethyl acetate extract of *Pentanema indicum* showed highest activity by inhibiting the growth of tested microbes at a concentration of 5mg/disc, (*Vibrio cholerae* - 17mm, *Staphylococcus aureus* – 15mm, *Pseudomonas fluorescens* – 13mm, *Klebsiella pneumoniae* – 13mm*)* followed by the chloroform extract of *Pentanema indicum* (*Vibrio cholerae* - 15mm, *Staphylococcus aureus* – 14mm, *Klebsiella pneumoniae* – 13mm*) Alpinia galanga* (Ethyl acetate extract) showed activity against tested microbes (*Pseudomonas fluorescens* – 15mm, *Bacillus aureus* – 14mm, *Bacillus subtilis* – 13mm*)*. The detailed results are tabulated and displayed. (Figure 1,2) (Table 1).



A. *C. aeruginosa and P. indicum* (*S. aureus*) **B.** *C. aeruginosa and P. indicum* (*E. faecalis*) , **C.** *C. aeruginosa and P. indicum* (*S. mitis*) , **D.** *C. aeruginosa and P. indicum* (*B. subtilis*) , **E.** *C. aeruginosa and P. indicum* (*B. cereus*) , **F.** *C. aeruginosa and P. indicum* (*E.coli*) , **G.** *C. aeruginosa and P. indicum* (*K. pneumoniae*) , **H.** *C. aeruginosa and P. indicum* (*P. aeruginosa*) , **I.** *C. aeruginosa and P. indicum* (*V. cholerae*) , **J.** *C. aeruginosa and P. indicum* (*P. fluorescens*)

Fig 1: Antibacterial activity of *C. aeruginosa* and *P. indicum*



A. *A. galanga and B. lacera* (*S. aureus*) **B.** *A. galanga and B. lacera* (*E. faecalis*) , **C.** *A. galanga and B. lacera* (*S. mitis*) , **D.** *A. galanga and B. lacera* (*B. subtilis*) , **E.** *A. galanga and B. lacera* (*B. cereus*) , **F.** *A. galanga and B. lacera* (*E.coli*) , **G.** *A. galanga and B. lacera* (*K. pneumoniae*) , **H.** *A. galanga and B. lacera* (*P. aeruginosa*) , **I.** *A. galanga and B. lacera* (*V. cholerae*) , **J.** *A. galanga and B. lacera* (*P. fluorescens*)

Fig 2: Antibacterial activity of *A. galanga* and *B. lacera*

Table 1: Zone diameter of inhibition (mm) of the selected plants crude extracts against selected 10 pathogens

Plant name	Extracts	Antibacterial activity (Zone of clearance in mm)									
		S.a	***E.f***	***S.m***	***B.s***	***B.a***	***E.c***	***K.p***	***P.a***	***V.c***	***P.f***
A. Galangal rhizome	He	7	7	-	8	9	7	-	7	-	10
	Ch	8	8	7	12	12	7	7	9	9	13
	Ea	10	10	-	13	14	11	10	11	11	15
	Me	7	8	-	8	9	7	7	7	7	11
B. Lacera whole plant	He	-	-	-	7	-	-	7	7	-	10
	Ch	-	-	-	-	-	-	-	-	-	9
	Ea	8	9	-	8	12	12	-	10	-	13
	Me	7	-	-	-	-	-	-	-	-	7
C. aeruginosa rhizome	He	-	7	-	-	-	-	-	-	-	-
	Ch	8	7	7	-	-	-	-	-	7	-
	Ea	-	-	-	-	-	-	-	-	-	-
	Me	7	7	-	-	-	-	-	7	7	-
P. indicum whole plant	He	11	10	8	-	-	-	7	-	13	9
	Ch	14	10	11	7	8	-	12	10	15	11
	Ea	15	10	11	10	9	-	13	12	17	13
	Me	7	-	-	-	-	-	-	-	7	-
Control Ciprofloxacin (5mg)		30	25	30	23	22	37	25	24	26	25

He- Hexane, Ch – Chloroform, Ea – Ethyl acetate, Me- Methanol

S. a – Staphylococcus aureus, E. f – Enterococcus faecalis, S. m – Streptococcus mitis, B. s – Bacillus subtilis, B. a – Bacillus aureus, E. c – Escherichia coli, K. p – Klebsiella pneumoniae, P. a – Pseudomonas aeruginosa, V. c – Vibrio cholerae, P. f – Pseudomonas fluorescens

Gas chromatography-mass spectrometry

The GC-MS of *P. indicum* (ethyl acetate) showed the existence of 23 secondary metabolites of which 1,2,3-Propanetriol, 1-acetate, is the major compound. (Figure 3) (Table 2).

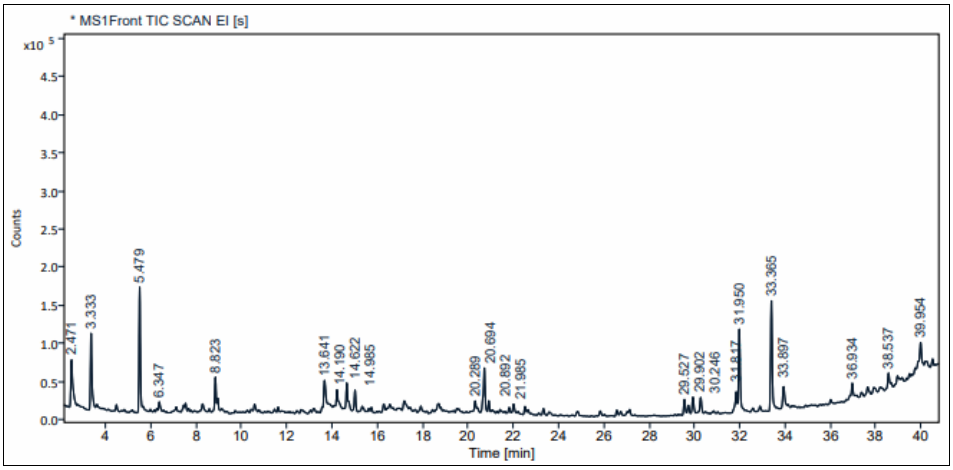


Fig 3: Chromatogram of *P. indicum*

Table 2: List of major compounds identified in the Ethyl acetate extract of *Pentanema indicum* whole plant extract through GC-MS

Sl. No	Compound	R.T.	Area %	Molecular formula	Molecular weight (g/mol)	Reported bioactivity	Reference
1	2-Butenoic acid, 2-methyl-	2.471	5.37	$C_5H_8O_2$	100.1158	antibacterial	Babu et al., 2023 [19]
2	Glycerin	3.333	7.73	$C_3H_8O_3$	92.09	antibacterial	Irnawati et al., 2024 [20]
3	1,2,3-Propanetriol, 1-acetate	5.479	15.50	$C_5H_{10}O_4$	134.1305	antibacterial	Saeet al., 2023ed
4	Ribitol	6.347	1.03	$C_5H_{12}O_5$	153.14	antibacterial	Price et al., 2017 [22]
5	4-Methoxychalcone	8.823	3.22	$C_{16}H_{14}O_2$	238.286	antibacterial	Luzhanin et al., 2022 [23]
6	Tenamfetamine	13.641	4.40	$C_{10}H_{13}NO_2$	179.22	antibacterial	Venkatraman et al., 2023 [24]
7	2-Propenoic acid, 3-phenyl-, ethyl ester, (E)-	14.190	1.57	$C_{11}H_{12}O_2$	176.2118	Antibacterial	Duraisamy et al., 2024 [25]
8	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	14.622	2.61	$C_{15}H_{22}$	202.3352	antimicrobial	Shareef et al., 2016 [26]
9	Pentadecane	14.985	2.95	$C_{15}H_{32}$	212.41	Antibacterial	Chuah et al., 2018 [27]
10	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	20.289	0.90	$C_{10}H_{12}O_3$	180.2005	Antibacterial	Palchykov et al., 2020 [28]
11	Ethyl p-methoxycinnamate	20.694	4.03	$C_{12}H_{14}O_3$	206.2378	antimicrobial	Lakshmanan et al., 2011 [29]
12	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-	20.892	1.32	$C_{11}H_{16}O_3$	196.2429	antibacterial	Francis et al., 2021 [30]

	2(4H)-one						
13	Cyclopropa [c, d] pentalen e-1,3-dione, hexahydro-4-(2-methyl-2	21.985	0.83	$C_{11}H_{18}O$	166.26	-	Ivankin et al., 2017 [31]
14	Phytol	29.527	1.60	$C_{20}H_{40}O$	296.5	antibacterial	Lee et al., 2016 [32]
15	2-(2-Carboxyethyl)-6,6-dimethyl-3-oxocyclohex-1-enecarboxylic acid	29.902	1.87	$C_{12}H_{16}O_5$	240.25	-	Dahdouh et al., 2023 [33]
16	10,13-Octadecadiynoic acid, methyl ester	30.246	1.78	$C_{19}H_{34}O_2$	290.440	antimicrobial	Elghwas et al., 2024 [34]
17	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	31.817	2.07	$C_{20}H_{40}O$	296.5	Antimicrobial	Sharmila *et al*., 2016 [35]
18	5,5,8a-Trimethyldecalin-1-one	31.950	12.22	$C_{13}H_{22}O$	194.31	antibacterial	Al-Tamimi et al., 2020 [36]
19	Styrene, 2,3,5,6-tetraethyl-4-vinyl-	33.365	17.71	$C_{18}H_{26}$	242.4	-	Malik et al., 2022 [37]
20	10,18-Bisnorabieta-8,11,13-triene	33.897	3.87	$C_{19}H_{28}$	256.4256	Antibacterial	Ghaly et al., 2020 [38]
21	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	36.934	1.80	$C_{37}H_{72}O_4$	581.0	Antimicrobial	Daniels et al., 2021 [39]
22	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-	38.537	2.27	$C_{27}H_{40}O_4$	428.6	Antibacterial	Jalil et al., 2022 [40]
23	Oleic acid, 3-(octadecyloxy)propyl ester	39.954	3.34	$C_{39}H_{76}O_3$	593.0189	Antimicrobial	Abubacker et al., 2014 [41]

Discussion

Microbes play havoc in human lives as they are known to cause infectious diseases. Controlling these pathogens is essential so as to control the spread of diseases. However, despite great efforts, they are uncontrollable as they are resistant to antibiotics. This is due to the overuse of antibiotics drugs (Manso et al., 2021) [13]. Mutation in the chromosomes and horizontal gene transfer within the bacteria is the cause for antibiotic resistance (Breijyeh et al., 2024) [14]. Undoubtedly antibiotics of plant origin which are rich in phytochemicals are extensively used to control pathogens (Abdallah et al., 2023) [15].

In this study, 4 plants viz., *Alpinia galanga* (L.) Willd, *Blumea lacera*, *Curcuma aeruginosa* Roxb, and *Pentanema indicum* were studied for the *in vitro* antibacterial activity against – *S. aureus*, *E. faecalis*, *S. mitis*, *B. subtilis*, *B. cereus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *V. cholerae* and *P. fluorescens* using different extracts. *Pentanema indicum* ethyl acetate extract exhibited highest activity against *Vibrio cholerae* - 17mm, *Staphylococcus aureus* – 15mm, *Pseudomonas fluorescens* – 13mm, *Klebsiella pneumoniae* – 13mm. This was followed by the chloroform extract of *P. indicum* against *Vibrio cholerae* - 15mm, *Staphylococcus aureus* – 14mm, *Klebsiella pneumoniae* – 13mm. This result could be compared with the studies carried out by Keer et al., (2021) [16] on the ethyl acetate and methanolic extract of *P. indicum* leaves against 4 bacteria viz., *B. subtilis*, *E. coli*, *S. aureus*, and *S. griseus*. The methanolic extract showed highest activity against *S. aures* with a zone of clearance of 20.00±0.92 mm while ethyl acetate showed highest activity against *E. coli* with a zone of clearance of 20.00±0.60 mm.

Ram et al., (2004) [17], carried out *in vitro* antimicrobial activity against 4 bacterial strains using ethanolic extracts of 23 medicinal plants from eastern ghats. Highest antibacterial activity was observed in *P. indicum* with 32 mm zone of inhibition against *M. luteus*, followed with 24 mm against *S. aureus*, 23 mm against *P. aeruginosa* and 20 mm against *M. roseus*. A similar study was performed by Ram et al., (2006) [18], using the essential oil of *P. indicum* var. *sivarajanianum* (L.) Ling against 4 pathogens. The study confirms that this plant possessed antibacterial activity with hexadecane, α-humulene, myrecene and β-thujone as the major compounds.

However, a very little *in vitro* antibacterial activity is gone into using *P. indicum.* This study proves that *P. indicum* is effective in controlling pathogenic bacteria.

Conclusion

India is rich in possessing a variety of traditional medicinal plants particularly in the eastern and western Ghats. From among the 4 medicinal plants, 3 plants exhibited antibacterial activity against 10 bacterial pathogens. Highest activity was recorded in the *P. indicum* (ethyl acetate) against *Vibrio cholerae* - 17mm, *Staphylococcus aureus* – 15mm, *Pseudomonas fluorescens* – 13mm, *Klebsiella pneumoniae* – 13mm. This was followed by the chloroform extract of *P. indicum* against *Vibrio cholerae* - 15mm, *Staphylococcus aureus* – 14mm, *Klebsiella pneumoniae* – 13mm. *Alpinia galanga* (ethyl acetate) showed good activity against tested microbes *Pseudomonas fluorescens* – 15mm, *Bacillus aureus* – 14mm, *Bacillus subtilis* – 13mm. It can be concluded that the medicinal plants can be used as potential antibacterial agents.

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