In Vitro Antibacterial Efficacy Of Some Ayurvedic Plants Against Biofilm Forming MDR Enteric Bacteria And GC-MS Analysis Of The Active Extract

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Abstract-Infectious diseases are still one of the leading causes of human mortality and morbidity across the globe. Among bacteria, emergence and spread of antimicrobial resistance (AMR) globally has threatens the successful treatment of infectious diseases. The continuous emergence of new types of resistant bacterial pathogens have decreased the efficacy of available antibacterial drugs. Evaluation of medicinal plants known for their biological activity against MDR bacteria is needed to explore and exploit the rich diversity of bioactive extracts of medicinal plants as an alternative antibacterial agent in combating AMR. In this study, methanolic extracts 10 medicinal plants were screened for antibacterial activity. The extracts were preliminary screened against strong biofilm forming MDR bacteria by agar well diffusion method at the concentration of 1.0 mg/mL of each plant extract. The zone of inhibition was measured for the comparative analysis of their antibacterial activity. Among tested plants, *Acoruscalamus* exhibited considerable antibacterial activity with mean of zone of inhibitions 15.7 mm against test isolates and highest zone of inhibition was found to be against *E. coli* isolates ECM4 (17.6±0.57). *Holarrhena antidysentrica* and *Hemidesmusindicus* showed moderate antibacterial activity. *Terminaliachebula*, *Punicagranatum* and *Plumbagozeylanica* showed relatively low activity against test isolates with mean of zone of inhibitions less than 12.66 mm. The MIC of extracts ranged from 0.125 mg/mL to 4 mg/mL against test bacteria. It was found that methanolic extract of *A. calamus* showed considerable antibacterial activity with lowest MIC 0.125 mg/mL. MIC against ESβL producing bacterial isolates ranged from 0.125 mg/mL to 2.0 mg/mL. The findings indicated that bioactive extracts might be effective in treating infection caused by MDR bacteria.

Keywords: Antimicrobial Resistance; Plant Extracts; Indian Medicinal Plants; Esβl; *Acorus calamus*.

Introduction

Infectious diseases are still one of the leading causes of human mortality and morbidity across the globe. Annual deaths worldwide due to AMR continue to climb to around 750,000 and are projected to reach as high as 10 million by the year 2050 (O'Neill, 2016). Emergence and spread of antimicrobial resistance (AMR) globally have threatens the successful treatment of infectious diseases (McEwen and Collignon, 2018). Global efforts are going on to understand drivers of AMR and to develop strategies to tackle AMR problem.Manychallenges both scientific and economic in antimicrobial development has caused slow development of new drugs (WHO, 2019a; WHO, 2019b). Various measures have been suggested to prevent rise and spared of AMR and to including use of alternative antibiotics in poultry and agriculture and possibly in combinational therapy. Medicinal plants traditionally used in Indian system of medicine (Ayurveda, Unani, and Siddha) are rich source of bioactive compounds and antimicrobial secondary metabolites.

Plant secondary metabolites are important for the adaptation in plants to environment they also ser serve as surveillance system. Secondary metabolites are the byproducts of primary metabolic pathways, and these are also responsible for specific tastes, odors, and colors of plant tissues. Such metabolites assist plant to cope with abiotic stresses. They are important for proper development and growth (Kessler and Kalske, 2018; Zaynab et al., 2018; Wink, 2020). Plant secondary metabolites commonly fall in one of these chemical classes, *viz.*terpenoids, phenolics and alkaloids. Terpenoids are the most diverse secondary metabolite groups, and it comprises of >50,000 known phytocompounds (Chassagne et al., 2019; Belcher et al., 2020).

Due to continuous emergence of new types of resistant bacterial pathogens the efficacy of available antibacterial drug is decreasing. On the other hand, plant-based screening studies have mainly utilized sensitive or less commonly resistant bacteria in test system. Such information become less relevant when new resistant strains develop. WHO has classifies these resistant bacterial pathogens in to different categories based on their pathogenicity and problematic nature (WHO, 2016).Extended spectrum β-lactamases (ESβL) enteric bacteria including *E. coli*, *Klebsiella*, Enterobacter etc. have been identified as one of the major problematic MDR bacteria (Adler et al., 2020). Evaluation of medicinal plants known for their biological activity and safe use in traditional medicine against such MDR bacteria is needed to explore and exploit the rich diversity of bioactive extracts of medicinal plants as alternative antibacterial agent in combating AMR. In the present study, Indian plants were evaluated against MDR bacterial strains and the most active extract of *A. calamus* was subjected to GC MS analysis to identify major compounds.

Material and Methods

Collection of plant material

The authentic plant samples were provided as gift from the Himalaya Drug Company, Dehradun, India. Some of the plant materials were purchased from local authorized supplier, Aligarh UP, India in January-March 2015. The taxonomic identification of the plant material was confirmed by Professor S. Hayat, Department of Botany, AMU, Aligarh (India). The voucher specimen has been submitted in the Department of Agricultural Microbiology, Faculty of Agricultural Sciences, AMU, Aligarh.

Preparation of methanolic plant extracts

The methanolic plant extracts were prepared by using method as described earlier by (Aqil et al., 2005). The plant materials were shade dried and powdered. Five hundred grams (500 g) of dried and powdered plant material was soaked in 2.5 liter of methanol (purity ≥ 99.8%) for 5 days with intermittent shaking. The extract was filtered through Whatman filter paper No.1 and concentrated to dryness under reduced pressure on rotatory evaporator (RE-2000A, Associated Scientific Technologies, Delhi, India) at 40°C and stored at 4°C for further study. The yield of crude methanolic plant extracts was obtained and calculated in percentage of total dry weight of starting material. The dried crude extracts were reconstituted in DMSO (<1%) to prepare stock/working solutions of desired concentrations needed for experiments.

Determination of antibacterial activity

The antibacterial activity of plant extracts was determined by agar well diffusion method described previously (Perez et al., 1990) with little modification as adopted earlier (Aqil et al., 2005). Briefly, 0.1 mL of freshly grown diluted inoculum (10^5 CFU/mL) of test organism was spread on Muller-Hinton agar plates. Wells of 8 mm diameter were punched into the agar medium and filled with plant extract of final concentration of 1.0 mg/mL and solvent blank (<1% DMSO) separately. The plates were incubated for overnight at 37°C. The antibacterial activity was evaluated by measuring the zone of inhibition against test organism. Antibiotic to which organism was sensitive was used as control.

Determination of MIC of plant extracts

The minimal inhibitory concentration (MIC) of plant extracts against bacterial strains was determined by broth microdilution susceptibility testing method as described by Eloff et al., (1998) using 2,3,5-triphenyltetrazohum chloride [TTC, tetrazolium red, purity min. 99%) as a growth indicator dye. The concentrations of plant extracts were two-fold serially diluted ranged from 0.0125 to 4 mg/mL in a sterile 96-well polystyrene microtiter plates 100µl of bacterial inoculum (≈1×10^6 CFU/mL) were added to each well. The covered microtiter plates were incubated for 18 h at 37°C. To indicate

bacterial growth, 40 µl of TTC dissolved in water (2 mg/mL w/v) were added to each well and incubated at 37°C for 30 min. The wells were examined for the color change to red, indicated the actively growing cells while no change in color indicate lack of bacterial growth. MIC was defined as the minimum concentration of plant extracts which inhibited the visible growth of test strains.

Phytochemical analysis of plant extracts by color test

Selected plant extracts were screened for the presence of major phytocompounds using standard methods as described below:

Alkaloids: Two mL of extract was taken in 5 mL distilled water and mixed with hydrochloric acid (2.0 M). Once the acid reaction stopped, 1 mL of Drangendorff's reagent was added. A red or orange precipitate indicates the presence of alkaloids (Kapoor et al., 1969; Wagner and Bladt, 1996).

Flavonoids: The method described by Kapoor et al., (1969), was used for detection of flavonoids. Hundred microliter extract (100 mg/mL) was dried over a water bath followed by the addition of 5-10 drops of concentrated hydrochloric acid. Thereafter, a pinch of Zn powder in reaction mixture was added. A pink or brown color indicates the presence of flavonoids.

Glycosides: Hundred microliter extract (100 mg/mL) was dissolved in distilled water and 1 mL of Sodium hydroxide solution (1%) was added. The development of yellow color indicates the presence of Glycosides (Odebiyi and Sofowora, 1978).

Phenols: Hundred microliter extract (100 mg/mL) was dissolved in distilled water and few drops of 10% aqueous ferric chloride solution were added. The presence of phenolic compounds will be indicated by development of blue green color (Fadeyi and Akpan, 1989).

Tannins: a few drops of 5% aqueous ferric chloride were added into 1 mL of extract followed by the addition of dilute sulphuric acid to the reaction mixture. On acidification, the development of blue black or green browncolor indicates the presence of tannins (Segelman and Farnsworth, 1969).

Saponin: Hundred microliter extract (100 mg/mL) was taken and few drops of 1% sodium carbonate solution was added. Mixture was shaken vigorously and left stand still for 3 minutes. A honeycomb like froth indicated the presence of saponins (El-Tawil, 1983).

Gaschromatography-mass spectrometry (GC-MS) of *A. calamus*

Methanolic extract of *A. calamus*was analyzed by Gas chromatography-mass spectrometer (Instrument model GCD 1800A, Hewlett Packard). The sample was injected into a split inlet at 260°C, with a split ratio 1:10. Helium (purity 99.999%) was used as a carrier, with a constant flow of 1.21 mL/min. The separation was achieved on HP-1 column (Thermo scientific), using the following temperature program: start at 700C and hold for 2 min, 5°C/min to 250°C and hold for 2 min, 10°C/min to 280°C and hold for 17.0 min (total run time 50 min). Elute was delivered to the mass spectrometer with Ion source temperature 230°C and interface temperature 270°C Data was acquired in Scan mode (m/z range 40–650). The compounds were identified by mass spectra comparison with libraries (Wiley Registry of Mass Spectral Data 7th ed. (McLafferty, 2005), and NIST/EPA/NIH Mass Spectral Library 05 (NIST/EPA/NIH, 2005). Relative amounts of components, expressed in percentages, were calculated by normalization measurement according to peak area in total chromatogram.

Results

Antibacterial activity of methanolic extracts of medicinal plants

A total of 10 medicinal plants extracts were collected for this study to evaluate the antibacterial activity. Methanolic extracts of all selected plants were primarily screened against MDR bacteria by agar well diffusion method at the concentration of 1.0 mg/mL of each plant extract. The zone of inhibition was measured, and data recorded is summarized in **Table-1**. *Acoruscalamus* exhibited considerable antibacterial activity with mean of zone of inhibitions 15.7 mm against test isolates and highest zone of inhibition was found to be against *E. coli* isolates ECM4 (17.6±0.57). *Holarrhena antidysentrica* and *Hemidesmusindicus* showed moderate antibacterial activity with mean of zone of inhibitions 14.96 mm and 13.49 mm against test isolates respectively and maximum zone of inhibition was found against KPMA19 (16.6±0.57mm) and ECMA2 isolates (15.3±1.52) respectively. *Terminaliachebula,*

Punicagranatum and *Plumbagozeylanica* showed relatively low activity against test isolates with mean of zone of inhibitions less than 12.66 mm. Four methanolic plants extracts namely *Cuminumcyminum*, *Elettaria cardamomum*, *Foeniculum vulgare* and *Psidiumguajava* showed no significant activity against bacterial isolates at tested concentration. On comparing mean of zone of inhibitions, the activity of above plants extracts was found highest in *A. calamus*followed by *H. antidysentrica>H. indicus>T. chebula>P. zeylanica>P. granatum*.

The methanolic plant extracts of *A. calamus*, *H. antidysentrica* and *H. indicus* demonstrated relatively high antibacterial activity by agar well diffusion assay in comparison with other plant extracts. These plant extracts were further investigated to evaluate their antimicrobial efficacy against all ESβL producing enteric bacteria in terms of MIC. The data presented in **Table-2** ranged from 0.125 mg/mL to 4 mg/mL against test bacteria. It was found that methanolic extract of *A. calamus* showed considerable antibacterial activity with lowest MIC 0.125 mg/mL. MIC against ESβL producing bacterial isolates ranged from 0.125 mg/mL to 2.0 mg/mL. On the other hand, *H. antidysentrica* and *H. indicus* exhibited moderate antibacterial activity. MIC ranged from 1 to ≥4 mg/mL against test isolates with lowest MIC 1.0 mg/mL. To determine comparative efficacy of bioactive plant extracts against ESβL producing enteric bacterial isolates, MIC_{50} and MIC_{90} were calculated using Probit regression analysis. **Table 3** showed thatmethanolic extracts of *A. calamus* showed considerable antibacterial potency against ESβL producing bacterial isolates with $MIC_{50}$and MIC_{90} (0.517 and 1.280 mg/mL) against *E. coli* isolates and for *Klebsiella* isolates, MIC_{50} and MIC_{90} values were 0.542 and 1.158 mg/mL respectively. The MIC_{50} and MIC_{90} for *H. antidysentrica* were found 1.248 and 2.268 mg/mL against *E. coli* isolates and 1.166 and 2.214 mg/mL against *Klebsiella* isolates, indicating moderate antibacterial activity of this plant extract. The methanolic plant extract of *H. indicus* was found least active against ESβL producing enteric bacteria with MIC_{50} and MIC_{90} values 2.052 and 3.464 mg/mL against *E. coli* isolates while, 2.0 and 3.752 mg/mL against *Klebsiella* isolates.

Comparative antibacterial efficacy of bioactive plant extracts was also determined by evaluating MIC range in terms of per cent of test isolates inhibited. *A. calamus* exhibited considerable MIC range in which 5.4% isolates inhibited at MIC as 0.25 mg/mL and 70.27% isolates exhibited MIC range of 0.5-1.0 mg/mL. 24.3% isolates showed MIC range 1.0-2.0 mg/mLas indicated in **Figure-1**. In *H. antidysentrica*, 29.72% isolates exhibited MIC range 0.5-1.0 mg/mL and 70.27% isolates showed MIC range 2.0-4.0 mg/mL. This is attributed to moderate MIC range of *H. antidysentrica* against ESβL producing enteric bacteria.*H. indicus* displayed highest MIC range in which most of the isolates (91.89%) exhibited MIC range 2.0-4.0 mg/mL and 2.7% isolates showed MIC even greater than 4 mg/mL. Based on lowest to highest MIC range, the antibacterial efficacy of selected bioactive plant extracts was found in order of *A. calamus>H. antidysentrica>H. indicus.*

Phytochemical analysis of plant extracts

Phytochemical analysis of all four methanolic plant extracts by color test is presented in **Table 4**. All extracts were found positive for the presence of flavonoids, phenols, tannins and saponins except *H. indicus*. Similarly, alkaloids were detected in all plant extracts. Glycosides were detected in *H. indicus*.

To detect the presence of potentially active phytocompound in methanolic extracts of *A. calamus*, GC-MS analysis was performed. The GC-MS analysis of *A. calamus* rhizome extract revealed the presence of 13 major components (**Table-5**). The compounds identified with % area of peak were α-asarone (66.23%), cis-linoleic acid (5.68%), lupeol acetate (2.26%), γ-asarone (2.12%), β-asarone (1.43%) etc. GC-MS chromatogram of methanolic extract of *A. calamus* is presented in **Figure-2**. Many other small peaks represent other phytocompounds in trace amount.

Table-1 Antibacterial activity of selected medicinal plants extracts against biofilm forming MDR bacteria by agar well diffusion assay.

Bacteria	**Antibacterial activity of plants extracts (*diameter of zone of inhibition in mm)**					
	Acoruscalamus	***Hemidesmusindicus***	***Holarrhenaantidysenterica***	***Plumbagozeylanica***	***Punicagranatum***	***Terminaliachebula***
ECMA2	14.33±0.57	15.33±1.15	15.33±0.57	12.66±1.15	13.0±1.73	10.0±0.57
ECM4	17.66±0.57	15.33±0.57	15.33±1.52	14.33±0.57	10.66±0.28	12.33±1.15
ECMW9	12.33±0.57	12.33±0.76	14.66±0.57	10.33±0.57	11.66±1.15	14.33±0.57
ECUA1	16.0±0.86	15.33±0.57	16.33±1.15	12.0±0.86	10.33±0.57	10.33±0.57
ECUA2	15.33±0.57	10.66±0.57	16.33±1.52	11.66±0.57	13.33±0.57	14.0±1.0
ECM49	17.33±0.57	14.33±1.15	16.33±0.57	11.33±0.57	10.66±0.57	12.66±0.57
ECG7	17.66±1.15	10.0±0.86	14.33±0.57	11.0±0.86	13.0±1.0	11.0±0.86
KPMA19	16.66±1.44	14.66±0.57	16.66±0.57	14.66±0.57	8.66±0.28	13.33±1.15
KCUA12	14.66±0.57	13.33±1.15	10.0±1.0	12.33±0.57	10.66±0.28	12.0±1.0
ENM32	15.0±0.86	12.33±0.57	15.66±0.57	10.66±0.28	9.33±0.57	13.66±0.57
ENM36	17.33±1.15	14.66±0.57	16.0±0.86	8.33±0.57	10.66±1.15	14.0±0.86
***E. coli* 25922**	15.0±0.86	14.66±0.57	16.0±0.57	12.0±0.86	12.33±0.57	14.0±1.0
Mean**	15.77^{b}	13.49^{c}	14.96^{b}	$11.74^{d,e}$	11.12^{e}	$12.6^{c,d}$

Note: *including well size; each well contains 1.0 mg/mL of plant extract and 0.1 mg/mL of active compound, -; indicates no zone of inhibition; No antibacterial activity was recorded in the crude methanolic extracts of *Cuminumcyminum* (Zeera), *Elettariacardamomum* (Elaichi), *Foeniculumvulgare* (Saunf) and *Psidiumguajava* (Amrud). **Mean values were compared using Duncan's multiple range test and letters indicate different significant groups at $p \leq 0.05$.

Table 2. MIC of selected methanolic plant extracts against ESβL producing enteric bacterial isolates.

Bacterial isolates	**Minimum inhibitory concentration (MIC) mg/mL**		
	A. calamus	***H. antidysenterica***	***H. indicus***
E. coli			
ECMA2	2	1	2
ECMA20	0.5	2	2
ECM 4	1	2	2
ECM8	2	4	>4
ECM16	0.5	2	4
ECM18	1	2	4
ECM49	2	2	2
ECMW5	1	2	2
ECMW6	0.5	1	2
ECMW9	2	2	4
ECMW21	2	4	4
ECMW30	1	2	4
ECM W31	1	2	4
ECMW41	1	2	2
ECUA1	1	2	4
ECUA2	2	2	2
ECUA8	0.5	2	4
ECGA4	0.125	1	1
ECGA7	1	2	4
ECUJ1	0.5	1	4
ECUJ2	0.5	1	2
ECUJ9	1	2	4
E. coli 25922	0.5	1	2
Klebsiella			
KPMA19	0.5	2	2
KPM27	0.25	1	1
KPM3	1	2	4
KPMA9	0.5	1	2
KPM S1	0.5	1	2
KPMA14	0.5	2	4
KPMA17	0.5	2	2
KPMEA17	2	2	4
KUJ12	2	4	4
KUJ13	0.5	1	2
KUJ16	1	2	4
Kp700603	2	2	4
E. cloacae			
ENM32	2	2	4
ENM36	1	1	2

Table 3. MIC_{50} and MIC_{90} of bioactive plant extracts against ESβL producing enteric bacterial isolates.

Bacterial isolates	Minimum inhibitory concentration (MIC) mg/mL			
	MIC_{50}	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**
A. calamus	0.517	1.280	0.542	1.158
H. antidysenterica	1.248	2.268	1.166	2.214
H. indicus	2.052	3.464	2.000	3.752

Table 4. Major groups of phytocompounds of selected methanolic plant extracts.

Name of plant	**Alkaloids**	**Flavonoids**	**Glycosides**	**Phenols**	**Tannins**	**Saponins**
A. calamus	+	+	-	+	+ (CT)	+
H. antidysenterica	+	+	-	+	+ (CT)	+
H. indicus	+	+	+	+	+ (CT)	-

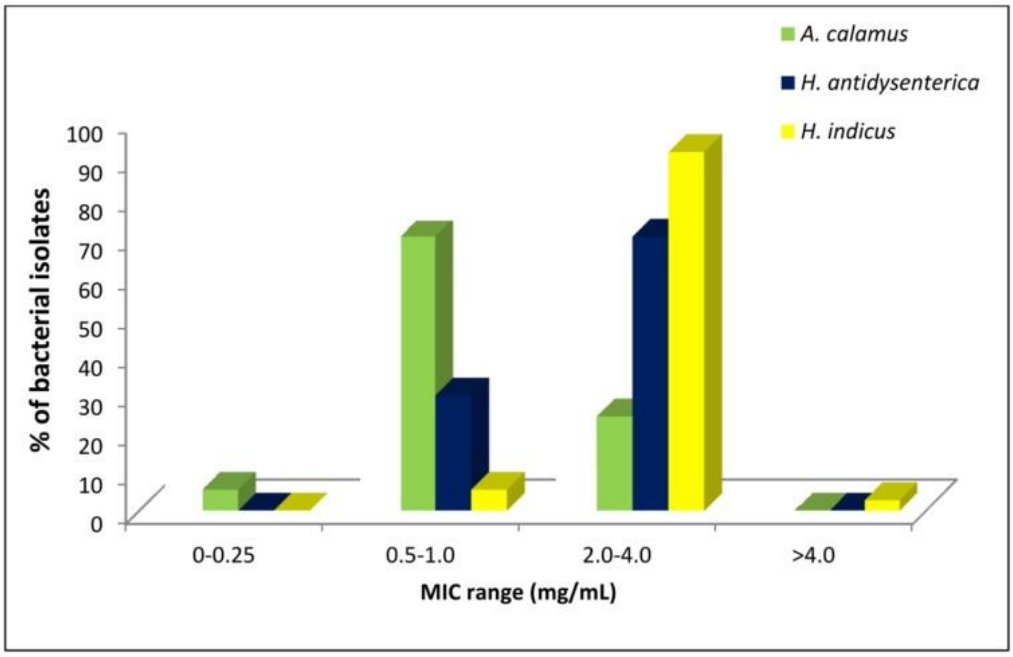
Note: CT represents catechin tannins

Table 5. Major components of methanolic extracts of A. calamus as identified by Gas chromatography–mass spectrometry.

Peak no.	**Retention time**	**Area %**	**Components**	**Chemical class**
12	17.43	66.23	α-Asarone	Phenylpropanoid
28	23.86	5.68	Cis-linoleic acid	Fatty acid
24	21.80	3.76	Tetra decanoic acid	Fatty acid
35	38.54	2.26	Lupeol acetate	Triterpene
10	16.33	2.12	γ-Asarone	Phenylpropanoid
25	23.29	1.85	Cis-linoleic acid methyl ester	Fatty acid methyl ester
32	35.84	1.67	γ-sitosterol	Phytosterol
21	19.16	1.47	Oplopanonyl acetate	Terpene
15	17.98	1.43	β-Asarone	Phenylpropanoid
3	14.61	1.39	Cis methyl isoeugenol	Phenol ether
5	15.61	1.42	Epishyobunone	Monoterpene
23	21.26	1.21	Hexadecanoic acid methyl ester	Fatty acid methyl ester
17	18.43	1.09	Tri-methyl benzyl alcohol	Phenol

Figures

Figure-1 MIC range of selected bioactive plant extracts against ESβL producing enteric bacterial isolates.



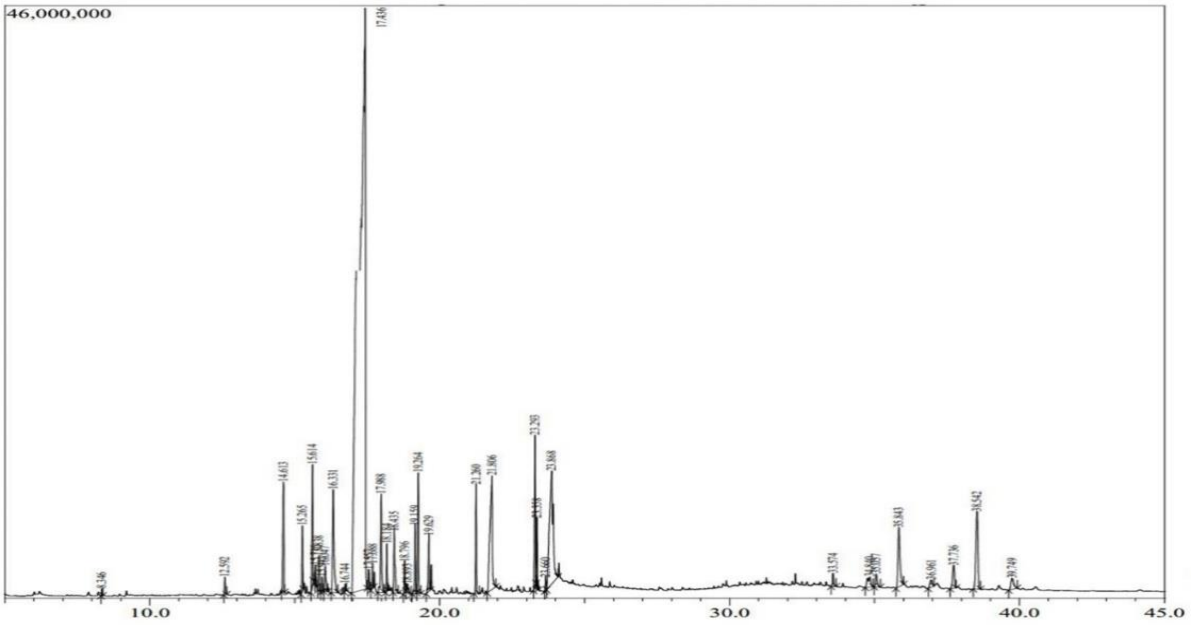


Figure-2 Gas chromatography–mass spectrometry (GC-MS) chromatogram of methanolic extract of A. calamus; Numbers above the peak refer to retention time.

Discussion

The emerging global scenario of multidrug resistance among pathogenic bacteria and poor discovery of new antibiotics with novel mode of action has led to the investigation of new combinations and possible alternative treatment strategies against MDR bacteria. Indian medicinal plants have been considered as a potential source of new antimicrobial agents. Since ancient times, medicinal plants have made significant contribution to human health. Many herbal formulations and decoctions have been used to cure various ailments and diseases. These herbal preparations have a diverse range of bioactive phytochemicals that can be explored for various biological activities such as antimicrobial efficacy, antiviral activity, antibiofilm activities, combinational effect with antimicrobials, antidiabetic etc. (Cowan et al., 1999; Dahanukar et al., 2000; Mukherjee et al., 2006; Borges et al., 2016). Plant-derived medicines are considered relatively safer than chemical antimicrobial (Gogtay et al., 2002).

In the present study, methanolic extracts of eleven plants, traditionally used in Indian medicine were screened for antibacterial efficacy against biofilm forming ESβL producing enteric bacteria. Of these, seven plant extracts exhibited varying level of inhibition against test isolates. The mean values of zone of inhibition were compared using Duncan's multiple range test. Overall activity of tested plant extracts is in order of *A. calamus>H. antidysentrica>H. indicus>T. chebula>P. zeylanica>P. granatum.* Moreover, higher activity was found mainly into methanolic extracts of *A. calamus, H. antidysentrica* and *H. indicus* while lower activity was observed in *T. chebula, P. zeylanica* and *P. granatum*.

In vitro efficacy of four methanolic plants extracts including *A. calamus*, *H. antidysentrica* and *H. indicus* were assessed in terms of MIC against ESβL producing enteric bacteria. The MIC of the methanolic extracts of individual plants varies against different test isolates. Comparative antibacterial efficacy of selectedplantsextractswas determinedbycalculatingMIC_{50}andMIC_{90}. Lower MIC_{50}andMIC_{90}indicatehigherantibacterialactivity.The$MIC_{50}$and $MIC_{90}$of *A. calamus*, *H. antidysentrica* and *H.indicus*wererangedfrom0.517to2.052mg/mLand1.280to3.464mg/mL respectively against*E. coli* isolates.*A. calamus*exhibited lowest MIC_{50} 0.542 mg/mL and MIC_{90} 1.158 mg/mL against *Klebsiella* spp.isolates followed by*H. Antidysentrica* (1.166 mg/mL and 2.214 mg/mL), and *H. indicus* (2 mg/mL and 3.752mg/mL).Antibacterialefficacyofbioactivemethanolicplantextractswasalso evaluatedbycomparativestudyonMICrangeintermsofpercentoftestisolates inhibited.*A. calamus*extractshowedlowestMICrangeagainsttest bacterialisolates followed by *H.antidysentrica*.While*H.indicus*extractexhibitedhighestMICrange2.0-4.0 mg/mL for 91.89% isolates.Overall, antibacterial efficacy of selected methanolic plants extracts in terms ofMIC,(highestpotencyandlowestMICvalues)againstESβLproducingenteric bacteria are in order of *A. calamus>H. antidysentrica>H. indicus*. These methanolic plants extracts exhibited antibacterial activity irrespective of the drug resistance pattern of the test bacteria. This reveals antibiotic resistance does not restrict antimicrobial potential of active plant extracts. The active compounds present in plant extracts might have different modes of actions with multiple target sites on test organisms (Omojate et al., 2014).

The results of the present study depicted that active methanolic plant extracts of *A. calamus, H. antidysentrica* and *H. indicus* exhibited a broad range of antimicrobial activity against multidrug resistant ESβL producing enteric bacteria.

Ourfindingsagreewithpreviousstudieshighlightingthepotent antimicrobial activity of traditional Indian medicinal plants including *A. calamus, H. antidysentrica* and *H. indicus* against ESβL producing and MRSA strains (Ahmad et al., 2001; Ahmad and Aqil. 2007). Other studies have also been described the antibacterial activity of crude methanolic extracts of medicinal plants against multidrug resistant bacteria associated with diseases in human(Bisi-Johnsonetal., 2017; Mishra et al., 2017). These findings suggest that potentially effective medicinal plant extracts could be used in treatment strategies against infectious diseases caused by drug resistant bacteria.

The active components of plant extracts such as alkaloids, flavonoids, terpenoides, glycosides and phenolic compounds interact with enzymes and proteins of the cell membrane causing efflux of protons toward cell exterior which finally disrupt the membrane accompanied by cell death. These phytoconstituents may also inhibit enzymes necessary for amino acids biosynthesis (Barbieri et al., 2017).Phytochemical investigation of bioactive plant extracts and their major active components were explored by GC-MS analysis. Incase of *A.calamus* GC-MS analysis predicted α-Asarone as major active constituents together with γ-Asarone, β-Asarone and terpenes. Consistent with this report, α-as a rone was found to be the major constituents of *A.calamus* rhizome that possess substantial antibacterial activity (Li and Wah, 2017).Moreover, this study is supported by the reports of other workers who have shown the antibacterial properties of *A. calamus* rhizome extracts (Kumar et al., 2014; Rawat et al., 2016).The study clearly demonstrated that plant extracts, especially *A. calamus*, are potent against drug resistant bacterial pathogens which could be exploited to tackle AMR problem after careful *in vivo* investigation.

References

Adler, A.; Katz, D.E. and Marchaim, D.The continuing plague of extended-spectrum β-lactamase–producing Enterobacteriaceae infections.*Infectious Disease Clinics*., 2016, 30(2):347-75.

Ahmad, I. and Aqil, F. In vitro efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug resistant enteric bacteria. *Microbiological Research.*,2007, 27,162(3):264-75.

Ahmad, I. and Beg, A.Z. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens.*Journal of Ethnopharmacology*., 2001, 1;74(2):113-23.

Aqil, F.; Khan, MS.; Owais, M. and Ahmad I. Effect of certain bioactive plant extracts on clinical isolates of β-lactamase producing methicillin resistant Staphylococcus aureus.*Journal of Basic Microbiology*., 2005, 1,45(2):106-14.

Barbieri, R.; Coppo, E.; Marchese, A.; Daglia, M.; Sobarzo-Sánchez, E.; Nabavi, S,F. and Nabavi, S,M. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiological research*, 2017, Mar. 1,196:44-68.

Belcher, M.S.; Mahinthakumar, J. and Keasling, J.D. New frontiers: harnessing pivotal advances in microbial engineering for the biosynthesis of plant-derived terpenoids. *Current opinion in biotechnology*, 2020, 65:88-93.

Bisi-Johnson, M.A.; Obi, C.L.; Samuelm, B.B.; Eloff, J.N. and Okoh, AI. Antibacterial activity of crude extracts of some South African medicinal plants against multidrug resistant etiological agents of diarrhoea. BMC complementary and alternative medicine, 2017, 17(1):321-30.

Borges, A.; Abreu, A.C.; Dias, C.; Saavedra, M.J.; Borges, F. and Simoes, M. New perspectives on the use of phytochemicals as an emergent strategy to control bacterial infections including biofilms.*Molecules*, 2016, 5, 21(7):877-918.

Cowan, M.M. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 1999, 1, 12(4):564-82.

Dahanukar, S.A.; Kulkarni, R.A. and Rege, N.N. Pharmacology of medicinal plants and natural products.*Indian Journal of Pharmacology*, 2000, 1,32(4):S81-118.

Eloff, JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *PlantaMedica*., 1998, 64(08):711-3.

El-Tawil, B.A.H. Chemical constituents of indigenous plants used in native medicine of Soudi Arabia. Arab *Gulf Journal of Scientific Research*, 1983, 1: 395-418.

Fadeyi, M.O. and Akpan, U.E. Antibacterial activities of the leaf extracts of Eugenia unifloraLinn.(Synonym Stenocalyxmichelli Linn.)*Myrtaceae.Phytotherapy Research*, 1989, 1,3(4):154-5.

Gogtay, N.J.; Bhatt, H.A.; Dalvi, S.S and Kshirsagar, N.A.The use and safety of non-allopathic Indian medicines.*Drug safety*, 2002, 1,25(14):1005-19.

Kapoor, L.D.; Singh, A.; Kapoor, S.L. and Srivastava, S.N.Survey of Indian medicinal plants for saponins, alkaloids and flavonoids.*Lloydia,*1969, 32: 397-302.

Kessler, A. and Kalske, A. Plant secondary metabolite diversity and species interactions. Annual Review of Ecology, Evolution, and Systematics, 2018, 49:115-38.

Kumar, V. Antimicrobial activity of rhizome extract of Acoruscalamus against different micro-organisms.Octa *Journal of Biosciences*., 2014, 1;2(1):59-63.

Li, K.S. and Wah CS. Antioxidant and antibacterial activity of Acoruscalamus. L leaf and rhizome extracts. *JurnalGiziKlinik Indonesia*. 2017, 1;13(4):144-58.

McEwen, S.A. and Collignon, P.J. Antimicrobial resistance: a one health perspective. *Microbiology spectrum*., 2018; 6(2):6-2.

McLafferty, F.W. Wiley Registry T Mof Mass Spectral Data, seventh ed. JohnWiley& Sons, Ltd., New York, NY, USA, 2005.

Mishra, M..; Rath, S.; Swain, S.S.; Ghosh, G.; Das, D. and Padhy, R.N. In vitro antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing MDR bacteria. *Journal of King Saud University-Science*, 2017, 1;29(1):84-95.

Mukherjee, P.K.; Maiti, K.; Mukherjee, K. and Houghton, P.J. Leads from Indian medicinal plants with hypoglycemic potentials.*Journal of Ethnopharmacology*, 2006, 15;106(1):1-28.

NIST/EPA/NIH. Mass Spectral Library with Search Program: (Data Version:NIST 05, Software Version 2.0d). National Institute of Standards and Technology, New York, NY, USA; 2005.

Odebiyi, O.O. and Sofowora, E.A. Phytochemical screening of Nigerian medicinal plants II. *Lloydia*, 1978, 41(3):234-46.

OmojateGodstime, C.; Enwa Felix, O.; JewoAugustina, O. and Eze Christopher, O. Mechanisms of antimicrobial actions of phytochemicals against enteric pathogens–a review. Journal of Pharmaceutical, *Chemical and Biological Sciences*., 2014, 2(2):77-85.

O'Neill, J. Tackling drug-resistant infections globally: final report and recommendations: Review on Antimicrobial Resistance, 2016.

Perez, C.; Pauli, M. and Bazerque, P.An antibiotic assay by the agar well diffusion method.*Acta. Biol. Med. Exp*. 1990, 15(1):113-5.

Rawat, S.; Jugran, A.K.; Bahukhandi, A.; Bahuguna, A. Bhatt, I.D.; Rawal, R.S. and Dhar, U. Anti-oxidant and anti-microbial properties of some ethno-therapeutically important medicinal plants of Indian Himalayan Region. *Biotechnology*, 2016, 1;6(2):154-66.

Segelman, A.B. and Farnsworth, N.R. Biological and phytochemical evaluation of plants. IV. A new rapid procedure for the simultaneous determination of saponins and tannins. Lloydia. 1969, 32(1):59.

Wagner H, Bladt S. Plant drug analysis: a thin layer chromatography atlas. Springer Science & Business Media; 1996.

Wang, L.; Jin, P.; Wang, J.; Jiang, L. Shan, T. and Zheng, Y. Methyl jasmonate primed defense responses against Penicilliumexpansum in sweet cherry fruit. Plant Molecular Biology Reporter, 2015; 33(5):1464-71.

WHO. Antibacterial agents in clinical development: an analysis of the antibacterial clinical development pipeline. Geneva, Switzerland: World Health Organization, 2019a.

WHO. Antibacterial agents in preclinical development: an open access database. Geneva, Switzerland: World Health Organization, 2019b.

WHO.International statistical classification of diseases and related health problems 10th revision (ICD-10). 2016. Available at: https://icd.who.int/browse10/2016/en.

Wink, M. Evolution of the angiosperms and co-evolution of secondary metabolites, especially of Alkaloids.Reference Series in Phytochemistry.Co-Evolution of Secondary Metabolites.Editors J.-M. Mérillon, and K. G. Ramawat (Cham, Switzerland: Springer International Publishing), 151–174, 2020

Zaynab, M.; Fatima, M.; Abbas, S.; Sharif, Y.; Umair, M.; Zafar, MH. And Bahadar, K. Role of secondary metabolites in plant defense against pathogens.*Microbial pathogenesis.*, 2018;124:198-202.