Malaysian Journal of Microbiology, Vol 20(1) 2024, pp. 58-66
DOI: http://dx.doi.org/10.21161/mjm.221452

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Antibiotic resistance modulation of *Clostridium perfringens* type D using indigenous plants extracts

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Received 15 February 2022; Received in revised form 31 August 2022; Accepted 27 October 2023

ABSTRACT

Aims: The study was aimed to explore the antimicrobial potential of ethanolic leaf extracts of *Eucalyptus globulus*, *Moringa oliefera*, *Syzygium cumini* and *Citrus limon* against antibiotic-resistant *Clostridium perfringens* type D (n=5).
Methodology and results: Antibiotic resistance pattern of *C. perfringens* type D isolates against tetracycline, gentamicin, ceftriaxone, amoxicillin and streptomycin was evaluated by disc diffusion method. Well diffusion and micro broth dilution methods were used to determine the anti-bacterial activity, sub-inhibitory concentrations and antibiotic resistance modulating effects of the plant extracts. Ethanolic extract of *E. globules* was selected to evaluate its modulatory impact and subjected to GC-MS analysis to separate and identify the phytochemicals. The results showed that the isolates were resistant to gentamicin (0 ± 0.00 mm), streptomycin (0 ± 0.00 mm), tetracycline (13.2 ± 2.28 mm) and ceftriaxone (0 ± 0.00 mm) while sensitive to amoxicillin (23.8 ± 1.30 mm) and tetracycline (13.2 ± 2.28 mm). *Eucalyptus globulus* exhibited the maximum anti-bacterial activity with a zone of inhibition (ZOI) of 14.6 ± 0.54 mm and minimum inhibitory concentration (MIC) (1500 ± 947.85 µg/mL). Other plant extracts (*M. oliefera*, *S. cumini* and *C. limon*) also showed anti-bacterial activity but couldn't modulate the resistance. The activity of ceftriaxone associated with *E. globulus* extract was improved with 20.2 ± 0.20 mm ZOI at 78.125 µg/mL sub-inhibitory concentration.
Conclusion, significance and impact of study: The study results indicate the possible use of the ethanolic extract of *E. globulus* alone or in combination with common antibiotics for the treatment of *C. perfringens* infections in small ruminants.

Keywords: Antibiotic resistance modulation, *Citrus limon*, *Clostridium perfringens* type D, enterotoxaemia, *Eucalyptus globulus*, *Moringa oliefera*, *Syzygium cumini*

INTRODUCTION

Clostridium perfringens is a Gram-positive rod, strict anaerobe and spore-forming bacterium. It is usually distributed in the environment, small intestine and colon of human beings and animals as normal flora (Jean *et al.*, 2004; Kiu and Hall, 2018). When the environment of the intestine changes due to sudden changes in diet or other factors, different strains of *C. perfringens* produce various toxins and cause enterotoxaemia, a common disease in animals (Hussain *et al.*, 2018). Globally, this infection has been reported as the third among the deadliest diseases in small ruminants (sheep and goats) (Khan *et al.*, 2019).

Toxinotyping of *C. perfringens* is based on the production of major toxins (alpha, beta, epsilon and iota) as A, B, C, D, E, F and G (Forti *et al.*, 2020). Overall, 17 toxins of *C. perfringens* have been identified. All toxinotypes of *C. perfringens* produce alpha (α) toxin which has sphingomyelinase and phospholipase activities (Takehara *et al.*, 2020). Beta (β) toxin is produced by B and C types and possesses necrotic and neurotoxic effects (Shrestha *et al.*, 2018). *Clostridium perfringens* type D produces alpha and epsilon toxins that cause kidney damage and dysentery in small ruminants (Hussain *et al.*, 2018; Bai *et al.*, 2020).

Antibiotics including penicillin, amoxicillin, tetracycline and erythromycin are the antibiotics of the first choice for the treatment of *C. perfringens* associated infections both in animals and humans (Gharieb *et al.*, 2021). However, due to the inappropriate and long-term use of antibiotics in the veterinary industry, the bacteria have become resistant to them (Rather *et al.*, 2017; Sayyar *et al.*, 2021). The antibiotics perturb the commensal microflora and provide the bacteria with a favourable environment

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ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(1) 2024, pp. 58-66
DOI: http://dx.doi.org/10.21161/mjm.221452

Table 1: *Clostridium perfringens* toxins genes primer sequences.

Toxin genes	*Primers	Sequences
Cpa	CPAlphaF	GCTAATGTTACTGCCGTTGA (5'-3')
	CPAlphaR	CCTCTGATACATCGTGTAAG (3'-5')
Cpb	CPBetaF3	GCGAATATGCTGAATCATCTA (5'-3')
	CPBetaR3	GCAGGAACATTAGTATATCTTC (3'-5')
Etx	CPEpsilonF	TGGGAACTTCGATACAAGCA (5'-3')
	CPEpsilonR2	AACTGCACTATAATTTCCTTTTCC (3'-5')
Iap	CPIotaF2	AATGGTCCTTTAAATAATCC (5'-3')
	CPIotaR	TTAGCAAATGCACTCATATT (3'-5')

cpa: Alpha toxin gene, cpb: Beta toxin gene, etx: Epsilon toxin gene and iap: Iota toxin gene. *Tm (Melting Temperature), 53 °C.

for pathogenesis and toxin production (Zimmermann and Curtis, 2019). The bacteria utilize various mechanisms to resist antibiotics, such as efflux systems, antibiotic degrading enzymes as well as disruption of cell membrane permeability (Ma and Ye, 2018). This adaptation of bacteria to antibiotics has become a global problem in the treatment of diseases. To overcome this issue, alternative treatments are being devised, including medicinal plants (Manandhar *et al.*, 2019), bacteriophages, etc. (Romero-Calle *et al.*, 2019).

Medicinal plants are rich sources of antimicrobial agents, that's why they are used in different countries as medicine (Yasmin *et al.*, 2020). The antimicrobial activity of plants has been attributed to a diverse range of phytochemicals (plant-derived compounds) which are synthesized as secondary metabolites (Al AlSheikh *et al.*, 2020). The secondary metabolites of plants include tannins, phenolic compounds, alkaloids and flavonoids (Manandhar *et al.*, 2019). Plants have been evaluated for their anti-bacterial action and the modulation of antibiotic resistance (Fankam *et al.*, 2017). It has been suggested that phytochemicals have the potential to overcome bacterial resistance mechanisms and improve the efficacy of antibiotics (Al AlSheikh *et al.*, 2020). Friedlein *et al.* (2021) reported that plant extracts, including allspice, cardamom, cinnamon, clove, coriander and ginger, have antimicrobial activity against *C. perfringens*. Plants produce a wide variety of secondary metabolites, like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins (Gonelimali *et al.*, 2018). These biomolecules are responsible for the plants' antimicrobial potential and synergistic activity. Possible modes of action of these compounds could be the destruction of the plasma membrane, inhibition of bacterial efflux pumps, inactivation of synthases involved in nucleic acid synthesis and disruption of the bacterial respiratory chain (Yuan *et al.*, 2021).

The present study was designed to determine the antibiotic resistance pattern of *C. perfringens* type D isolates against commonly used antibiotics for treatment of enterotoxaemia in small ruminants and the modulator effect of four indigenous medicinal plants (*E. globulus*, *M. oleifera*, *C. limon* and *S. cumini*) on antibiotic-resistant isolates. The contents of the extract were analyzed by gas chromatography-mass spectrometry (GC-MS) to identify the compounds with anti-bacterial potential.

MATERIALS AND METHODS

Clostridium perfringens strains

Clostridium perfringens type D (n=5) isolates were procured from the PG Microbiology Laboratory, Institute of Microbiology, UVAS, Lahore. Bacteria from bead stock culture were revived on Perfringens agar medium (TSC) (pH 7.6 ± 0.2) and confirmed by Gram staining, spore staining and biochemical testing (Wani *et al.*, 2018). *Clostridium perfringens* isolates were further characterized based on *cpa* (α), *cpb* (β), *etx* (ε) and *iap* (ι) genes by polymerase chain reaction (PCR) (Hussain *et al.*, 2018). Specific primers for toxin genes PCR have been stated in Table 1.

Antibiotic resistance testing

The disc diffusion method was used to evaluate antibiotic resistance patterns following a previously described method with minor modifications (Sapico *et al.*, 1972). The bacterial suspension was adjusted equal to 0.5 McFarland standards and inoculated on Perfringens agar media (TSC) plates, followed by swabbing. Commercially available antibiotic discs as per CLSI standards (amoxicillin 25 µg, ceftriaxone 30 µg, gentamicin 10 µg, streptomycin 10 µg and tetracycline 30 µg) were placed on plates and then incubated at 37 °C for 24 h under anaerobic conditions in a CO_2 incubator. The diameters (mm) of ZOI were measured and compared with the British Society for Antimicrobial Chemotherapy (BSAC).

Preparation of ethanolic extracts of plants

The leaves of *M. oleifera*, *C. limon*, *E. globulus* and *S. cumini* were collected from the agricultural field (Lahore division of Punjab, Pakistan) in December 2019. The plants were identified at the Botany Department, Government College University Lahore, Pakistan. The reference numbers allotted to *E. globulus*, *M. oleifera*, *C. limon* and *S. cumini* were GC. Herb. Bot. 3643, GC. Herb. Bot. 3644, GC. Herb. Bot. 3641 and GC. Herb. Bot. 3642, respectively. The preparation of ethanolic extracts was carried out using the Soxhlet apparatus (CG-1368) (Harborne *et al.*, 1999). The leaves were collected, washed, disinfected, rinsed with distilled water and dried under shade. Fine powder of dried leaves was prepared

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

by using a pestle and mortar. The powder (100 g) of all the plants was put into the Soxhlet apparatus, thimble and loaded into the main chamber of the Soxhlet extractor. The extractor was set on the round bottom flask containing 500 mL ethanol (solvent) and a condenser was attached. The solvent was heated by using a heating mantle for 24 h; vapors moved up the distillation arm and then into the chamber holding thimble. Once the chamber was filled, it was drained by a siphon side arm back down to the distillation flask. The cycles were repeated many times, and then it was compressed under reduced pressure in a rotary evaporator. The dried mass weight was recorded. Stock solutions of plant extracts (100 mg/mL of each) were prepared in 1mL dimethyl-sulfoxide (DMSO) and stored at 4 °C until further processing.

Evaluation of the anti-bacterial activity of indigenous plants

Anti-bacterial activity of plant extracts was evaluated using a previously described well diffusion method with minor modifications (Ahmad *et al.*, 1998). Fifty (50) mg/mL of plant extract stock solution was used for the assessment of anti-bacterial activity. The bacterial suspension (0.5 McFarland standards) was inoculated on Perfringens agar media (TSC) plates. Wells were punched, sealed with molten agar and inoculated with 50 µL of plant extract. The plates were incubated at 37 °C for 24 h in a CO_2 incubator and the diameter of ZOI was measured in mm.

Determination of MIC

The micro broth dilution method was used to determine the MICs of plant extracts following a previously described method with minor modifications (CLSI, 2006). Reinforced Clostridial Medium (RCM) (100 µL) was poured in 1-12 wells in a flat-bottom 96-well plate. Then the stock solution of plant extract (100 µL) was poured into the first well and diluted (two-fold serial dilution) up to the 10^{th} well. The bacterial suspension (100 µL) equal to 0.5 McFarland standards was added in each well up to the 11^{th} well. Optical density was recorded at zero time at 600 nm using an ELISA reader (BIOBASE). Plates were incubated at 37 °C for 24 h in a CO_2 incubator and then again, optical density was recorded.

Evaluation of antibiotic resistance modulating the activity of plant extracts

Agar well diffusion method was used to evaluate the modulating activity of plant extracts against antibiotic-resistant *C. perfringens* type D isolates. A culture of 0.5 McFarland standards was inoculated on sterile Perfringens agar medium (TSC) plates and wells were punched by using a well borer. Each plate had four wells in which DMSO blank (50 µL), antibiotic solution alone (50 µL), plant extract solution alone (50 µL) and the mixture of antibiotic + plant extract (1:1, 50 µL) was poured. Four different sub-inhibitory concentrations of plant extracts, including 78.125, 39.062, 19.531 and 9.765 µg/mL were used. Plates were incubated at 37 °C for 24 h in a CO_2 incubator. The diameters of zones of inhibition were measured in mm.

Gas chromatography and mass spectrometry (GC-MS) of ethanolic extract of *E. globulus*

To determine the contents of ethanolic extract of *E. globulus,* GC-MS analysis was performed by following a previously described method (Pan *et al.*, 2019). CARBOWAX capillary column, along with helium as carrier gas was employed for the analysis. The injector was heated at 260 °C and the extract was injected at a 1 µL/min rate. Biologically active compounds in the extracted sample were determined by comparative analysis of retention time with standard compounds.

Statistical analysis

Data obtained in all experiments were analyzed through One-way analysis of variance (ANOVA) followed by Duncan's Multiple Range post hoc test using Statistical Package for Social Sciences (SPSS) 20.0 software.

RESULTS

***Clostridium perfringens* type D isolates**

The isolates were confirmed by macroscopic and microscopic characteristics, biochemical tests and PCR. The bacterial cells were observed as Gram-positive, bacilli and have sub-terminal spores. All the isolates were catalase and oxidase negative and fermented sucrose, fructose, maltose, glucose and lactose sugar. The double zone hemolysis was observed on blood agar. Opaque halo around the colonies was observed in lecithinase testing. Toxin genes, including alpha (cpa) and epsilon (etx) were identified by PCR.

Antibiotic resistance pattern

The isolates were subjected to antibiotic resistance testing by the disc diffusion method. The highest mean ZOI was recorded for amoxicillin (23.8 ± 1.30 mm), followed by tetracycline (13.2 ± 2.28 mm), gentamicin, streptomycin and ceftriaxone (0.0 ± 00 mm). The ZOI of antibiotics was compared with BSAC standards, which showed that *C. perfringens* type D isolates were resistant to tetracycline, gentamicin, ceftriaxone and streptomycin while sensitive to amoxicillin (Table 2).

Anti-bacterial activity of indigenous plants extracts

Anti-bacterial activity of four different indigenous plant extracts was evaluated against *C. perfringens* type D isolates. The total yield of ethanolic extracts was calculated in mL relative to the dry plant matter (Figure 1). A maximum 20% yield was obtained from *C. limon* extraction, followed by *E. globulus* (18%), *M. oleifera*

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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DOI: http://dx.doi.org/10.21161/mjm.221452

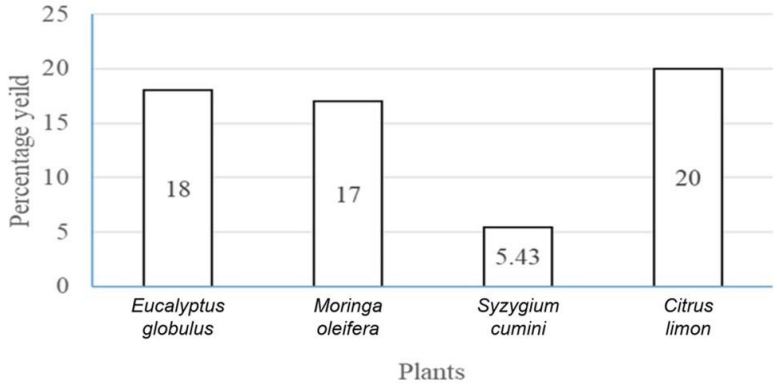

Figure 1: Percentage yield of *Eucalyptus globulus*, *Moringa oleifera*, *Syzygium cumini* and *Citrus limon*.

Table 2: Standard zone of inhibition (mm) of different antibiotics.

Antibiotics	Antibiotics concentration (µg)	Standard zone of inhibition (mm)			Results (Mean ZOI)
		Sensitive	Intermediate	Resistant	
Tetracycline	30	≥19	15-18	≤14	13.2 ± 2.28 mm
Amoxicillin	25	≥17	14-16	≤13	23.8 ± 1.30 mm
Ceftriaxone	30	≥18	15-17	≤14	0 ± 0.00 mm
Gentamicin	10	≥15	13-14	≤12	0 ± 0.00 mm
Streptomycin	10	≥10	7-9	≤6	0 ± 0.00 mm

Table 3: Anti-bacterial activity of indigenous plant extracts.

Plant extracts	Zone of inhibition (mm)
Eucalyptus globulus	14.6 ± 0.54^{b}
Citrus limon	12.6 ± 1.51^{b}
Syzygium cumini	11.2 ± 0.83^{ab}
Moringa oliefera	5.80 ± 7.94^{a}

[a,b] Mean values share different superscripts differ significantly while same superscripts differ non-significantly. mm: millimeter.

Table 4: Minimum inhibitory concentration (MIC) values of plant extracts.

Plant extract	Means of MIC values (µg/mL) ± Standard deviation
Eucalyptus globulus	1500 ± 947.85^{b}
Moringa oliefera	5000 ± 0.00^{c}
Citrus limon	208.33 ± 90.21^{a}
Syzygium cumini	703.125 ± 393.22^{ab}

[a,b,c] Mean values share different superscripts differ significantly while same superscripts differ non-significantly. µg/mL: microgram per milliliter.

(17%) and *S. cumini* (5.43%). All the plant extracts were effective in reducing the growth of *C. perfringens* type D. Highest mean ZOI was recorded for *E. globulus* (14.6 ± 0.54 mm), followed by *C. limon* (12.6 ± 1.51 mm), *S. cumini* (11.2 ± 0.83 mm) and *M. oliefera* (5.8 ± 7.94 mm). The minimum ZOI of *M. oleifera* was significantly (P<0.05) different from *C. limon* and *E. globules*, while insignificantly (P>0.05) different from *S. cumini* (Table 3).

Evaluation of MIC of plant extracts

Higher and lower MIC values of all plant extracts were recorded. The MIC value of *M. oleifera* was 5000 ± 0.00 µg/mL, followed by *E. globulus* (1500 ± 947.85 µg/mL), *S. cumini* (703.125 ± 393.22 µg/mL) and *C. limon* (208.33 ± 90.21 µg/mL). The MIC value of *C. limon* was significantly different from *E. globulus* and *M. oleifera*, and insignificantly different from *S. cumini*. There were insignificant differences (P>0.05) observed between the MIC values of *E. globulus* and *S. cumini*. Significant differences (P<0.05) were observed among the MIC values of *E. globulus*, *M. oleifera* and *C. limon* (Table 4).

Modulation of the antibiotics resistant *C. perfringens* Type D by plant extracts

Sub-inhibitory concentrations (78.125, 39.062, 19.531 and 9.765 µg/mL) of *E. globulus* extract were used to

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(1) 2024, pp. 58-66
DOI: http://dx.doi.org/10.21161/mjm.221452

Table 5: Antibiotic resistance modulatory activity of *E. globulus* at sub-inhibitory concentrations against *C. perfringens* Type D.

Eucalyptus globulus sub-inhibitory concentration (µg/mL)	Zone of inhibition (ZOI) (mm)		
	Gentamicin 10 µg	Streptomycin 10 µg	Ceftriaxone 30 µg
78.125	11.3 ± 0.26[a]	5.1 ± 0.20[b]	20.2 ± 0.20[c]
39.062	10.2 ± 0.20[a]	5.1 ± 0.15[b]	20.1 ± 0.15[c]
19.531	10.1 ± 0.10[a]	4.2 ± 0.20[b]	19.2 ± 0.2[c]
9.765	9.1 ± 0.1[a]	4.1 ± 0.10[b]	19.1 ± 0.15[c]

[a,b,c] Mean values share different superscripts differ significantly while have same superscripts differ non-significantly. mm: millimetre.

Table 6: Analysis of volatile components of *E. globulus* leaves by GC-MS.

Sr. No	RT (min)*	Compound	Percent area
1	6.210	1-8 cineol	17.62
2	6.431	B-Myrecene	1.73
3	6.599	Globulol	4.20
4	6.877	Gamma-terpenene	2.74
5	6.927	Methane 1,2,3 triol	3.79
6	6.273	P-Cymene	5.22
7	9.142	Sabinol	2.57
8	9.325	Cryptone	6.28
9	9.697	Limonene	10.13
10	11.048	Piperitone	17.77
11	11.974	Spathulenol	4.06
12	20.159	a-terpineol	4.53
13	20.433	a-pinene	19.36

*RT, Retention time.

modulate the resistance against antibiotics such as tetracycline, gentamicin, streptomycin and ceftriaxone. The results are shown as ZOI in Table 5. The extract has significantly improved the activity of ceftriaxone, gentamicin and streptomycin-resistant *C. perfringens* Type D at its subinhibitory concentrations. However, an important modulating effect was observed with ceftriaxone, which exhibited maximum ZOI (20.2 ± 0.20 mm) at 78.125 µg/mL sub-inhibitory concentration. No modulating effect was noted with gentamicin and streptomycin in the presence of that extract. A significant difference ($P<0.05$) was observed in ZOI values among the antibiotics.

Gas chromatography and mass spectrometry (GC-MS) of ethanolic extract of *E. globulus*

GC-MS analysis was performed to obtain the mass spectra of volatile compounds of *E. globules* (Figure 2). A total of 13 compounds were identified, mainly including globulol, gamma-terpenene, p-cymene, piperitone, spathulenol, a-terpinol, cryptone, 1-8 cineol, limonene, a-pinene. Each volatile component of the *E. globulus* extract was well separated and identified. a-pinene was abundant, constituting 19.36% of the total volatile content, followed by piperitone (17.77%), 1-8 cineol (17.62%) and limonene (10.13%) (Table 6).

DISCUSSION

Clostridium perfringens type D is the causative agent of enterotoxaemia in sheep and goats, occasionally in cattle or other animals (Garcia *et al.*, 2015). It produces potent toxins (alpha and epsilon). The pathogenicity of the disease is mainly because of the production of epsilon toxin (ETX) (Xin and Wang, 2019).

In the present study, *C. perfringens* isolates were evaluated for their antimicrobial resistance pattern and the modulatory potential of four different indigenous plant extracts was checked. The isolates were confirmed by colony characters, Gram and spore staining and biochemical testing. Identification of alpha and epsilon toxins by PCR confirmed the isolates as *C. perfringens* type D.

Different antibiotics, including penicillin, amoxicillin, tetracycline and erythromycin, are used to treat *C. perfringens* type D infections. But over time, the bacteria have developed resistance to antibiotics because of their long-term and inappropriate use (Ngamwongsatit *et al.*, 2016). Several studies have reported resistance by *C. perfringens* type D against antibiotics. Mohiuddin *et al.* (2020) reported 57% and 86% resistance to ciprofloxacin and erythromycin, respectively, by *C. perfringens* type A and D isolates from sheep feces and goats. According to Efuntoye *et al.* (2011), maximum resistance to penicillin and ampicillin was observed. The findings of the present study revealed 100% resistance against gentamicin, ceftriaxone and streptomycin (0.00 mm). In contradiction,

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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DOI: http://dx.doi.org/10.21161/mjm.221452

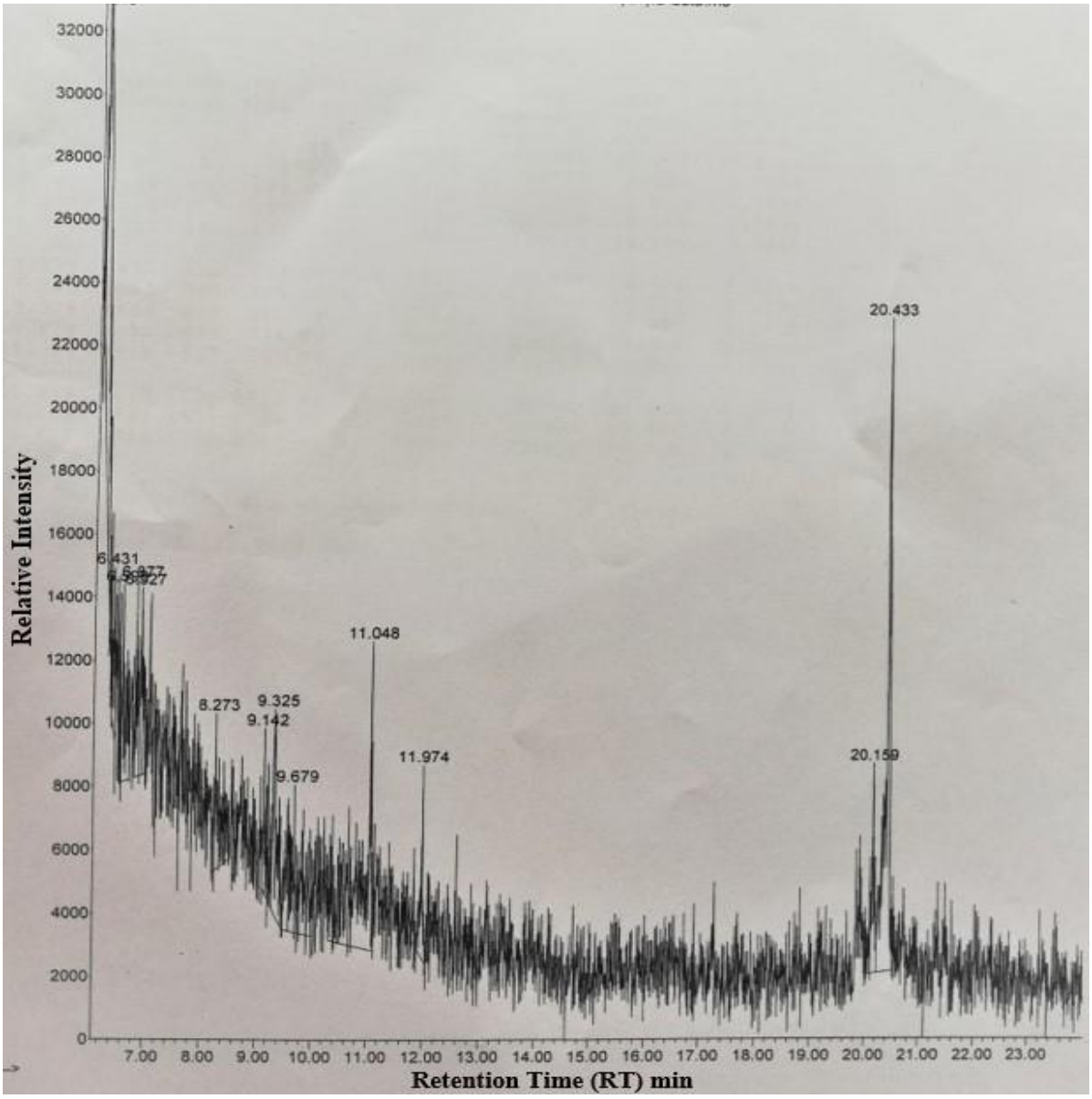

Figure 2: Mass spectra of volatile compounds from the ethanolic extract of *E*. *globulus* leaves using GC-MS.

in a previous study, isolates of C. perfringens were sensitive to gentamicin (20 mm), streptomycin (13 mm) and tetracycline (32 mm) (Taj *et al.*, 2018). This contradiction with our results could be due to differences in region, sampling conditions and inappropriate use of antibiotics.

The development of antibiotic resistance in bacteria has compelled scientists to look for alternative strategies to resolve this problem. Medicinal plants have been used for centuries to treat various ailments and are now considered the best alternatives to antibiotics. In the present study, ethanolic extracts of *E. globulus*, *M. oliefera*, *C. limon* and *S. cumini* plant leaves were used for their anti-bacterial activity against *C. perfringens*. These plants were chosen in view of their easy availability for animals in Pakistan.

The results showed that the extracts of *C. limon*, *S. cumini*, *M. oleifera* and *E. globulus* had anti-bacterial activity against *C. perfringens* type D isolates. These results are in accordance with similar previous studies (Al Fadel *et al.*, 2015; Silva *et al.*, 2016). In another study, the anti-bacterial potential of ethanolic, methanolic and acetone extracts of *M. oliefera* was evaluated and reported, which is in line with our findings (Carmen *et al.*, 2018). All the plant extracts were able to inhibit the growth of *C. perfringens* type D. However, *E. globulus* extract showed maximum modulation of antibiotic resistance against ceftriaxone. Modulation findings were similar to a

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

previous report which showed that a combination of Eucalyptus honey and antibiotic (cefotaxime) gave a synergistic effect for *C. perfringens* with 10.00 ± 0.58 mm ZOI (Hegazi *et al.*, 2014).

The phytochemicals of the *E*. *globulus* ethanolic extract were separated and identified by GC-MS analysis. Compounds including a-pinene, piperitone, a-terpineol, Gamma-terpenene, 1-8 cineol, globulol and limonene were recorded in abundance. Similar results were reported by Daroui-Mokaddem *et al*. (2010). According to previous studies, all of these compounds have modulatory effects. Piperitone improves the antimicrobial activity of some drugs, such as furazolidone. This mechanism of action has been attributed to the disruption of membrane permeability that increases the intake of antibiotics in the cell (Alexopoulos *et al.*, 2019). Similarly, 1-8 cineol and Gamma-terpenene exhibit their synergistic activity by acting on the bacterial plasma membrane (Şimşek and Duman, 2017). Sreepian *et al.* (2022) reported the synergistic effects of limonene with gentamicin against clinical isolates of MRSA and MSSA.

Phytochemicals are small organic bioactive hydrophobic compounds that are considered as naturally occurring antibiotics. According to some previous findings, one of the possible modes of action of these compounds could be the destruction of the plasma membrane of a bacterial cell, facilitating the influx of antibiotics inside the bacteria (Leite *et al.*, 2007). This mechanism of action has been associated with some terpenoids that cause damage to bacterial plasma membranes (Saleem *et al.*, 2010). The extracts can also modulate the resistance by inhibiting bacterial efflux pumps, increasing the intracellular accumulation of the antibiotics (Stavri *et al.*, 2007). These mechanisms could be the possible reason for the modulation of antibiotic resistance against ceftriaxone by *C. perfringens* type D isolates. In a previous study, the inhibitory effect of α-pinene along with β-pinene and eugenol was observed, and maximum anti-bacterial potential was observed against endocarditis caused by Gram-positive bacteria (Leite *et al.*, 2007).

CONCLUSION

In this study, the antibiotic resistance pattern of *C*. *perfringens*, as well as anti-bacterial activity and resistance modulation potential of indigenous plants, were determined. As a result, it was observed that all the plants used in this study have anti-bacterial activity, but only *E. globulus* was able to modulate the antibiotic resistance of *C. perfringens* against streptomycin, gentamicin and most significantly in ceftriaxone. Hence, it is concluded that plants have phytochemical compounds that have the ability to modulate antibiotic resistance. Therefore, combining antibiotics and plant extracts could be a better approach for controlling various antibiotic-resistant infections in the future.

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