Pertanika J. Trop. Agric. Sci. 48 (6): 2033 - 2055 (2025)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Metabolomics Profile of *Ziziphus mauritiana* and Its Anti-*Vibrio* Activity Using ^{1}H NMR Spectroscopy Coupled with Multivariate Data Analysis

Azizul Isha[1]*, Noraznita Sharifuddin[2], Mazni Abu Zarin[3], Yaya Rukayadi[1], and Ahmad Faizal Abdull Razis[1]

[1]Natural Medicines and Products Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Aquatic Animal Health and Therapeutics Laboratory, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Laboratory of Vaccine and Biomolecules, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Ziziphus mauritiana is a medicinal plant commonly used in conventional therapies since it has health-beneficial effects, as it is rich with many biologically active compounds. However, scientific research on this plant's metabolite profile is insufficient. The goals of this study are to establish chemical profiles of *Z. mauritiana* leaves that were extracted using a variety of polarity solvents. ^{1}H NMR-metabolomics was employed to investigate the correlation between chemical markers and anti-*Vibrio* activities. A portion of the powdered sample of *Z. mauritiana* was extracted using different solvent systems (hexane, chloroform, acetone, and 70% (v/v) ethanol). The profiles of metabolites in each extract obtained were determined by NMR spectroscopy. The correlation between the identified metabolites and anti-*Vibrio* properties of *Z. mauritiana* was interpreted using the metabolomics approach. The *Z. mauritiana* extracts were successfully clustered using principal component analysis according to their metabolite profiles. Acetone and 70% (v/v) ethanol were determined to have good extraction efficiency. The anti-*Vibrio* activity was shown to be highly correlated with the polar metabolites of the 70% (v/v) ethanol extract in the partial least squares analysis. The 70% (v/v) ethanol extracts exhibit a more diverse spectrum than the other extracts, with a total of 42 metabolites that include carbohydrates, amino acids, organics, and fatty acids. The rapid examination of the

ARTICLE INFO

Article history:
Received: 19 February 2025
Accepted: 06 November 2025
Published: 25 November 2025

DOI: https://doi.org/10.47836/pjtas.48.6.19

E-mail addresses:
azizul_isha@upm.edu.my (Azizul Isha)
noraznita@upm.edu.my (Noraznita Sharifuddin)
mazniaz@upm.edu.my (Mazni Abu Zarin)
yaya_rukayadi@upm.edu.my (Yaya Rukayadi)
madfaizal@upm.edu.my (Ahmad Faizal Abdull Razis)
*Corresponding author

metabolite composition of *Z. mauritiana* in relation to its various solvent extractions with a specific anti-*Vibrio* biological activity has been facilitated by the implementation of NMR-metabolomics.

Keywords: Anti-*Vibrio* activity, ^{1}H-NMR-based metabolomics, multivariate data analysis, *Ziziphus mauritiana*

INTRODUCTION

Viral infections continue to pose a substantial public health threat due to their transmission. The *Vibrio* species are Gram-negative rods that are motile and found in freshwater and estuarine water environments (Alam et al., 2009; Ramalingam & Ramarani 2006). *V. vulnificus, V. parahaemolyticus, V. campbellii, and V. harveyi* are the most common *Vibrio* species causing infections in people. The pathogenicity of *Vibrio* strains can be harmful to humans or marine animals using a broad repertory of virulence factors encoded by virulence genes (Schroeder et al., 2017). It may present three main syndromes of clinical illness, including primary septicemia, wound infections, and gastroenteritis (Yun & Kim, 2018). According to Baker-Austin & Oliver (2018), the transmission of *Vibrio* to humans can occur by the ingestion of undercooked marine products or contamination of water.

Antibiotics and other antimicrobial agents have been extensively employed to effectively cure, manage, and avoid bacterial infections in humans as well as animals (Saga & Yamaguchi, 2009). *Vibrio* infections are treated with the following antibiotics: tetracycline, chloramphenicol, expanded-spectrum cephalosporins (ceftazidime), quinolones (doxycycline or fluoroquinolone), trimethoprim-sulphamethoxazole, gentamicin, levofloxacin, and ciprofloxacin (Elmahdi et al., 2016; Malla et al., 2014; Wong et al., 2015). Nevertheless, the environment has been contaminated with antimicrobial-resistant *Vibrio* species because of the ubiquitous application of antibiotics in aquaculture, farming, livestock fields, and pharmaceuticals (Heuer et al., 2009; Gao et al., 2017; Lulijwa et al., 2020). Antibiotic resistance is a major cause of therapeutic failures and significant morbidity and death. Consequently, urgent action is required to avert the proliferation of resistant bacteria. Natural anti-*Vibrio* agents derived from natural resources could be the subject of future research and development efforts.

Given the growing concern regarding the resistance of bacteria to commercial antibiotics in the pharmaceutical industry, it is imperative to investigate alternative plant sources and consider *Z. mauritiana* as a potential source of antimicrobial medications (Febriza et al., 2022; Jain et al., 2019. It is a plant that is indigenous to the Southeast Asia's Indo-Malaysia and commonly known as bidara in Malaysia. The genus *Ziziphus*, belonging to the family *Rhamnaceae*, comprises approximately 170 species and is widely utilized to treat of health issues (Abdallah et al., 2016). The leaves have traditionally been used to alleviate a variety of ailments, including diarrhea, nausea, vomiting, asthma, fever, and dermatitis (Abalaka et al., 2010). The roots are utilized to prevent and treat cutaneous

diseases (Adzu et al., 2001). People consume the immature foliage as a vegetable and use it to treat liver disorders, fever, and asthma (Dahiru & Obidoa, 2007).

Previous studies reported that *Z. mauritiana* leaves have phytochemical compounds, which act as anti-*Vibrio* activity. Febriza et al. (2022) found that a *Z. mauritiana* leaf inhibited the growth of *V. cholera*. Another study by Jain et al. (2019) claimed that extracts from *Z. mauritiana* leaf had anti-*Vibrio* activity against *V. parahemolyticus.* Secondary metabolites, including alkaloids, tannins, phenolic substances, and flavonoids, have been identified in *Z. mauritiana* leaves (Najafi, 2013). The fruits are abundant in vitamin C and contain 20–30% sugar, 2.5% more protein, and 12.8% carbohydrates (Priyanka et al., 2015). Furthermore, research has indicated that the bark exhibits cytotoxic activity to a range of cancer cell lines (Pisha et al., 1995). It has also been noted that *Z. mauritiana* has certain therapeutic advantages, including antioxidant, anticancer, antidiarrheal, antibacterial, anti-inflammatory and hypoglycemic activities (Butt et al., 2021; Dahiru et al., 2010; Goyal et al., 2012; Prakash et al., 2020; Ramar et al., 2022; Verma et al., 2018). There is limited scientific research on the anti-*Vibrio* potential of *Z. mauritiana*, and most of the existing studies focus on *V. cholerae* and *V. parahemolyticus*. Therefore, there is a need for further research to investigate the potential anti-*Vibrio* effects of *Z. mauritiana* leaf against different *Vibrio* spp. for a comprehensive understanding of this plant's antimicrobial properties.

By combining NMR with multivariate data analysis, the metabolomic profiles of numerous plant species and traditional phytomedicines can be determined (Choi et al., 2007). Multivariate methods, such as PCA, PLS-DA, and OPLS-DA, have been utilized to evaluate the intricate sets of NMR data. Through a combination of anti-*Vibrio* activity, ^{1}H NMR data and a metabolomics approach, the biomarkers and mechanism of action exerted by *Z. mauritiana* leaves extracted should be better understood. Hence, the purpose of this work is to identify the chemical profiles of *Z. mauritiana* leaves extracted by varying polarity solvents and to correlate the chemical markers to anti-*Vibrio* activities using the ^{1}H NMR-metabolomics approach. The findings of this investigation may offer novel prospects for the effective treatment of *Vibrio* infections.

MATERIALS AND METHODS

Materials

Extractions were conducted using solvents of analytical grade, including ethanol, n-hexane, acetone, and chloroform. NMR solvents such as deuterated methanol-d_4 (CD_3OD, 99.8%), nondeuterated potassium dihydrogen phosphate (KH_2PO_4, pH 6.0), deuterium oxide (D_2O, 99.9%), and trimethylsilylpropionic acid-d_4 sodium salt (TSP) were supplied by Merck (Darmstadt, Germany). Deionized water was obtained from a PURELAB Chorus 2 system (ELGA Lab Water, USA). The American Type Culture Collection (Manassaa, VA, USA) was the source of *V. vulnificus* (ATC.33147)*, V. parahaemolyticus* (ATC.17802), *V.*

campbellii (ATC.BAA-1116) and *V. harveyi* (ATC.35084). Mueller Hinton agar (MHA; Difco, Sparks, USA) and Mueller Hinton broth (MHB; Difco, Sparks, USA) were used as media for the antibacterial assays. Dimethyl sulfoxide (DMSO) was obtained from Sigma-Aldrich.

Leaves of *Z. mauritiana* Harvesting and Preparation

Leaves of *Z. mauritiana* were harvested at the Universiti Putra Malaysia campus. The mature leaves collected from the *Z. mauritiana* tree were taken from the uppermost branches, where they received ample sunlight. The harvested leaf samples were rinsed with flowing distilled water and delicately dried using paper towels. The samples were subsequently stored in an ultra-low temperature freezer overnight in sealed receptacles. The frozen samples were dried in a freeze-dryer (Scanvac Labogene) for 72 h and pulverised into fine powder using a grinder (Waring, 32 BL80, New Hartford, NY, USA). Until the subsequent analysis, the powder samples were stored in an ultra-low temperature freezer.

Preparation of *Z. mauritiana* Leaves Extracts

The *Z. mauritiana* freeze-dried leaves powder (10 g) was extracted using sonication in 200 mL of the respective solvents, including 70% (v/v) ethanol, acetone, chloroform and hexane (20 min, 25°C). The crude extracts were obtained by evaporating the combined filtered supernatants with a rotary evaporator after the process was re-extracted twice. A total of six replicates for each solvent were prepared.

Antibacterial Assay

To prepare a stock solution of each plant extract, 100 mg of the extract was dissolved with 1 ml of DMSO. For each stock solution, 100 µL of the stock solution was mixed with 900 µL of pure water to make a 1% test solution (10 mg/mL).

Disc Diffusion Assay

An agar diffusion assay was implemented to evaluate antibacterial activity (Rukayadi et al., 2009). *Vibrio* isolates were cultured on Mueller–Hinton agar medium that contained 1% NaCl from the culture stock. Subsequently, pure colonies were employed to generate 0.5 McFarland turbidity. To inoculate fresh Petri dishes, a cotton swab was used. The extracts of *Z. mauritiana* leaves were dissolved with DMSO to make a 10 mg/mL concentration. *Vibrio* isolates were cultured on Mueller–Hinton agar medium that contained 1% NaCl from the culture stock. Subsequently, pure colonies were employed to generate 0.5 McFarland turbidity. Inhibition zones were observed after the inoculated dishes were incubated at 37°C for 24 hours. Six replicates were utilised in each experiment, and the inhibition zone

diameter was determined in millimetres (mm) for each experiment. Tetracycline (5 µg) was used as a standard drug against the tested *Vibrio* strains.

Determination of Minimum Inhibitory Concentration (MIC) Values

The MIC for *Vibrio* isolates that are susceptible to the *Z. mauritiana* leaves extracts was determined using the broth microdilution method. To produce test concentrations ranging from 10 to 0.0195 mg/mL for each solvent extract derived from a 10 mg/mL stock of plant extracts, a series of two-fold serial dilutions were prepared using filtered sterile distilled water. Each well of the 96-well plates was filled with a 100 µL aliquot of double-strength Mueller-Hinton broth containing 1% NaCl. Subsequently, 50 µL of the extract was added in descending order, along with 50 µL of the test bacteria suspension. 50 µL of tetracycline, 50 µL of the test microorganisms, and 100 µL of Mueller-Hinton broth with 1% NaCl are present in the positive control well. The negative control well is composed of 50 µL of the test microorganisms, 50 µL of filtered sterile distilled water, and 100 µL of Mueller-Hinton broth with 1% NaCl. After the test extract was added to the inoculated plates, they were incubated aerobically at 37°C for 24 hours to evaluate the MIC. The minimal concentration of the test sample that resulted in complete inhibition of bacterial growth is denoted by the MIC value. The MICs were obtained in triplicate.

Determination of Minimum Bactericidal Concentration (MBC) Values

A 10 µL suspension sample was transferred to an MHA plate for sub-culture in order to ascertain the MBC from each of the 12 wells used in the MIC test. The MBC value of the test extract was calculated after the inoculation plates had been incubated at 37°C for 24 hours. The MBC value was the minimum concentration at which no bacterial growth was observed. The MBCs were obtained in triplicate.

NMR Measurement and Multivariate Data Analysis

The ^{1}H NMR spectra were run at 25˚C using a 500 MHz Varian INOVA NMR spectrometer. The samples were prepared as per the earlier published approach (Kim et al., 2011). A 1:1 mixture of CD_3OD and KH_2PO_4 (pH 6.0) in D_2O containing 0.1% TSP sodium salt was added to a 2.0 mL Eppendorf tube containing 50 milligrams of each extract. 0.75 mL of volume was introduced. The mixture was vortexed for one minute, ultrasonically for twenty minutes, and centrifuged for ten minutes at 10,000 rpm (7,826 G-force) at 27°C. The ^{1}H NMR measurement was conducted by promptly transferring the supernatant (0.6 mL) to a 5 mm NMR tube and employing a pre-saturation sequence.

The acquired spectra were analysed with Chenomx software (v.5.1, Alberta, Canada) with a consistent configuration used across all spectra. The spectral intensities were grouped

into bins of similar width (0.04) within the range of 0.50–10.00. The areas between 4.70 and 4.90, which correspond to water and 3.23 and 3.36, which represent residual methanol, were omitted.

The multivariate data analysis was employed to examine the variation in metabolite contents of *Z. mauritiana* leaves extracted with various solvents, including acetone, chloroform, hexane, and ethanol (70% (v/v) using SIMCA-P software (v. 13.0, Umetrics, Umeå, Sweden). Principal component analysis (PCA) was used to establish the categorization properties of the four different solvent samples, as well as the metabolites that influenced their variation. The samples were grouped according to their similarity in the score plot of PCA, while the variables' contributions to the observed differences among the samples were illustrated in the loading plot. Pareto scaling was selected in this analysis.

A Partial Least Squares (PLS) analysis, which is a kind of multivariate regression, was used to look at how the biological activities of certain metabolites relate to the responses of chosen Vibrio strains. This technique helps uncover the correlations between these two datasets, providing insights into which metabolites might be influencing the activity of the bacteria. The *X*-variables used in the PLS analysis were NMR chemical shifts, while the *Y*-variable represented the inhibition zone value as determined by the disc diffusion method. The accuracy and predictiveness of the PLS model were confirmed through the use of a model validation technique, such as a permutation test.

The identification of metabolites was conducted by comparing the distinctive signals recognized in the ^{1}H NMR spectra of the extract derived from *Z. mauritiana* leaves with those documented in the literature, the Chenomx database (Chenomx NMR Suite 7.7 library) and the human metabolome database (www.hmdb.ca; accessed on 2nd December 2024). Additionally, the 2D *J*-resolved was utilized to aid in the identification of metabolites.

RESULTS AND DISCUSSION

Antibacterial Activity of Extracts of *Z. mauritiana* Leaves

The disk diffusion susceptibility test was used to screen the *Z. mauritiana* leaves extracted with different solvents for measurement of antimicrobial activity against *Vibrio* strain. The extracts' potency was assessed by the extent of the inhibition zone that emerged as a result of the anti-*Vibrio* effect. The extract is more efficacious and has a higher anti-*Vibrio* activity when the zone of inhibition is larger. In Table 1, the inhibition zones of *Z. mauritiana* leaves against *Vibrio* strains (*V. vulnificus, V. parahaemolyticus, V. campbellii* and *V. harveyi*) are illustrated. The leaves were extracted with various solvents, including 70% (v/v) ethanol, acetone, chloroform, and hexane. The ethanol with 70% (v/v) had the best effect on all *Vibrio* strains. At a concentration of 10 mg/mL, it had inhibition zones of 15.66 mm for *V. parahaemolyticus*, 10.46 mm for *V. vulnificus*, 24.18 mm for *V. campbellii* and 19.53 mm for *V. harveyi* from the different extract solvents.

Table 1
Inhibition zones of Z. mauritiana *leaves extracted with different solvents against Vibrio strains*

***Vibrio* Strain**	**Tetracycline (5 µg)**	**Inhibition Zone Diameter (mm)**			
		70% (v/v) Ethanol	**Acetone**	**Chloroform**	**Hexane**
V. vulnificus (ATC.33147)	22.00±0.00	15.66±0.16	11.60±0.19	9.46±0.38	9.48±0.40
V. parahaemolyticus (ATC.17802)	17.00±0.00	10.46±0.17	9.55±0.30	7.53±0.16	7.46±0.33
V. campbellii (ATC.BAA-1116)	24.55±0.30	24.18±1.85	18.00±0.71	13.57±1.42	11.33±0.88
V. harveyi (ATC.35084)	16.33±0.88	19.53±0.34	12.62±0.35	10.42±0.34	9.43±0.24

Diameter of inhibition zones in mm (including disc). Values are expressed as means ± standard deviation (SD)

It can be observed that the inhibition zone increases with the polarity of the extracts as it increases from hexane to ethanol. The antibacterial activities of 70% (v/v) ethanol and acetone extract against the *Vibrio* strain were significantly enhanced. It is likely a result of the ability of high-polarity solvents to dissolve a greater number of secondary metabolites (Kebede & Shibeshi, 2022). Extraction of active metabolites and their possible antibacterial effects are affected by the solvent's polarity.

MIC and MBC Values of Extracts of *Z. mauritiana* Leaves

The anti-*Vibrio* activity of the *Z. mauritiana* leaves that were extracted using different solvents was further investigated by determining the MIC and MBC values, in addition to the total activity. Table 2 displays the MIC and MBC values for the extracts that were tested on the four *Vibrio* strains. The results show the level of growth inhibition exhibited by the various extracts against the *Vibrio* strains.

Table 2
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values (mg/mL) of different solvent extracts of Z. mauritiana *leaves against Vibrio strains*

***Vibrio* spp.**		**70% (v/v) Ethanol**	**Acetone**	**Chloroform**	**Hexane**
V. vulnificus (ATC.33147)	MIC	0.625	2.500	2.500	2.500
	MBC	1.250	2.500	2.500	5.000
V. parahaemolyticus (ATC.17802)	MIC	0.313	1.250	1.250	2.500
	MBC	1.250	2.500	2.500	2.500
V. campbellii (ATC.BAA-1116)	MIC	0.625	2.500	2.500	2.500
	MBC	0.625	2.500	2.500	5.000
V. harveyi (ATC.35084)	MIC	1.250	2.500	2.500	2.500
	MBC	1.250	2.500	2.500	5.000

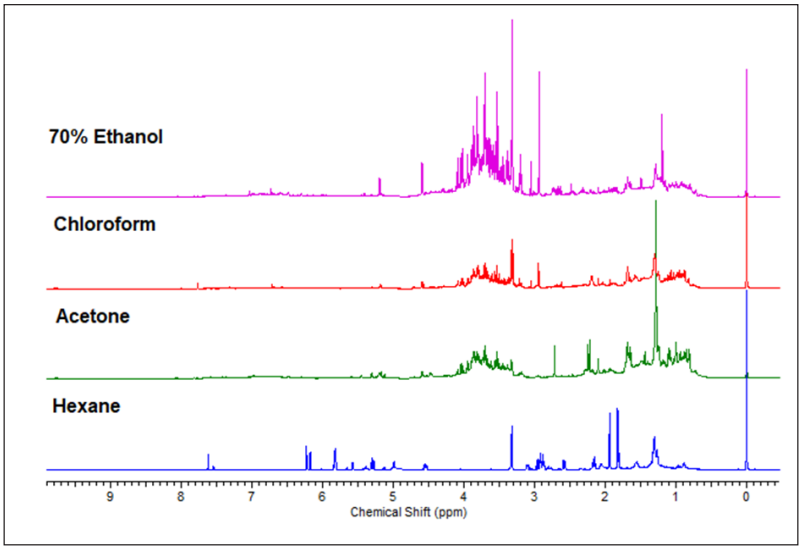

Figure 1. Representative 500 MHz ^{1}H NMR spectra of different solvent extracts of *Z. mauritiana* leaves

The MIC and MBC values of the 70% (v/v) ethanol extracts varied from 0.313 to 1.250 mg/mL, respectively. Compared to the *V. harveyi* strain, which demonstrated a MIC and MBC of 1.250 mg/mL, the 70% (v/v) ethanol extract exhibited greater efficacy against *V. parahaemolyticus* (MIC 0.313 mg/mL; MBC 1.250 mg/mL), *V. campbellii* (MIC 0.625 mg/mL; MBC 0.625 mg/mL), and *V. vulnificus* (MIC 0.625 mg/mL; MBC 1.250 mg/mL). The acetone and chloroform extracts have MICs and MBCs of 1.250–2.500 mg/mL and 2.500 mg/mL, respectively. Nevertheless, MIC and MBC of the hexane extracts were 2.500 mg/mL and 2.500–5.000 mg/mL, respectively.

Tamokou et al. (2017) reported that plant extracts or their components are considered highly potent if they exhibit MIC values below 100 µg/ml. They are considered significantly active if the MIC is from 100 to 512 µg/ml, moderately active if the MIC is between 512 and 2048 µg/ml, and inactive if the MIC exceeds 2048 µg/ml. Hence, the current findings demonstrate that the extracts from *Z. mauritiana* leaves exhibit a moderate to significant level of activity against the *Vibrio* strains that were determined. Furthermore, some polar metabolites found in the leaves' extracts have significant anti-*Vibrio* properties and could contribute strongly to the overall plant's anti-*Vibrio* activity.

^{1}H NMR Spectra of Extracts of *Z. mauritiana* Leaves and Its Metabolites Identification

The ^{1}H NMR analysis has been used to identify the metabolites of *Z. mauritiana* leaves extracted with different solvents (70% (v/v) ethanol, acetone, chloroform and hexane). Figure 1 illustrates representative ^{1}H NMR spectra of the extracts of *Z. mauritiana* leaves. A total of 42 metabolites were detectable in *Z. mauritiana,* comprising carbohydrates, fatty

acids, sesquiterpene lactones and amino acids. Table 3 and Figure 2 display the identified metabolites and their corresponding distinctive signals obtained from different extracts of *Z. mauritiana* leaves. These metabolites were identified by comparing them with those documented in the literature, the Chenomx database (Chenomx NMR Suite 7.7 library), and the human metabolome database. The 2D *J*-resolved experiment was additionally employed to support the identified metabolites (Figure S1-Supplementary data).

Table 3
NMR signal assignments for metabolites identified in the ^{1}H and 2D NMR spectra of Z. mauritiana *leaves extract, in addition with their corresponding multiplicity (s: singlet; d: doublet; t: triplet; m: multiplet) and scalar coupling constant (J(Hz)) values*

Peak No.	Metabolites	^{1}H Chemical Shifts (multiplicity, J)
1	Alanine	1.50 (d, J= 10.0 Hz), 3.16 (d, J=2.5 Hz)
2	Acetic acid	1.94 s
3	Proline	4.09 (dd, J = 1.5 Hz, 1.0 Hz)
4	Succinic acid	2.49 s
5	Asparagine	4.04 (dd, J = 1.5 Hz, 0.5 Hz), 2.98 (dd, J=3.5 Hz, J=3.5 Hz)
6	Choline	4.07 s, 3.22 s
7	Betaine	3.27 s
8	Sucrose	3.68 s, 4.06 (t, J= 3.5 Hz), 4.14 (d, J= 8.0 Hz), 5.41 (d, J= 4.5 Hz)
9	Formic acid	8.47 s
10	Valine	1.00 (d, J=6.5 Hz), 1.03 d (J=6.5 Hz)
11	Glycine	3.53 s
12	Oleic acid	2.33 (t, J=5.0 Hz)
13	Glucose	5.20 (d, J=5.0 Hz), 3.49 (dd, J=5.0 Hz, J=5.0 Hz)
14	Quercetin	6.31 (d, J=2.5 Hz), 6.48 s
15	Kaempferol	7.77 (d, J=5.0 Hz), 6.41 s
16	Myricetin	6.52 (d, J=5.0 Hz), 7.04 s
17	Catechin	4.60 (d, J=10.0 Hz), 6.99 (d, J=5.0 Hz), 3.95 (m)
18	Fructose	4.19 (d, J=10.0 Hz)
19	Malic acid	4.24 (dd, J=5.0 Hz, J=10.0 Hz), 4.31 (dd, J=5.0 Hz, J=5.0 Hz)
20	Fumaric acid	6.55 s
21	Lactic acid	1.35 (d, J=10.0 Hz)
22	Arginine	3.22 (t, J=10.0 Hz)
23	*γ*-Aminobutyric acid	3.02 (t, J=5.0 Hz)
24	Hydroxybenzoic acid	7.82 (d, J=5.0 Hz)
25	Chlorogenic acid	3.88 (dd, J=5.0 Hz, J=5.0 Hz)
26	*N,N*-Dimethylglycine	2.94 s
27	Ascorbic acid	4.54 (d, 5.0 Hz)
28	Betulin	0.96 s, 0.94 s, 0.92 s
29	(*E*)-Aconitic acid	6.72 s, 3.58 s

Table 3 *(continue)*

Peak No.	Metabolites	^{1}H Chemical Shifts (multiplicity, J)
30	(*Z*)-Aconitic acid	6.22 s, 3.55 s
31	Phosphorylcholine	3.23 s
32	4-Hydroxyisoleucine	1.88 m, 3.81 (d, J=10.0 Hz)
33	Aspartic acid	2.85 (dd, J=10.0 Hz, J=10.0 Hz)
34	Leucine	2.66 m
35	Quinic acid	3.05 s
36	Caffeoylquinic acid	2.20 (dd, J=10.0 Hz, J=10.0 Hz), 3.96 (dd, J=5.0 Hz, J=5.0 Hz)
37	Orientin	6.14 s
38	1-*O*-ethyl-β-glucoside	1.19 (t, J=10.0 Hz)
39	Rutin	1.10 (d, J=5.0 Hz)
40	*L*-Rhamnitol	1.27 (t, J=5.0Hz)
41	*β*-Pinene	2.46 m
42	Glutamic acid	3.79 (dd, J=1.5 Hz, J=2.5)

The signals observed in the aliphatic area of δ 0.50–3.00 were used to assign the following compounds: valine, acetic acid, succinic acid, oleic acid, lactic acid, *γ*-aminobutyric acid, *N*,*N*-dimethylglycine, betulin, 1-*O*-ethyl-*β*-glucoside, rutin, aspartic acid, leucine, quinic acid, *L*-rhamnitol and *β*-pinene. Alanine was detected with the doublet at δ 1.50 and δ 3.16, whereas the signals at δ 2.98 (dd) and δ 4.04 (dd) were detected as asparagine. Additionally, the signals at δ 1.88 (m) and δ 3.81 (d) were assigned to 4-hydroxyisoleucine, whereas the signals at δ 2.20 (dd) and δ 3.96 (dd) were detected as caffeoylquinic acid.

In the sugar area (δ 3.00-5.50), sucrose, glucose, and fructose were discovered. Other metabolites present in the sugar area were proline, choline, betaine, glycine, malic acid, arginine, chlorogenic acid, ascorbic acid, phosphorylcholine, quinic acid, glutamic acid and *γ*-aminobutyric acid. The signals exhibited on the singlet at δ 3.58 and δ 6.72 were used to attribute (*E*)-aconitic acid, while (*E*)-aconitic acid was detected at δ 3.55 and δ 6.22. The signals at δ 3.95 (m), δ 4.60 (d) and δ 6.99 (d) were ascribed to catechin.

In the aromatic region (δ 5.50-8.50), formic acid, fumaric acid, hydroxybenzoic acid and orientin were identified with the signals at δ 8.47 (s), δ 6.55 (s), δ 7.82 (d) and δ 6.14 (s), respectively. Quercetin was observed with the signals at δ 6.31 (d) and δ 6.48 (s), respectively. Meanwhile, the signals at δ 6.41(s) and δ 7.77 (d) were ascribed to kaempferol. The identified peaks of myricetin were detectable at δ 6.52 (d) and δ 7.04 s, respectively.

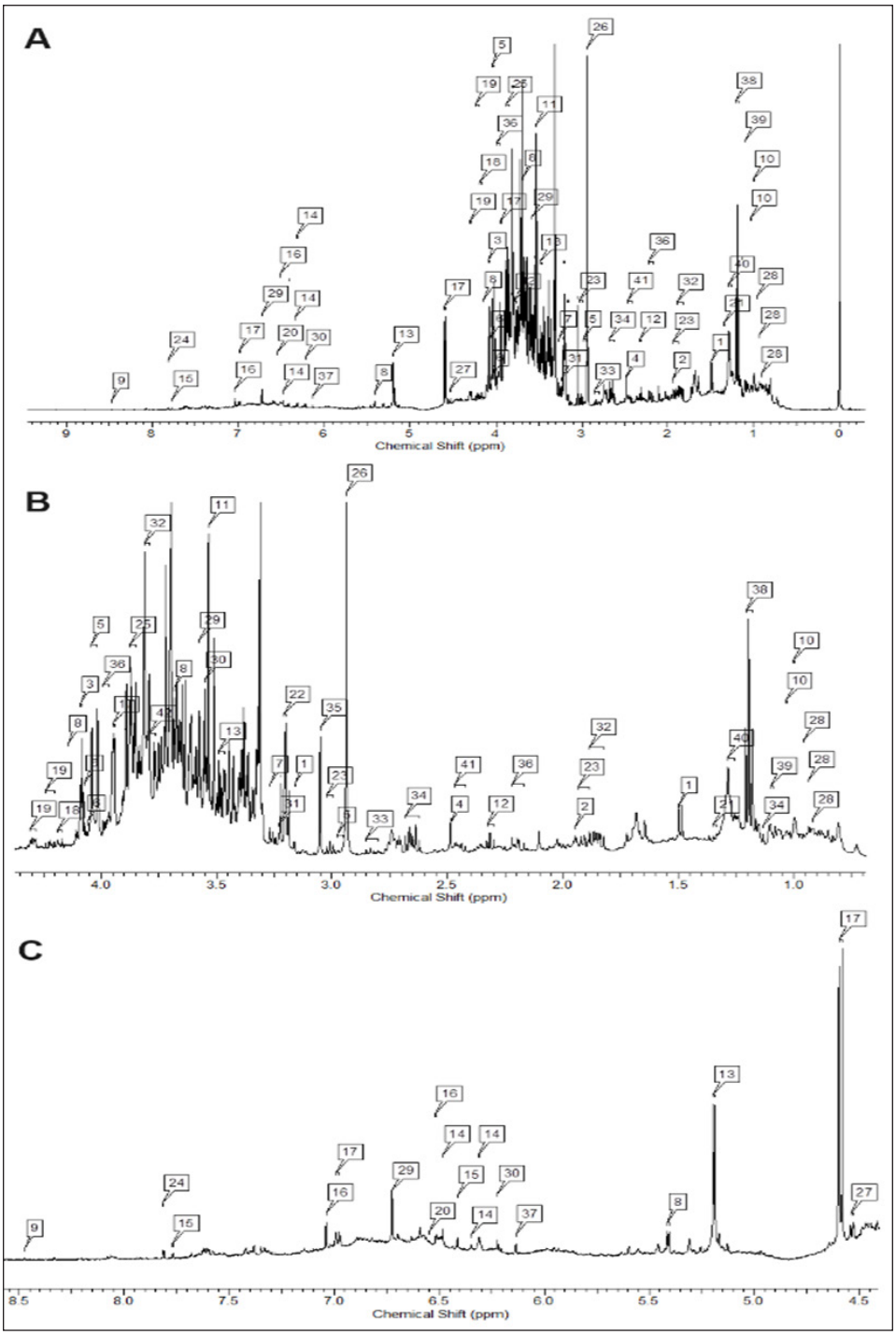

Figure 2. (A) ^{1}H NMR spectrum of *Z. mauritiana* leaves extract. (B) An expanded ^{1}H NMR spectrum between 0.50 and 4.30. (C) An expanded ^{1}H NMR spectrum between δ 4.40 and 8.51. Refer Table 1 for metabolite number interpretation

Discrimination of Extracts of *Z. mauritiana* Leaves by PCA Model from NMR Analysis

The changes of metabolite content in the four solvent extracts, i.e., hexane, chloroform, acetone, and 70% (v/v) ethanol of *Z. mauritiana* leaves were further evaluated using MVDA. PCA was applied to understand the clustering features of the four different extract samples and the metabolites contributing to the variability. PCA is an unsupervised technique that is advantageous for the identification of patterns and the fingerprinting of metabolites in data sets that lack any prior knowledge of sample information or classification (Kosmides et al., 2013). The utilization of PCA in MVDA is to recognize the pattern and cluster of the samples depending on their variance by exposing the samples to different principal components (PCs). PCA uses the PC to project original data based on a specific feature, enabling a simple evaluation of sample variability. The PCA score plot displays how samples are grouped together, while the loading plot shows how much each variable contributes and how they relate to the differences between samples (Son et al., 2008). In this study, PCA was applied to the NMR data of the four different solvent extracts of *Z. mauritiana* leaves to evaluate the differences in their metabolite contents. The score plot was created to assess the differences among the four different solvents, while the loading plot showed the metabolite signals that might help explain the differences between the clusters. Pareto scaling was selected in this analysis to reduce the influence of variables with large magnitudes while preserving the overall variance.

As shown in Figure 3A, the PCA score plot of four different solvent extracts of *Z. mauritiana* leaves obtained from ^{1}H NMR data spectra show different metabolite profiles. PC1 exhibited the greatest sample variation, followed by PC2. The PCA score plot demonstrates that PC1 accounted for 54.2% of the data's variation, while PC2 explained 30.8% of it. The different extracts of *Z. mauritiana* leaves were clustered into three groups. The chloroform and hexane extracts were combined, resulting in negative scores for PC1 and PC2, respectively. The acetone and 70% (v/v) ethanol extracts were clearly distinguished, with positive and negative PC2 scores, respectively.

Based on the PCA loading plot displayed in Figure 3B, the chloroform and hexane extracts contain higher levels of acetic acid, aspartic acid, (*Z*)-aconitic acid, lactic acid, ascorbic acid and orientin. The acetone extract was separated by having a higher content of hydroxybenzoic acid, kaempferol, (*E*)-aconitic acid, quercetin, proline, caffeoylquinic acid, alanine, succinic acid, oleic acid, *L*-rhamnitol, rutin, valine and botulin. Meanwhile, 1-*O*-ethyl-*β*-glucoside, 4-hydroxyisoleucine, arginine, asparagine, betaine, catechin, chlorogenic acid, choline, formic acid, fructose, fumaric acid, glucose, glutamic acid, glycine, leucine, malic acid, myricetin, *N,N*-dimethylglycine, phosphorylcholine, quinic acid, sucrose, *β*-pinene and *γ*-aminobutyric acid were found more abundant in 70% (v/v) ethanol extract. The metabolite distribution among these four different solvent samples extracted could be due to the polarity of these metabolites. Abdusalam et al. (2022) also

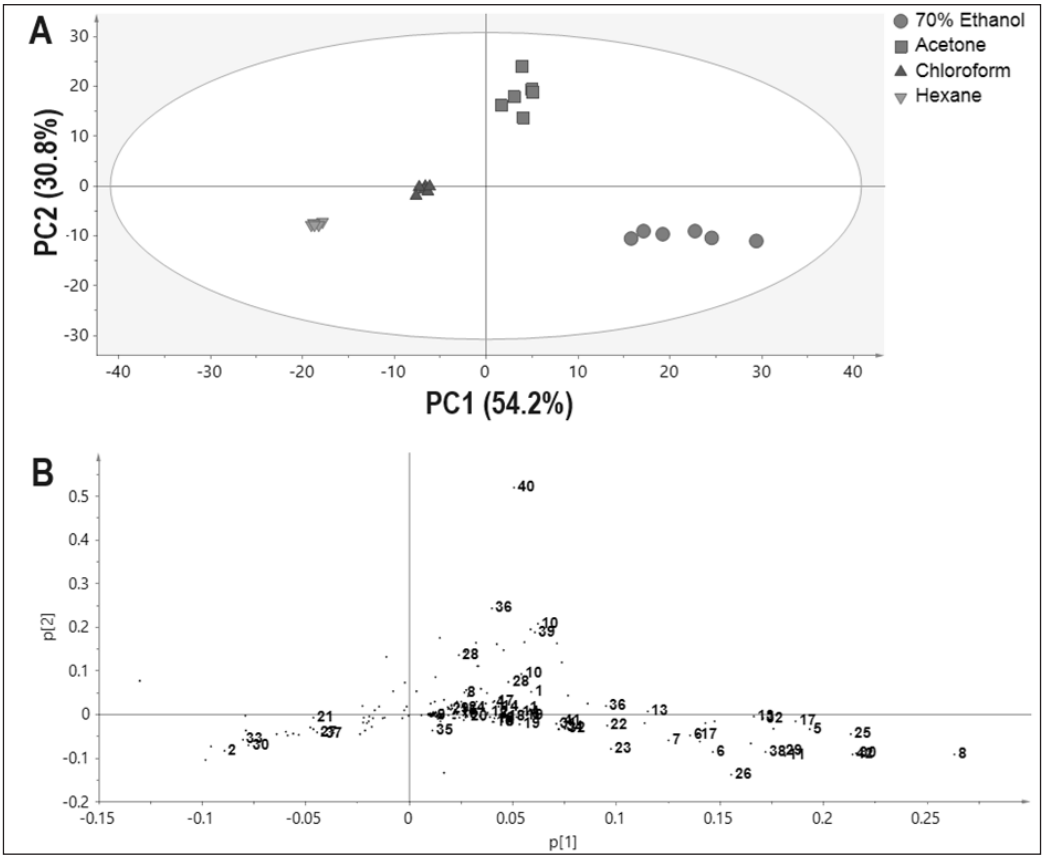

Figure 3. The PCA score (A) and loading (B) plots of different extracts of *Z. mauritiana*. Refer Table 1 for metabolite number interpretation

reported that the influence of the separation on the sample in PCA analysis is based on the polarity of the metabolites, i.e., their hydrophobic and hydrophilic characteristics.

A partial least squares (PLS) analysis was carried out to evaluate the relationship between the biological activities of the extract-identified metabolites and the selected *Vibrio* strains. *X* variables are represented by the NMR signals, while *Y* variables are represented by the inhibition zone value obtained through the disc diffusion method of anti-*Vibrio* activities against *Vibrio* species. The PLS model was validated through *R2* and *Q2* cumulative internal cross-validation. The *R2* metric quantifies the viability of a model, whereas *Q2* measures the model's prediction accuracy.

Theoretically the model's performance improves when the *R2* and *Q2* values approach 1. Indicating that the model shows a goodness-of-fit and predictive quality (Eriksson, 2006). The autofit analysis of the PLS model for *Z. mauritiana* extracts proved that the resulting model is effectively characterized by four principle components (PCs), as proven

by a cumulative goodness of fit (*R2Y*) of 0.965. Additionally, the model demonstrates strong predictive ability, as indicated by the cumulative *Q2* value of 0.936. Permutation tests were utilized to validate the PLS model. The cross-validation and permutation tests showed no PLS model overfitting (Figure S2-Supplementary data). The *Y*-intercepts of *Q2* and *R2* were below 0.5 and 0.05, respectively, signifying that the PLS model was valid and not indicative of overfitting (Maulidiani et al., 2013). Based on these results, the PLS model shows an adequate fit of the model. The PLS biplot (Figure 4) showed that the 70% (v/v) ethanol extract of *Z. mauritiana* leaves had closer anti-*Vibrio* activity against *V. parahaemolyticus, V. harveyi, V. vulnificus* and *V. campbelli.*

Among the compounds in the 70% (v/v) ethanol extract contributing to the anti-*Vibrio* activity of the *Vibrio* strains were alanine, arginine, asparagine, betaine, caffeoylquinic acid, catechin, chlorogenic acid, choline, formic acid, fructose, fumaric acid, *γ*-aminobutyric acid, glucose, glutamic acid, glycine, hydroxybenzoic acid, kaempferol, malic acid, myricetin, phosphorylcholine, proline, quercetin, quinin acid, sucrose, *N*,*N*-dimethylglycine, leucine, (*E*)-aconitic acid, (*Z*)-aconitic acid, and 4-hydroxyisoleucine. These metabolites have been

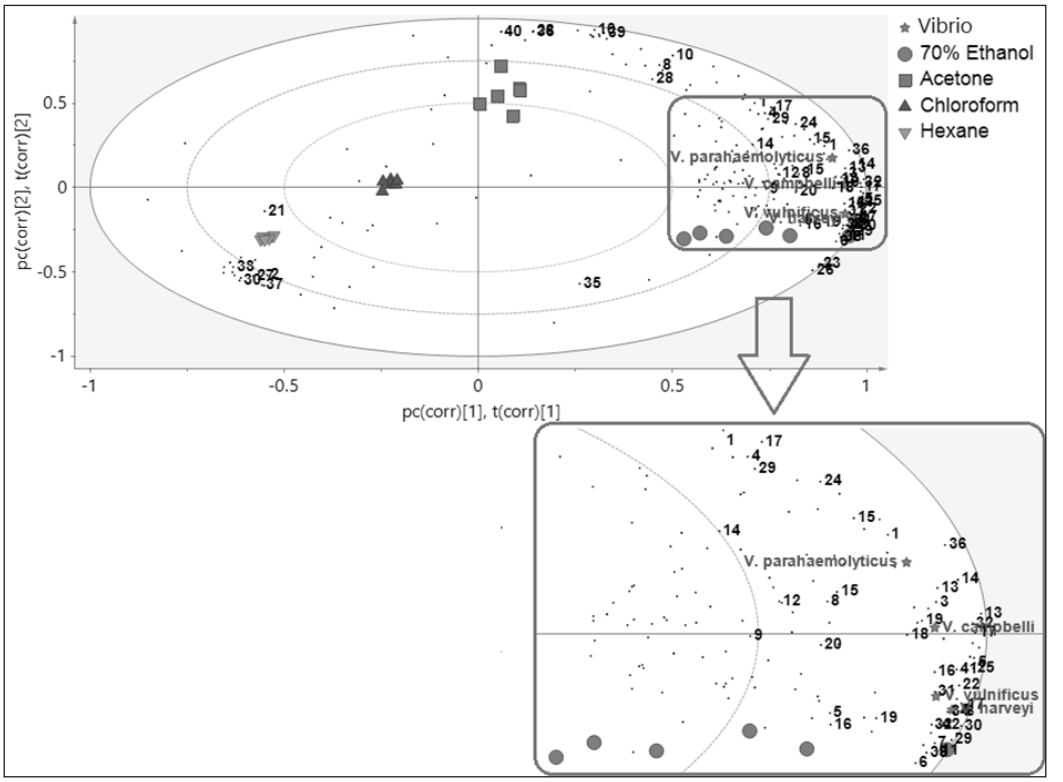

Figure 4. The PLS biplot illustrates the correlation between the metabolites found in various *Z. mauritiana* extracts and their antibacterial activity against selected *Vibrio* species. Refer Table 1 for metabolite number interpretation

shown to be antibacterial against numerous bacteria, including *Vibrio* species (Cho et al. 1998; Duranti et al., 2020; He et al., 2011; Lu et al., 2021; Mine & Boopathy, 2011; Ng et al., 2017; Otsuka et al., 2008; Roy et al., 2022; Taguri et al., 2006).

Several secondary metabolites showed significant antibacterial biological activity. Hydroxybenzoic acid has been demonstrated to be efficient against some bacteria. Cho et al. (1998) demonstrated that hydroxybenzoic acid had antibacterial properties against many types of Gram-positive and Gram-negative bacteria strains, such as *V. vulnificus*. The other metabolites of this plant, such as quercetin, catechin, myricetin, kaempferol, malic acid, formic acid and fumaric acid, were also reported to have antibacterial activity. Quercetin has been reported to exhibit high antibacterial action against *V. parahaemolyticus* (Roy et al., 2022)*, V. harveyi* (Vikram et al., 2010) and *V. campbelli* (Phuong et al., 2020). It was discovered that catechin and myricetin were responsible for inhibiting the growth of many microorganisms, including *V. parahaemolyticus, V. vulnificus* and *Aeromonas hydrophila* (Taguri et al., 2006). Kaempferol and some derivatives exhibit efficacy against *Vibrio* species, demonstrating significant antibacterial capabilities (Christopoulou et al., 2008; Habbu et al., 2009; Martini et al., 2004; Otsuka et al., 2008). Malic acid effectively inhibits infections caused by *Vibrio* species, including *V. harveyi* and *V. parahaemolyticus* (Mine & Boopathy 2011; Ng et al., 2017; Tomotake et al., 2006).

Formic acid has been shown to be a relatively effective antimicrobial. Mine & Boopathy (2011) investigated the MIC of four organic acids (formic, acetic, propionic, and butyric acids) on *V. harveyi* and discovered that formic acid was the most effective inhibitor. Fumaric acid serves as a potent antibacterial agent. Prior studies have demonstrated that fumaric acid displays potent antibacterial properties against many spoilage pathogens, such as *Campylobacter jejuni*, *Salmonella typhimurium, Escherichia coli, Listeria monocytogenes* and *Staphylococcus aureus* (Kim et al., 2001, He et al., 2011). Quinic acid has demonstrated significant antibacterial efficacy against *Staphylococcus aureus* (Bai et al., 2018) and *Pseudomonas aeruginosa* (Lu et al., 2021).

Gamma-aminobutyric acid (GABA) is found in several microbes, plants, and humans (Duranti et al., 2020). In plants, it participates in processes such as pH regulation, nitrogen storage, and growth (Shelp et al., 2012). Additionally, it serves as a plant defence mechanism against biotic stress caused by insects and necrotrophic fungi (Bown & Shelp, 2016; Seifi et al., 2013). GABA is predominantly synthesised via the irreversible α-decarboxylation of *L*-glutamic acid. The enzyme glutamic acid decarboxylase catalyses this reaction (Michaeli et al., 2011). Within the mitochondrial matrix, GABA undergoes metabolism through the GABA shunt, which is associated with various physiological processes, including the influx of carbon into the tricarboxylic acid cycle (TCAC), modulation of cytosolic pH, osmoregulation, and the generation of energy and signalling. The GABA shunt's relationship with the TCAC ultimately links carbon and nitrogen metabolism in plants (Ramos-Ruiz et

al., 2019; Shelp et al., 2017). Phong et al. (2023) determined the GABA content in lactic acid bacteria strains from seven types of Nem Chua products and tested their antibacterial efficacy against *Bacillus subtilis*. They found that the lactic acid bacteria strains contain GABA and possess antibacterial activity against *Bacillus subtilis*.

Our results suggested that the metabolites including amino acids, organic acids and fatty acid had a synergistic effect on enhancing the anti-*Vibrio* activity. It can be indicated that the combined effect of these metabolites is greater than the sum of their individual effects, leading to a more potent and effective action against *Vibrio* bacteria. This synergy can be achieved by targeting different mechanisms within the bacteria, such as disrupting cell membranes or inhibiting biofilm formation.

CONCLUSION

The study examined the phytochemical profile of *Z. mauritiana* leaf extracts, identifying various metabolites, including carbohydrates, amino acids, and both organic and fatty acids. The extract obtained using 70% ethanol exhibited elevated levels of these compounds. Additionally, it demonstrated anti-*Vibrio* activity against *V. parahaemolyticus*, *V. harveyi*, *V. vulnificus*, and *V. campbelli*, thereby supporting its traditional applications. Further investigation is required to elucidate *Z. mauritiana*'s role in antimicrobial agents, particularly regarding its effects on signalling pathways and network pharmacology. The findings highlight the potential of *Z. mauritiana* for traditional use.

ACKNOWLEDGEMENT

This work was funded by Universiti Putra Malaysia under the Geran Inisiatif Putra Muda [GP-IPM/2022/9731700].

REFERENCES

Abalaka, M.E, Daniyan, S.Y. & Mann, A. (2010). Evaluation of the antimicrobial activities of two Ziziphus species (*Ziziphus mauritiana* L. and *Ziziphus spinachristi* L.) on some microbial pathogens. *African Journal of Pharmacy and Pharmacology,* 4(4), 135-139.

Abdallah, E.M., Elsharkawy, E.R. & Ed-dra, A. (2016). Biological activities of methanolic leaves extract of *Ziziphus mauritiana*. *Bioscience Biotechnology Research Communications, 9*(4), 605-614. https://doi.org/10.21786/bbrc/9.4/6

Abdusalam, K.B., Lee, S.Y., Mediani, A., Akhtar, M.T., Buzgaia, N., Rukayadi, Y., Ismail, I.S. & Shaari, K. (2022). ^{1}H NMR-based metabolomics profiling of *Syzygium grande* and *Oenanthe javanica* and relationship between their metabolite compositions and antimicrobial activity against Bacillus species. *Records of Natural Products, 16*(2), 128-143. http://doi.org/10.25135/rnp.258.21.01.1927

Adzu, B., Amos, S., Wambebe, C. & Gamaniel, K. (2001). Antinociceptive activity of *Ziziphus* spina-christi root bark extract. *Fitoterapia, 72*(4), 344–350. https://doi.org/10.1016/S0367-326X(00)00289-6

Alam, M., Chowdhury, W.B., Bhuiyan, N.A., Islam, A., Hasan, N.A., Nair, G.B., Watanabe, H., Siddique, A.K., Huq, A., Sack, R.B., Akhter, M.Z., Grim, C.J., Kam, K.M., Luey, C.K., Endtz, H.P., Cravioto, A. & Colwell, R.R. (2009). Serogroup, virulence, and genetic traits of *Vibrio* parahaemolyticus in the estuarine ecosystem of Bangladesh. *Applied and Environmental Microbiology, 75*, 6268–6274. https://doi.org/10.1128/AEM.00266-09

Bai, J., Wu, Y., Zhong, K., Xiao, K., Liu, L., Huang, Y., Wang, Z. & Gao,0 H. (2018). A comparative study on the effects of quinic acid and shikimic acid on cellular functions of *Staphylococcus aureus*. *Journal of Food Protection, 81*(7), 1187-1192. https://doi.org/10.4315/0362-028X.JFP-18-014

Baker-Austin, C. & Oliver, J.D. (2018). *Vibrio vulnificus:* New insights into a deadly opportunistic pathogen. *Environmental Microbiology, 20*(2), 423–430. https://doi.org/10.1111/1462-2920.13955

Bown, A.W. & Shelp, B.J. (2016). Plant GABA: Not just a metabolite. *Trends in Plant Science, 21*, 811–813. https://doi.org/10.1016/j.tplants.2016.08.001

Butt, S.Z., Hussain, S., Munawar, K.S., Tajammal, A. & Muazzam, M.A. (2021). Phytochemistry of *Ziziphus mauritiana*; Its nutritional and pharmaceutical potential. *Scientific Inquiry and Review, 5*(2),1–15. https://doi.org/10.32350/sir.52.01

Cho, J.Y., Moon, J.H., Seong, K.Y. & Park, K.H. (1998). Antimicrobial activity of 4-hydroxybenzoic acid and trans 4-hydroxycinnamic acid isolated and identified from rice hull. *Bioscience, Biotechnology and Biochemistry, 62*, 2273–2276. https://doi.org/10.1271/bbb.62.2273

Choi, H.K., Yoon, J.H., Kim, Y.S. & Kwon, D.Y. (2007). Metabolomic profiling of Cheonggukjang during fermentation by ^{1}H NMR spectrometry and principal components analysis. *Process Biochemistry, 42*, 263-266. https://doi.org/10.1016/j.procbio.2006.07.014

Christopoulou, C., Graikou, K. & Chinou, I. (2008). Chemosystematic value of chemical constituents from *Scabiosa hymettia* (Dipsacaceae). *Chemistry & Biodiversity, 5,* 318-323. https://doi.org/10.1002/cbdv.200890029

Dahiru, D. & Obidoa, O. (2007). Pretreatment of albino rats with aqueous leaves extract of *Ziziphus mauritiana* protects against alcohol-induced liver damage. *Tropical Journal* of *Pharmaceutical Research, 6*(2), 705-710. https://doi.org/10.4314/tjpr.v6i2.14649

Dahiru, D., Mamman, D.N. & Wakawa H.Y. (2010). *Ziziphus mauritiana* fruit extract inhibits carbon tetrachloride-induced hepatotoxicity in male rats. *Pakistan Journal of Nutrition, 9*(10), 990–993. https://doi.org/10.3923/pjn.2010.990.993

Duranti, S., Ruiz, L., Lugli, G.A., Tames, H., Milani, C., Mancabelli, L., Mancino, W., Longhi, G., Carnevali, L., Sgoifo, A., Magolles, A., Ventura, M., Ruas-Madiedo, P. & Turroni, F. (2020). *Bifidobacterium adolescentis* as a key member of the human gut microbiota in the production of GABA. *Scientific Reports, 10,* 1–13. https://doi.org/10.1038/s41598-020-70986-z

Elmahdi, S., DaSilva, L.V. & Parveen, S. (2016). Antibiotic resistance of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in various countries: A review. *Food Microbiology, 57,* 128–134. https://doi.org/10.1016/j.fm.2016.02.008

Eriksson, L. (2006). *Multi- and Megavariate Data Analysis.* 2nd ed. Umea, Sweden: Umetrics AB.

Febriza, A., Faradiana, S. & Abdullah, M.F. (2022). Antibacterial Effects of *Ziziphus mauritiana* (Lam) Leaf Extract Against *Vibrio* cholerae. *Herb-Medicine Journal, 5*(3), 9-13. https://doi.org/10.30595/hmj.v5i3.14307

Gao, H., Zhang, L., Lu, Z., He, C., Li, Q. & Na, G. (2017). Complex migration of antibiotic resistance in natural aquatic environments. *Environmental Pollution, 232*, 1–9. https://doi.org/10.1016/j.envpol.2017.08.078

Goyal, M., Nagori, B.P. & Sasmal, D. (2012). Review on ethnomedicinal uses, pharmacological activity and phytochemical constituents of *Ziziphus mauritiana* (*Z. jujuba* Lam., nonMill). *Spatula DD, 2*(2), 107-106. https://doi.org/10.5455/spatula.20120422080614

Habbu, P.V., Mahadevan, K.M., Shastry, R.A. & Manjunatha, H. (2009). Antimicrobial activity of flavanoid sulphates and other fractions of *Argyreia speciosa* (Burm.f) Boj. *Indian Journal of Experimental Biology, 47*, 121-128.

He, C.L., Fu, B.D., Shen, H.Q., Jiang, X.L. & Wei, X.B. (2011). Fumaric acid, an antibacterial component of Aloe Vera L. *African Journal of Biotechnology, 10*, 2973-2977. https://doi.org/10.5897/AJB10.1497

Heuer, O.E., Kruse, H., Grave, K., Collignon, P., Karunasagar, I. & Angulo, F.J. (2009). Human health consequences of use of antimicrobial agents in aquaculture. *Clin Infectious Diseases, 49*, 1248–1253. https://doi.org/10.1086/605667.

Jain, P., Haque, A., Islam, T., Alam, Md. A., & Reza, H. M. (2019). Comparative evaluation of *Ziziphus mauritiana* leaf extracts for phenolic content, antioxidant and antibacterial activities. *Journal of Herbs, Spices & Medicinal Plants*, *25*(3), 236–258. https://doi.org/10.1080/10496475.2019.1600627

Kebede, B. & Shibeshi, W. (2022). In vitro antibacterial and antifungal activities of extracts and fractions of leaves of *Ricinus communis* Linn against selected pathogens. *Veterinary Medicine and Science,* 8, 1802–1815. https://doi.org/10.1002/vms3.772

Kim, H.K., Choi, Y.H. &Verpoorte, R. (2011). NMR-based plant metabolomics: where do we stand, where do we go? *Trends in Biotechnology, 29*(6), 267–275. https://doi.org/10.1016/j.tibtech.2011.02.001

Kim, K.Y., Davidson, P.M. & Chung, H.J. (2001). Antibacterial activity in extracts of Camellia japonica L. petals and its application to a model food system. *Journal of Food Protection, 64*, 1255-1260. https://doi.org/10.4315/0362-028X-64.8.1255

Kosmides, A.K., Kamisoglu, K., Calvano, S.E., Corbett, S.A. & Androulakis, I.P. (2013). Metabolomic fingerprinting: Challenges and opportunities. *Critical Reviews in Biomedical Engineering*, *41*(3), 205-221. https://doi.org/10.1615/critrevbiomedeng.2013007736.

Lu, L., Zhao, Y., Yi, G., Li, M., Liao, L., Yang, C., Cho, C., Zhang, B., Zhu, J., Zou, K. & Cheng, Q. (2021). Quinic acid: A potential antibiofilm agent against clinical resistant *Pseudomonas aeruginosa*. *Chinese Medicine, 16*, Article 72. https://doi.org/10.1186/s13020-021-00481-8

Lulijwa, R., Rupia, E.J. & Alfaro, A.C. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: A review of the top 15 major producers. *Reviews in Aquaculture, 12,* 640–663. https://doi.org/10.1111/raq.12344

Malla, S., Dumre, S.P., Shakya, G., Kansakar, P., Rai, B., Hossain, A., Nair, G.B., Albert, M.J., Sack, D., Baker, S. & Rahman, M. (2014). The challenges and successes of implementing a sustainable antimicrobial

resistance surveillance programme in Nepal. *BMC Public Health,* 14, 1–7. https://doi.org/10.1186/1471-2458-14-269

Martini, N.D., Katerere, D.R. & Eloff, J.N. (2004). Biological activity of five antibacterial flavonoids from *Combretum erythrophyllum* (Combretaceae). *Journal of Ethnopharmacology, 93*, 207–212. https://doi.org/10.1016/j.jep.2004.02.030

Maulidiani, M., Abas, F., Khatib, A., Shitan, M., Shaari, K. & Lajis, N.H. (2013). Comparison of partial least squares and artificial neural network for the prediction of antioxidant activity in extract of Pegaga (Centella) varieties from ^{1}H nuclear magnetic resonance spectroscopy. *Food Research International, 54*, 852-860. https://doi.org/10.1016/j.foodres.2013.08.029

Michaeli, S., Fait, A., Lagor, K., Nunes-Nesi, A., Grillich, N., Yellin, A., Bar, D., Khan, M., Fernie, A.R., Turano, F.J. & Fromm, H. (2011). A mitochondrial GABA permease connects the GABA shunt and the TCA cycle and is essential for normal carbon metabolism. *The Plant Journal, 67*(3), 485-498. https://doi.org/10.1111/j.1365-313X.2011.04612.x

Mine, S. & Boopathy, R. (2011). Effect of organic acids on shrimp pathogen, *Vibrio harveyi*. *Current Microbiology, 63*, 1–7. https://doi.org/10.1007/s00284-011-9932-2

Najafi, S. (2013). Phytochemical screening and antibacterial activity of leaves extract of *Ziziphus mauritiana* Lam. *International Research Journal* of *Applied* and *Basic Sciences, 4*(11), 3274–3276. https://doi.org/10.9734/JAMPS/2021/v23i830252

Ng, W.K., Lim, C.L., Romano, N. & Kua, B.C. (2017). Dietary short-chain organic acids enhanced resistance to bacterial infection and hepatopancreatic structural integrity of the giant freshwater prawn, *Macrobrachium rosenbergii. International Aquatic Research, 9,* 293–302. https://doi.org/10.1007/s40071-017-0177-y

Otsuka, N., Liu, M.H., Shiota, S., Ogawa, W., Kuroda, T., Hatano, T. & Tsuchiya, T. (2008). Anti-methicillin resistant Staphylococcus aureus (MRSA) compounds isolated from *Laurus nobilis*. *Biological & Pharmaceutical Bulletin, 31,* 1794-1797. https://doi.org/10.1248/bpb.31.1794

Phong, H.X., Viet, L.Q., Chau, L.M., Long, B.H.D., Thanh, N.N., Phat, D.T. & Truong, L.D. (2023). Isolation and selection of lactic acid bacteria with the capacity of producing γ-aminobutyric acid (GABA) and antimicrobial activity: Its application in fermented meat product. *Current Nutrition & Food Science, 19*, 831-37. https://doi.org/10.2174/1573401319666221115111236

Phuong, N.N.M., Le, T.T., Camp, J.V. & Raes, K. (2020). Evaluation of antimicrobial activity of rambutan (*Nephelium lappaceum* L.) peel extracts. *International Journal of Food Microbiology, 321*, Article 108539. https://doi.org/10.1016/j.ijfoodmicro.2020.108539

Pisha, E., Chai, H., Lee, I.S., Chagwedera, T.E., Farnsworth, N.R., Cordell, G.A., Beecher, C.W., Fong, H.H., Kinghorn, A.D., Brown, D.M., Wani, M.C., Wall, M.E., Hieken, T.J., Gupta, T.K.D. & Pezzuto, J.M. (1995). Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. *Nature Medicine, 1*, 1046–1051. https://doi.org/10.1038/nm1095-1046

Prakash, O., Usmani, S., Singh, R., Singh, N., Gupta, A. & Ved, A. (2020). A panoramic view on phytochemical, nutritional, and therapeutic attributes of *Ziziphus mauritiana* Lam.: a comprehensive review. *Phytotherapy Research, 35*(1), 63–77. https://doi.org/10.1002/ptr.6769

Priyanka, C., Kumar, P., Bankar, S.P. & Karthik, L. (2015). In vitro antibacterial activity and gas chromatography–mass spectroscopy analysis of *Acacia karoo* and *Ziziphus mauritiana* extracts. *Journal of Taibah University for Science, 9*(1), 13-19. https://doi.org/10.1016/j.jtusci.2014.06.007

Ramalingam, K. & Ramarani, S. (2006). Pathogenic changes due to inoculation of gram-negative bacteria *Pseudomonas aeruginosa* (MTCC 1688) on host tissue proteins and enzymes of the giant freshwater prawn, *Macrobrachium rosenbergii* (De Man). *Journal* of *Environmental Biology, 27,* 199–205.

Ramar, M.K., Henry, L.J.K., Ramachandran, S., Chidambaram, K. & Kandasamy, R. (2022). *Ziziphus mauritiana* Lam attenuates inflammation via downregulating NFκB pathway in LPS-stimulated RAW 264.7 macrophages & OVA-induced airway inflammation in mice models. *Journal of Ethnopharmacology, 295,* Article 115445. https://doi.org/10.1016/j.jep.2022.115445

Ramos-Ruiz, R., Martinez, F. & Knauf-Beiter, G. (2019). The effects of GABA in plants. *Cogent Food & Agriculture, 5*(1). https://doi.org/10.1080/23311932.2019.1670553

Roy, P.K., Song, M.G., Jeon, E.B., Kim, S.H., Park, S.Y. (2022). Antibiofilm efficacy of quercetin against *Vibrio parahaemolyticus* biofilm on food-contact surfaces in the food industry. *Microorganisms, 10*(10), Article 1902. https://doi.org/10.3390/microorganisms10101902

Rukayadi, Y., Lee, K., Han, S., Yong, D., Hwang, J.K. (2009). In vitro activities of panduratin A against clinical Staphylococcus strains. *Antimicrobial Agents and Chemotherapy, 53,* 4529–4532. https://doi.org/10.1128/AAC.00624-09

Saga, T. & Yamaguchi, K. (2009). History of antimicrobial agents and resistant bacteria. *Japan Medical Association Journal*, 52, 103–108.

Schroeder, M., Brooks, B.D. & Brooks, A.E. (2017). The complex relationship between virulence and antibiotic resistance. *Genes, 8*(1), Article 39. https://doi.org/10.3390/genes8010039

Seifi, H.S., Curvers, K., Vleesschauwer, D.D., Delaere, I., Azis, A. & Höfte, M. (2013). Concurrent overactivation of the cytosolic glutamine synthetase and the GABA shunt in the ABA-deficient sitiens mutant of tomato leads to resistance against Botrytis cinerea. *New Phytologist, 199*(2), 490–504. https://doi.org/10.1111/nph.12283

Shelp, B.J., Bown, A.W. & Zarei, A. (2017). γ-aminobutyrate (GABA): A metabolite and signal with practical significance. *Botany, 95*(11)*,* 1015–1032. https://doi.org/10.1139/cjb-2017-0135

Shelp, B.J., Bozzo, G.G., Trobacher, C.P., Zarei, A., Deyman, K.L. & Brikis, C.J. (2012). Hypothesis/review: Contribution of putrescine to 4-aminobutyrate (GABA) production in response to abiotic stress. *Plant Science, 193–194,* 130-135. https://doi.org/10.1016/j.plantsci.2012.06.001

Son, H.S., Kim, K.M., van den Berg F., Hwang, G.S., Park, W.M., Lee, C.H. & Hong, Y.S. (2008). ^{1}H nuclear magnetic resonance-based metabolomic characterization of wines by grape varieties and production areas. *Journal of Agricultural and Food Chemistry*, *56*(17), 8007-8016. https://doi.org/10.1021/jf801424u

Taguri, T., Tanaka, T. & Kouno, I. (2006). Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure. *Biological and Pharmaceutical Bulletin, 29*(11), 2226—2235. https://doi.org/10.1248/bpb.29.2226

Tamokou, J.D.D., Mbaveng, A.T. & Kuete, V. (2017). Antimicrobial activities of African medicinal spices and vegetables. In: Kuete, V. (eds), *Medicinal spices and vegetables from Africa: Therapeutic potential against metabolic inflammatory infectious and systemic diseases.* (pp 207-237). Cambridge: Academic Press.

Tomotake, H., Koga, T., Yamato, M., Kassu, A. & Ota, F. (2006). Antibacterial activity of citrus fruit juices against *Vibrio* species. *Journal of Nutritional Science and Vitaminology (Tokyo), 52*(2), 157-160. https://doi.org/10.3177/jnsv.52.157

Verma, S.S., Verma, R.S., Verma, S.K., Yadav, A.L. & Verma, A.K. (2018). Impact of salt stress on plant establishment, chlorophyll and total free amino acid content of ber (*Ziziphus mauritiana* Lamk.) cultivars. *Journal of Pharmacognosy and Phytochemistry, 7*(2), 556–559.

Vikram, A., Jayaprakasha, G.K., Jesudhasan, P.R., Pillai, S.D. & Patil, B.S. (2010). Suppression of bacterial cell–cell signalling, biofilm formation and type III secretion system by citrus flavonoids. *Journal of Applied Microbiology, 109*(2), 515-527. https://doi.org/10.1111/j.1365-2672.2010.04677.x

Wong, K.C., Brown, A.M., Luscombe, G.M., Wong, S.J. & Mendis, K. (2015). Antibiotic use for *Vibrio* infections: important insights from surveillance data. *BMC Infectious Diseases, 15,* Article 226. https://doi.org/10.1186/s12879-015-0959-z

Yun, N.R. & Kim, D.M. (2018). *Vibrio* vulnificus infection: a persistent threat to public health. *The Korean Journal of Internal Medicine, 33*(6), 1070-1078. https://doi.org/10.3904/kjim.2018.159

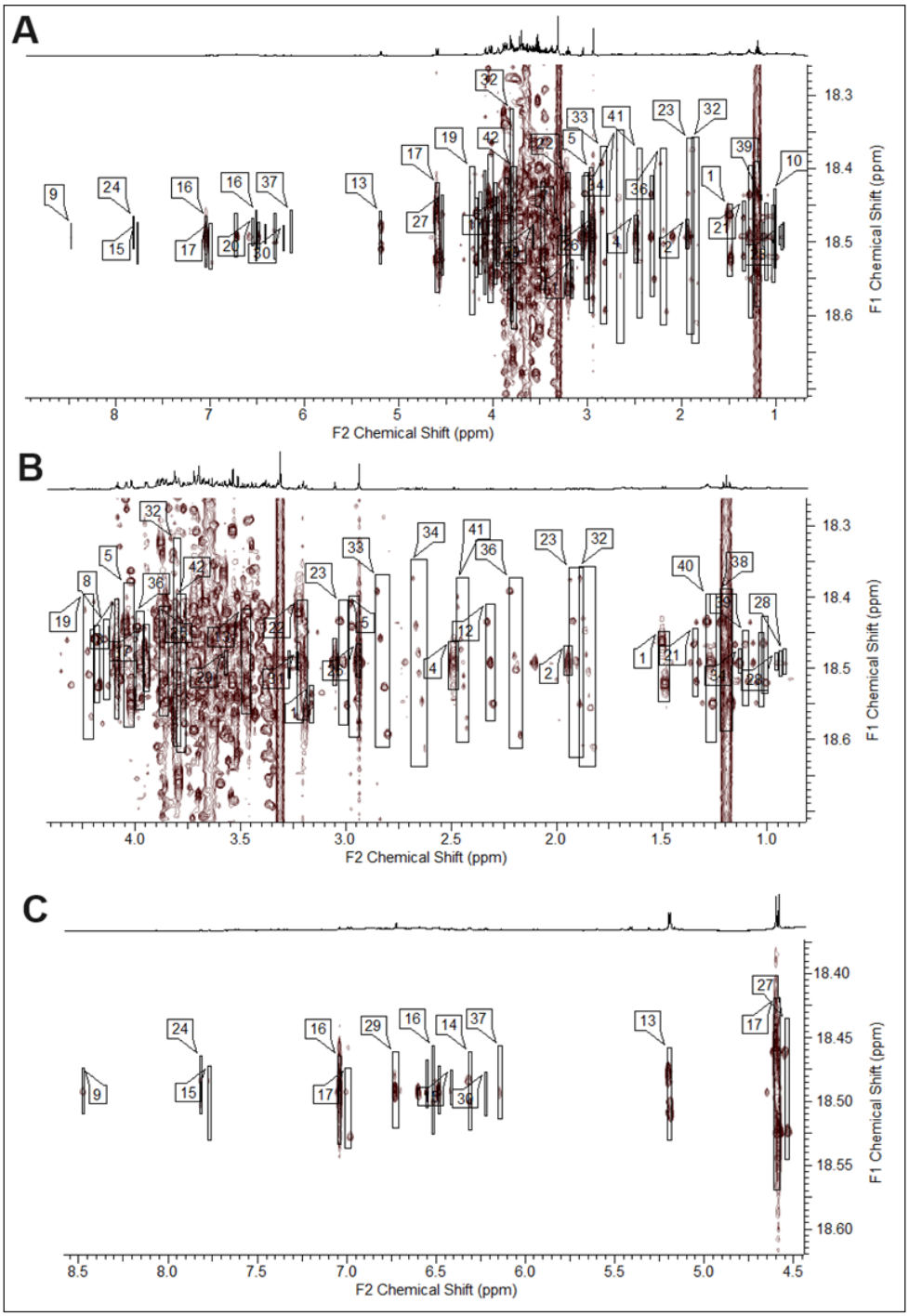

Figure S1. 2D *J*-resolved experiments of *Z. mauritiana* leaves extract: (A) in the region from *δ* 0.85 to 9.00; (B) in the region from *δ* 0.80 to 4.40; (C) in the region from *δ* 4.45 to 8.55. Refer Table 1 for metabolite number interpretation

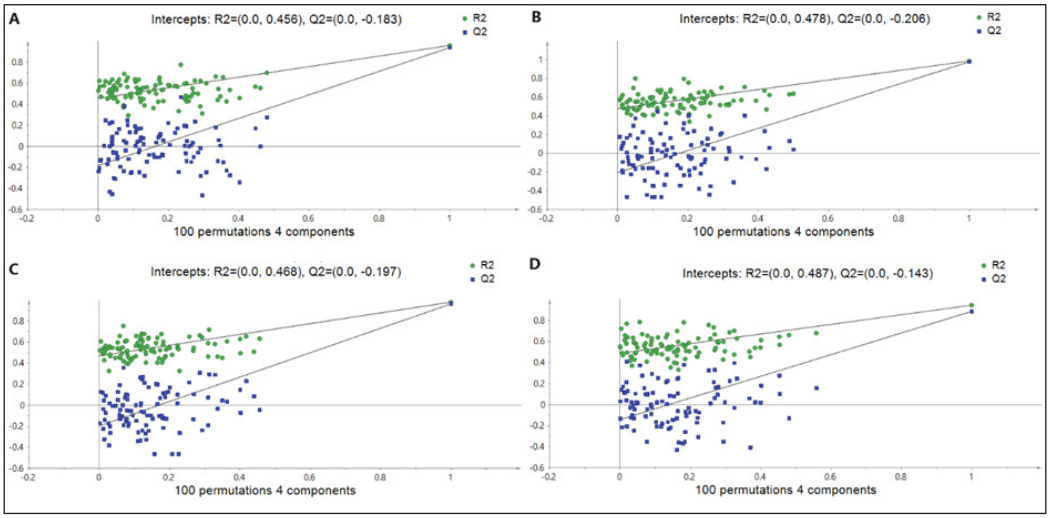

Figure S2. The permutation plots of the developed PLS model generated for the anti-*Vibrio* activity of *Z. mauritiana* extract against (A) *V. parahaemolyticus* (B) *V. harveyi* (C) *V. vulnificus* (D) *V. campbellii*