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Anti-vibriocidal activity and gas chromatography mass spectrometry (GC-MS)-based chemical composition of *Mirabilis jalapa* leaf methanolic extract

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Abstract
Background: Plant produces tremendous diversity of secondary metabolites or chemical constituents which are of great importance to researchers in pharmacology field. The objective of this study was to evaluate the anti-vibriocidal activity and to determine chemical composition of methanolic leaf extract of *Mirabilis jalapa* L., the plant belongs to the family *Nyctaginaceae*.
Results: The preliminary qualitative investigation of *M.jalapa* leaf methanolic extract revealed the presence of Steroids, Coumarins, Alkaloids, Tannins, Glycosides and Phenols. The antibacterial activity was determined against *Vibrio parahaemolyticus* with zone of inhibition of 18.66 mm at 100 µg/mL concentration and Minimum Inhibitory Concentration (MIC) was found to be 62.5mg/mL. The chemical compounds or plant secondary metabolites ascertained based on molecular weights (*m/z*) obtained from gas chromatography-mass spectrometry chromatograms. The obtained spectrum peaks of GC-MS analysis was compared with stored database of National Institute Standard and Technique (NIST) library. A total of 29 chemical compounds were identified in GC-MS analysis, among these the highest peak area percentage was shown by Mome Inositol (46.89%) followed by Decanoic acid (16.64%) and its derivatives. Apart from these, two Phthalic Acid Esters (PAE's) were also found in this study which is believed to be synthesized by plants, fungi, algae and bacteria according to the recent studies.
Conclusion: Evidently, some of these chemical compounds reported several biological activities which support the ethanomedicinal use of *M.jalapa* in treatment of various human ailments and diseases. The identified components in this study support the presence of phytopharmaceutical and ethanomedicinal versality of *M.jalapa* that could be used in the formulation of antimicrobial drugs.

Keywords: Medicinal plant, phytochemicals, *Mirabilis jalapa*, GC-MS, *Vibrio parahaemolyticus*

Introduction
Indian subcontinent has abundant wealth of medicinal and aromatic plants. Since ancient time, several drugs or medicines have been formulated using plant materials [1]. Plants produce tremendous diversity of secondary metabolites called phytochemicals or bioactive compounds which are found in almost all the parts of the plants such as stem, bark, leaves, flower, fruits, seeds and root etc. [2]. Phytochemical constituents such as alkaloids, anthraquinone glycosides, carbohydrates, cardiac glycosides, coumarins, flavonoids, phenols, saponins, steroids, tannins and terpenoids etc., have been isolated from plants and investigated their pharmacological activities thoroughly [3].
Several research studies reported that naturally derived compounds have no or fewer side effects than other synthetic medicines [3]. Many synthetic, non-natural drugs exhibit adverse side effects and some are unable to treat the terminal stages of diseases such as cancer. Several phyto constituents found in plants showed effective pharmacological activities and devoid of side effects [4]. Natural medicines are widely used in many regions of the world to treat many diseases and ailments in humans and non-human animals [5]. Still there are several substances with medicinal or pharmacological activities yet to be revealed in plant kingdom [6].
The preliminary phytochemical screening analysis and quantitative estimation of various phytochemicals do not provide insights into the presence of pharmacologically significant secondary metabolites or chemical constituents. Spectroscopic and chromatographic screening methods can provide the preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological studies.

The determination of phytochemical constituents is primarily done by combining relatively expensive and laborious techniques such as gas chromatography (GC) and liquid chromatography (LC) combined with mass spectroscopy (MS) are very sensitive to detect the pharmacologically active chemical constituents in plant extracts. In recent years, GC-MS has firmly established due to its prominence and reliability to detect metabolomics profiling of both plant and non-plant species, but this method is not suitable for detection of volatile compounds in the sample [7].

GC-MS is one of the best, fastest and most accurate techniques for detecting a wide variety of compounds such as alcohols, alkaloids, nitro compounds, long chain hydrocarbons, organic acids, steroids, esters, amino acids, etc. Conventional separation techniques for natural products have low separation efficiencies and high costs. More importantly, the active ingredient is usually of low content and is lost during the separation process due to irreversible adsorption on the solid support. Recoveries of isolated compounds may be too low for further activity testing. Over the past decade, GC-MS/MS and UPLC-MS/MS have been widely used for the separation and rapid identification of compounds in natural products. However, it is not possible to determine which compounds in a complex mixture respond to which activity using chromatographic mass spectrometry alone [8].

Mirabilis jalapa L. (*Nyctaginaceae*) is a perennial herb and traditionally used medicinal plant for the treatment for various diseases. It is popularly known as four 'O' clock plant and exhibit incomplete dominance for flower colour [5]. Traditionally *Mirabilis jalapa* used for treatment of gastrointestinal disorders, recovery of external wounds, amenorrhea and dysmenorrhea in women and also for the treatment of jaundice and hepatitis [9]. The roots of this plant has hypoglycaemic and hypolipidemic activities in animals [10]. Studies revealed that *Mirabilis jalapa* contain a purgative alkaloid i.e, trigonelline, oxymethyl anthraquinones, arabinose, galactose and ß-sitosterol [11]. Phytochemical investigation and spectroscopy analysis of *Mirabilis jalapa* extracts exhibited numerous bioactive compounds such as proteins, flavonoids, alkaloids, tri-terpenes and steroids. However several studies also found presence of Alpha and Beta amyrins, arabinose, dopamine, daucosterol, campesterol were reported in the *Mirabilis jalapa* extracts [12].

A bacterial disease AHPND (Acute Hepatopancreatic Necrosis Disease) is caused by *Vibrio parahaemolyticus* originally known as Early Mortality Syndrome (EMS), a major threat to the shrimp aquaculture industry. This bacterial species carry an extra chromosomal plasmid pVA1, which encodes binary toxins such as Pir A and Pir B, which are homologous to Photorhabdus insect-related (Pir) toxins. *V. parahaemolyticus* lacks these binary toxins is a non-virulent bacterium. This bacterial species initially colonizes the stomach of the shrimp and infects the hepatopancrease [13].Virulent *Vibrio parahaemolyticus* which can encode binary toxins can also trasmit to the humans through uncooked or raw sea food which is infected with *Vibriosis* and cause the acute gastroenteritis [14]. Initially all Vibrio species infects the stomach of shrimp and cause lethargy, empty stomach and midgut, sloughing of tubule epithelial cells, discoloration of hepatopancreas, damage of R (resorptive), F (fibrillar), B (blister) and E (embryonic) cells of hepatopancreas and hemocytic infiltration in later stages of infection [15].

There are several traditional treatment methods are available to control the shrimp *vibriosis*. Antibiotics such as Azithromycin, Oxytetracycline, oxolinic acid, and florfenicol etc. are used to inhibit *Vibrio parahaemolyticus*, but inappropriate and extensive use of antibiotics leads to the development of antibiotic resistance to almost all the antibiotics and also leaving adverse impact on environment [16]. There is a need to replace the antibiotics with appropriate biogenic, eco-friendly methods to achieve the sustainable aquaculture practices. One of the eco-friendly technologies to reduce the adverse environmental impact is using the phytochemical constituents or secondary metabolites derived from plants [17].

Thus, this study is proposed to investigate the preliminary phytochemical screening and antibacterial activity against *Vibrio parahaemolyticus* of methanolic leaf extract of *Mirabilis jalapa*. The GC-MS analysis is also carried out to investigate the phytochemical constituents.

Methods

Identification and collection of leaves: The plant *Mirabilis jalapa* (Figure 1) identified and confirmed through literature. Fresh leaves were collected from Adikavi Nannaya University, Rajamahendravaram, and Andhra Pradesh, India.

Preparation of leaf *Mirabilis jalapa* extract: The collected leaves were shade dried under sunlight for 3 days and made into fine powder using electrical blender. 25 grams of flower powder was packed and kept it in soxhlet apparatus. 300 mL of methanol was used as solvent and continued up to 7 cycles. The extracted solutions was collected, dried and weighed separately.

Qualitative phytochemical investigation of leaf extracts

The methanolic extracts obtained from *Mirabilis jalapa* leaves were subjected to qualitative phytochemical investigation to determine the plant active constituents with alkaloids, anthraquinone glycosides, carbohydrates, cardic glycosides, coumarins, flavonoids, phenols, saponins, steroids, tannins and terpenoids phytochemical identification tests.

Anti-Vibriocidal efficiency of *Mirabilis jalapa* methanolic leaf extract:

The *Mirabilis jalapa* methanolic extract synthesized from the aqueous leaf extract of *Mirabilis jalapa* was tested for anti-vibriocidal activity on *Vibrio parahaemolyticus* (MTCC 451). The anti-vibriocidal activity of the *Mirabilis jalapa* methanolic extract was determined by the well diffusion assay and micro broth dilution method using marine nutrient agar (MNA) medium. In this method, 10^5 CFU/mL of pathogenic organisms, *Vibrio parahaemolyticus* was taken from pure cultures and was swabbed on Marine nutrient agar (MNB) plates using sterile cotton swab. An approximately six wells with depth of 2.5 mm were made on agar media using sterile gel puncture. The 6 wells were earmarked and WL1 was taken as negative control, and in WL2 20 µl of antibiotic (Azithromycin 20 mg/mL) was added. WL2 to WL6 were added with 25 µl, 50 µl, 75 µl and 100 µl of *Mirabilis jalapa* methanolic extract (20mg/mL) respectively and incubated at 37 ºC for 24 hours. The experiment was carried out in triplicates under aseptic conditions. The mean±SD values of zone of inhibition were calculated [18].

Micro dilution broth was performed to determine the minimum inhibitory concentration (MIC) of *Mirabilis jalapa* methanolic extract according to the *Loo et al.* 2018. This assay was carried out in 96-well micro titer plate using

standard broth dilution method. The bacterial inoculum of pathogen was adjusted to the concentration of 10^5 CFU / mL. Column-1 is taken as negative control and filled with 100 µl of Maine nutrient broth in micro titer plate. 100 µl of *Mirabilis jalapa* methanolic extract stock solution (20mg/mL) was added to the column 1 & 2, and column-11 and 12 served as negative control. Except negative control, 50µl (10^5 CFU / mL) of pathogen was added to the all other columns and plates were incubated at 37 ºC for 24 hours. After incubation the wells were observed for bacterial growth. Formation of turbidity indicates the growth of bacteria. The experiment was run in triplicates and the mean ± SD values of MIC was calculated [19].

Evaluation of Anti-oxidant activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay: Free radical scavenging activity of biogenic silver nanoparticles was determined using DPPH assay. *Mirabilis jalapa* methanolic extract prepared at different concentrations – 20, 40, 60, 80 and 100 µg/mL were agitated thoroughly and added to 2mL of 3 x 10^{-5} M DPPH in methanol was added to all test tubes. The solutions were incubated at 37 ºC in dark chamber for about 1 hour and the absorbance was recorded at 517nm wavelength using UV-Vis spectroscopy. The DPPH scavenging activity of each concentration was calculated by given below formula [20]. Ascorbic acid was used as standard.

DPPH scavenging effect % Inhibition=$A_0 - A_1 / A_0 \times 100$

Where
A_0 =the absorbance of control
A_1 = the absorbance of standard

Gas Chromatography and Mass Spectroscopy (GC-MS) analysis of *Mirabilis jalapa* methanolic extract

Gas Chromatograph: A Shimdzu GC-2010 Plus gas chromatograph was fitted with a 2 mm straight deactivated direct injector liner and a 15 m Alltech EC-5 column (250 µI.D., 0.25 µ film thickness). A split injection was used for sample injection and the split ratio was set to 10:1. The oven temperature program started at 35 °C, held for 2 minutes, then ramped at 20 °C per minute to 450 °C and held for 5 minutes. Helium carrier gas was set at a flow rate of 2 ml/min (constant flow mode).

Mass Spectrum: The software GCMS Solution Ver.2 is used to connect directly to a capillary column metal quadruple mass filter pre-rod mass spectrometer in electron ionization (EI) mode. 2.6 were used for all analyses. Low-resolution mass spectra were acquired at a resolution of 1000 (20% height resolution) with 0.3 s per scan from *m/z* 25 to *m/z* 1000 with a 0.2 s delay between scans. High-resolution mass spectra were acquired at a resolution of 5000 (20% height resolution), scanning the magnet from *m/z* 65 to m/z 1000 at 1 sec/scan.

Mass spectrometry library search

Identification of the compounds' constituents consisted of matching the recorded spectra to the mass spectra of the NIST Library V 11 database provided by the instrument software. A GC/MS metabolomics database was used for similarity searches by retention index.

Results: Preliminary phytochemical analysis of *M. jalapa* methanolic leaf extracts revealed the presence of Steroids, Coumarins, Alkaloids, Tannins, Glycosides and Phenols represented in Table 1. These phytochemical constituents found to be has broad spectrum biological activities.

In the present study, the antivibriocidal activity of *Mirablis jalapa* leaf methanolic extract exhibited efficient antibacterial activity against *Vibrio parahaemolyticus* with zone of inhibition. 18.66 mm at 100 µg (Figure 2 a & b) and the control (Azithromycin) had shown 20.33mm at 100 µg concentration. The minimum inhibitory concentration (MIC) of methanolic leaf extract of *Mirablis jalapa* against *Vibrio parahaemolyticus* is found to be 62.5 mg/ml (Table 2) in the present study.

It is evident from (Figure 4) that the *Mirabilis jalapa* methanolic leaf extract exhibited effective DPPH free radical scavenging activity and compared with the ascorbic acid standard (Figure 3). The concentration dependent % of DPPH inhibition of plant extract was observed in this study. The maximum inhibition at 500 µg/ml was 84.77% and the inhibitory concentration 50 (IC_{50}) value was 63.4287 µg/ml indicating the 63.42 µg/ml concentration of methanolic extract required to inhibit the 50% of DPPH concentration.

Gas chromatography coupled with mass spectrometry (GC-MS) employed to detect the chemical constituents of *Mirabilis jalapa* methanolic leaf extract. The obtained GC-MS spectrum peaks represented in Figure 5. A total of 29 phytochemicals were identified in GC-MS analysis, among these the highest peak area percentage was shown by Mome Inositol (46.89%) followed by Decanoic acid (16.64%) and its derivatives (Table 3). Two important PAE's were also identified in GC MS analysis of this study, which are Bis (2-ethylhexyl) phthalate (1.47%) and Diethyl phthalate (0.53%).

Discussion

Medicinal plants have been used for a long time and their use is widespread in both developed and developing countries. The potentiality of plants as a source of new drugs is still largely unexplored. Medicinal plants are used by all population groups, either directly as folk remedies or indigenous medical systems, or indirectly in modern medicine formulations. Many plants used in traditional medicine are effective in treating various ailments caused by pathogenic organisms. Studies have shown that medicinal plants have broad spectrum biological activities [2].

Phytochemical evaluation of *Mirabilis jalapa* leaf methanolic extract revealed presence of various phytochemicals including polyphenols which are having pharmacological activities. Steroids are major group of plant secondary metabolites and exhibit immense chemical diversity. These plant derived steroids has hypocholesterolemic activity, anti-cancer activity, anti-inflammatory, anti-leishmanial, anti-microbial, immunomodulatory, neuroprotective, and anti-genotoxicity, anti-metastatic, trypanocidal, hypoglycaemic and cholinesterase inhibitory activities [21]. Coumarins are one for the important plant hormones involved in regulation of plant growth and the basic skeleton is made up with benzene and α-pyrone with different substituents on nucleus. These coumarins have anti-cancer, anti-bacterial, anti-coagulant, anti-tuberculosis, anti-fungal, anti-inflammatory, and anti-cholinesterase activities [22]. Alkaloids are major group of biologically important secondary metabolites involved in plant defense mechanism and also with anti-malarial, anti-cholinergic, anti-diabetic, anti-inflammatory, anti-oxidant, anti-depressant, hepatoprotective activity, nootropic, anti-psychotic, anti-hypertensive, anti-diuretic, anti-cancer, anti-stress, anti-arrhythmic, anti-microbial and anti-nociceptive

activities [23]. Tannins are phenolic compounds of plants, which are polymeric proantocyanidins and either oligomeric or gallolyl esters. These tannins have anti-carcinogenic, anti-viral, anti-cancer, anti-bacterial, anti-inflammatory, anti-oxidant, anti-diabetic, α-glucosidase and tyrosinase inhibitory activities [24]. Glycosides are unique group of plant secondary metabolites resembles the structure of steroid saponins with having various pharmacological activities such as antiviral, anti-tumour, and inhibit Na+, K+-ATPase activity and also increase myocardial contraction [25]. Polyphenols or phenolic compounds are one of the widespread secondary metabolites in plants which are essential for development, growth and reproduction of plants. These compounds have important pharmacological activities such as anti-oxidant, anti-bacterial, anti-diabetic, anti-hemorrhoidal, anti-rheumatic, anti-proliferative, anti-depressant and neuroprotective activities [26].

Vibriosis is a bacterial disease caused by gram negative, rod shaped, halophilic and fermentative bacteria belongs to the family *Vibrionaceae* which can infect both shell fish and fin fish and cause huge mortality in natural inhabitants, hatcheries and rearing ponds of commercial aquaculture systems [27]. *Vibriosis* has been described as red leg disease, bacterial septicaemia, AHPND (Acute hepatopancreatic necrosis diseases) and luminescent *vibriosis* etc. Different species of *vibrio* causing *Vibriosis* reported from various studies on shrimp among them *Vibrio parahaemolyticus* is shown high prevalence and 100% mortality [13]. In a study, ethanolic leaf crude extracts of *Mirablis jalapa* exhibited zone of inhibition with 34.33 mm and 51.33 mm at 250 µl (20mg/mL) concentration against *Salmonella typhi* and *Bacillus cereus* [11]. Another study reported, ethanolic and methanolic leaf extracts of *Mirablis jalapa* showed efficient antimicrobial activity against *Pseudomonas aeurginosa*, *Proteus mirabilis* and *Salmonella typhi* with MIC ranged from 300mg/mL to 500mg/mL concentration of plant extract [10]. A study on anti-bacterial activity of *Mirablis jalapa* leaf extracts test on 7 clinical pathogens revealed methanolic extract shown highest activity in well diffusion assay. In contrast, the aqueous extract showed low MIC values against all the tested pathogens [28]. A study reported that zones of inhibition of Green tea extracts against *V. parahaemolyticus* ranged from 10 to 13 mm depending on tea products [29].

Antioxidants are natural or synthetic compounds that slow or prevent damage to cells caused by free radicals or species that cause oxidative stress in cells. Antioxidants must be low in concentration and able to scavenge free radicals or reactive oxygen/nitrogen species. In recent years, several spectrophotometric methods have been used to measure the antioxidant capacity of both natural and synthetic substances are developed [30]. The DPPH free radical scavenging activity was determined in this study. A study reported the of *Mirabilis jalapa* root and aerial parts exhibited 1679µg/ml and 3723 µg/ml IC_{50} values respectively which is higher than the leaf IC_{50} of our present study [31].

Among the identified chemical constituents or secondary metabolites, Imidazole, 2-amino-5-[(2-carboxy)vinyl compound has Antimicrobial and anti-inflammatory activities [32]. Cyclohexanone is used as precursor molecule for the synthesis of various radiopharmaceuticals and synthesis of 6-aminohexanoic acid [33]. Cetylpyridinium chloride or Sixteen alkyl pyridine chloride widely used in formulations of textile softeners, disinfectants, cosmetics and pharmaceuticals [34]. The hexadecanoic acid is a saturated fatty acid, having broad spectrum biological activities such as, antimicrobial, antioxidant, anticancer and anti-haemolytic activity [36]. 1-decanol, a long chain fatty alcohol with bactericidal activity and membrane-damaging activity [37]. 13-Octadecenoic acid is used as Pesticide. Octadeca-9, 12-dienoic acid antimicrobial Skin treatment. Cis-11-Octadecenoic acid and 9-cis-Octadecenoic acid are derivatives of octadecanoic acid has antimicrobial, antifungal and antiviral activities. Gamma-Sitosterol has antidiabetic, antimicrobial and cytotoxicity activities [38]. Mome Inositol is a polysaccharide chain with six hydroxyl groups abundantly found in several medicinal plants. The GC-MS analysis of leaf methanolic fraction of *Mirabilis jalapa* in the present study obtained highest peak are for Mome inositol (46.89%). This compound has anti-proliferative activity against MCF-7, HepG2, Hs27 and MDA-MB-231cell lines [39]. Methyl alpha-D-mannopyranoside or alpha MM, has efficient inhibitory activity against *Escherichia coli* and *Proteus mirabilis* [40]. Cis-11-eicosenoic acid, has efficient antibacterial activity against *Propionibacterium acnes* [41]. Phytol has significant role in management of obesity, diabetes, fatty liver disease, and cardiovascular diseases [42]. Methyl stearate has Anti-inflammatory activity, Antihelmintic activity, Antinociceptive activity and also has inhibitory activity on GABA aminotransferase and Gastrin [43]. Cis-vaccenic acid, a omega-7 fatty acid has antibacterial and hypolipidemic activities [44]. 1, 2- Benzene Dicarboxylic Acid, in combination with Mono 2- Ethylhexyl Ester showed potential cytotoxic activity against HepG2 and MCF- 7 cell lines and has been shown less cytotoxic activity on normal cell lines (HaCaT and NIH 3T3) [45]. Phthalic acid esters also known as PAE's are belongs to liphophilic molecule class, which are widely used as plasticizers and additives because of their mechanical flexibility and extensibility. Synthesized PAE's are causing tremendous hazard to the environment. Recent studies suggesting, PAE's are also synthesized by plants, algae, bacteria and fungi as secondary metabolites and possesses allelopathic, insecticidal, antimicrobial and other biological activities [46]. In contrast to synthesized PAE's, the two compounds Diethyl phthalate and Bis(2-ethylhexyl) phthalate found in this study were also identified through GC-MS analysis in many of the studies and are believed to be involved in enhancing competitiveness of microorganisms, plants, and algae to accommodate biotic and abiotic stress. In algae these PAE's are synthesized possibly via Shikimic pathway [47, 46]. Diethyl phthalate has acetylcholinesterase, neurotoxic activity and antimicrobial activities [35].

Fig 1: *Mirabilis jalapa* plant

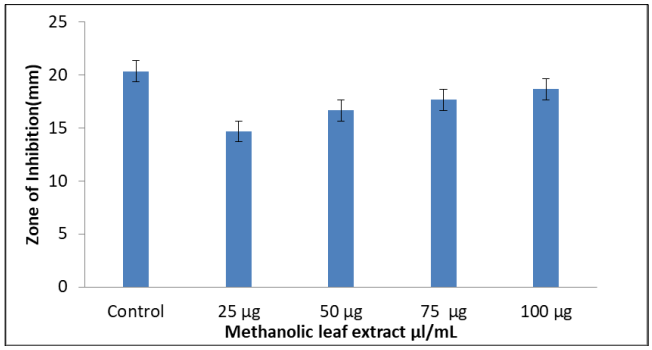


Fig 2(a): The well diffusion assay of *Mirabilis jalapa* methanolic leaf extract against *Vibrio parahaemolyticus*.

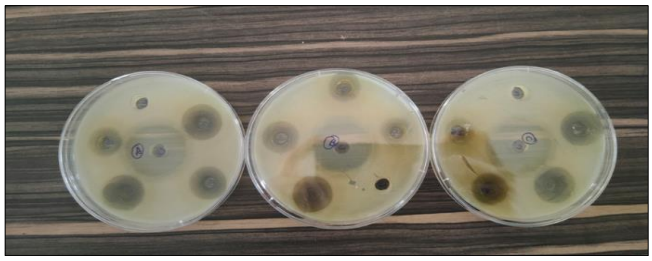


Fig 2(b): The well diffusion assay of *Mirabilis jalapa* methanolic leaf extract against *Vibrio parahaemolyticus*.

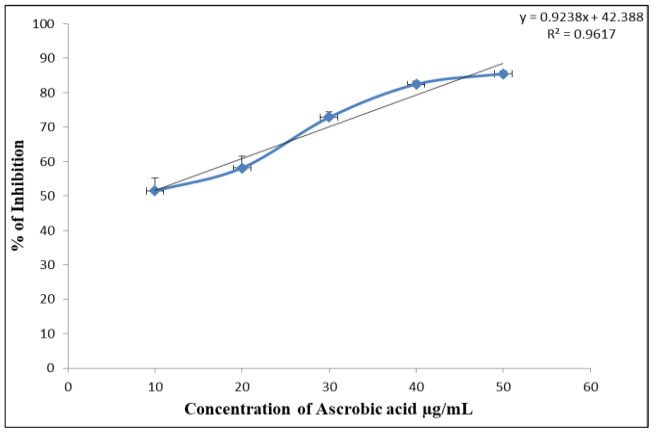


Fig 3: The standard Ascorbic acid for DPPH assay

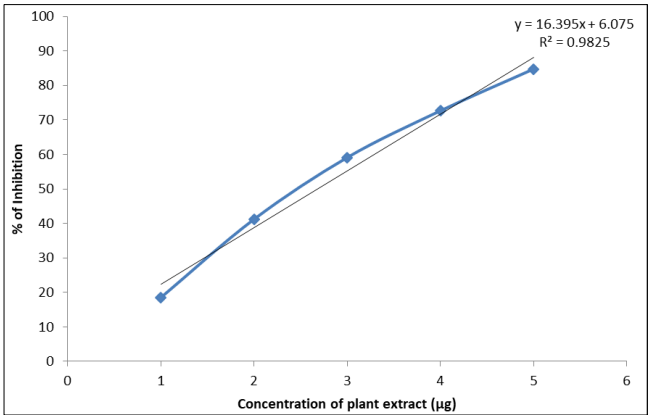


Fig 4: DPPH assay for plant extract

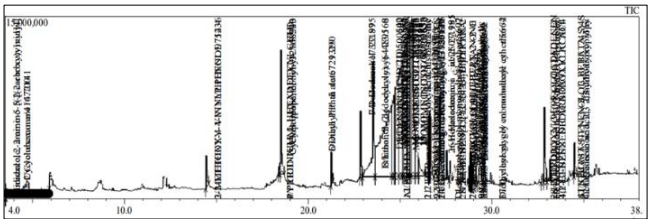


Fig 5: GC-MS chromatogram of Methanolic leaf extract of *Mirabilis jalapa*

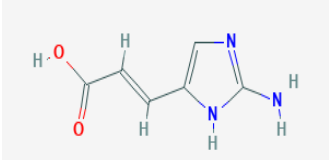
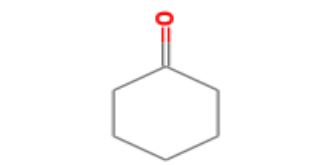
Table 1: Preliminary qualitative phytochemical investigation of *Mirabilis jalapa* leaf aqueous extract

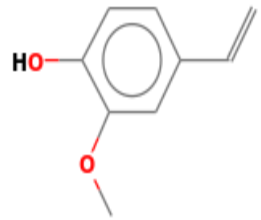
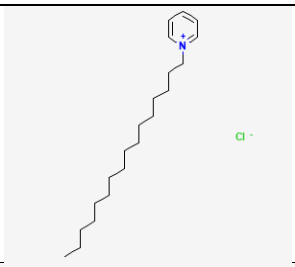
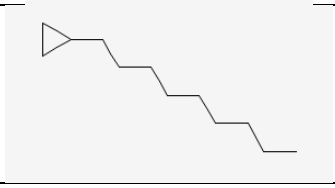
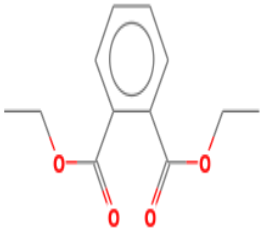
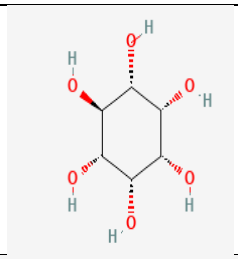
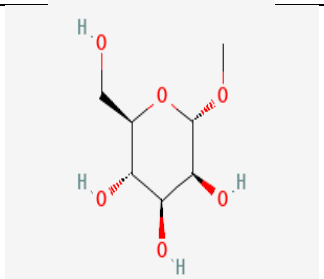
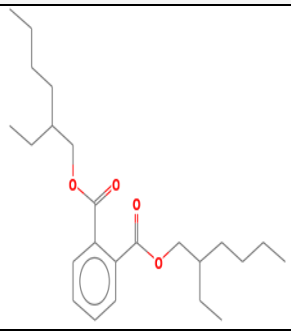
S. No	Phytochemical constituents	Methanolic leaf extract of MJ
1	Steroids	Present
2	Anthraquinones	Absent
3	Carbohydrates	Absent
4	Coumarins	Present
5	Alkaloids (Mayer's)	Absent
6	Alkaloids (Wagner's)	Present
7	Flavonoids	Absent
8	Tannins	Present
9	Terpenoids	Absent
10	Saponins	Absent
11	Glycosides	Present
12	Phenols	Present

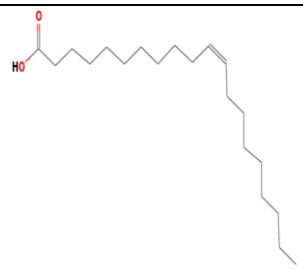
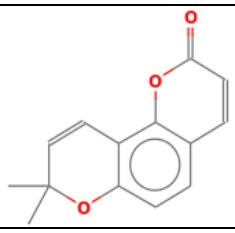
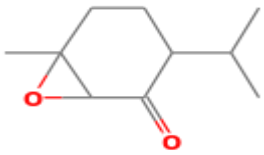
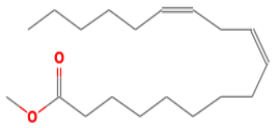
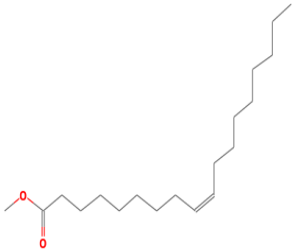
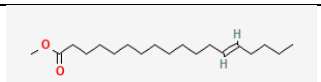
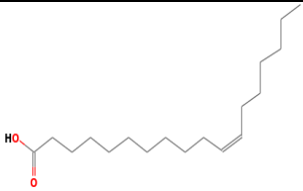
Table 2: The Minimum Inhibitory Concentration (MIC) determined against *Vibrio parahaemolyticus* using methonolic leaf extract of *Mirabilis jalapa.*

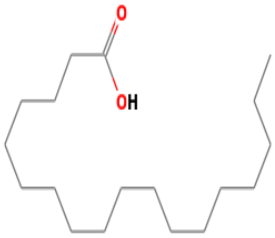
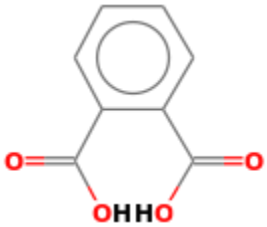
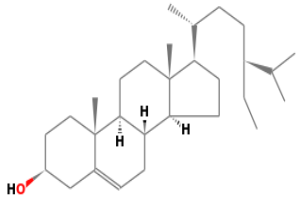
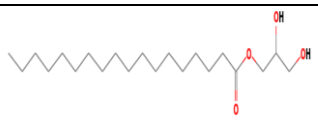
S. No	Minimum Inhibitory Concentration (MIC)
1	62.5 µg/mL

Table 3: Bioactive compounds from methanolic leaf extract of *Mirabilis jalapa* identified using GC-MS analysis

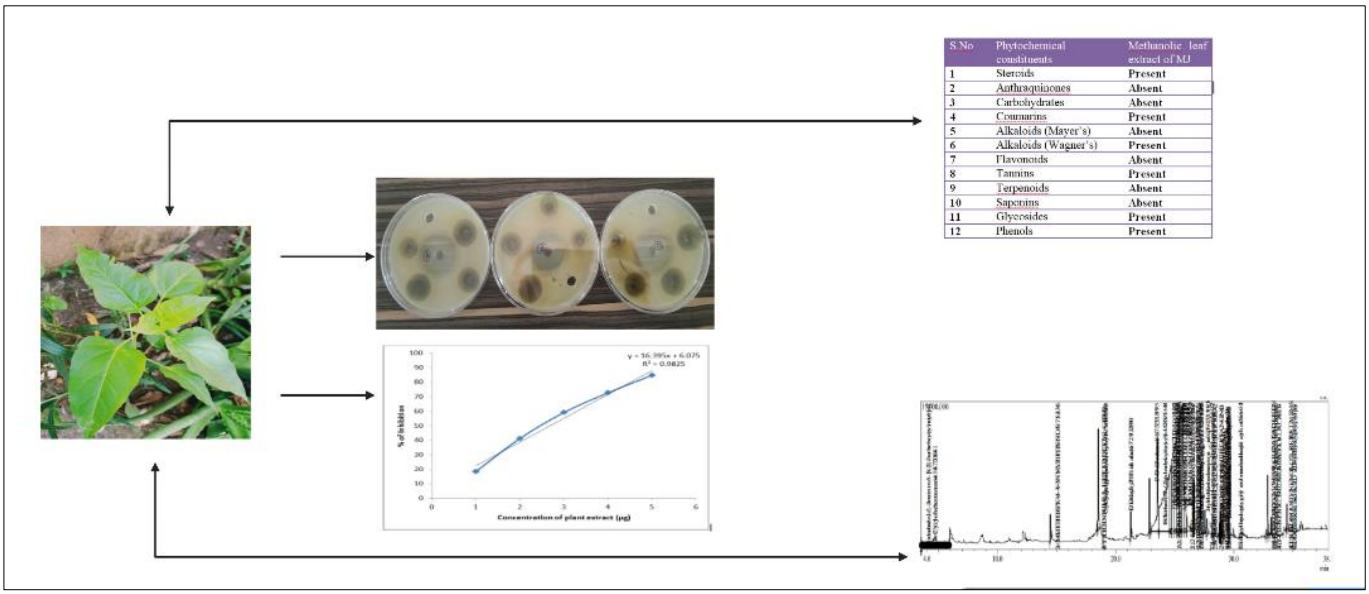
S. No	Name of the compound	Retention time	Area%	Molecular weight	Molecular formula	Molecular structure
1.	Imidazole, 2-amino-5-[(2-carboxy)vinyl	3.529	0.14	153.14	$C_6H_7N_3O_2$	
2.	Cyclohexanone	4.700	3.29	98.14	$C_6H_{10}O$	

3.	2-methoxy-4-vinylphenol	14.484	0.77	150.17	$C_9H_{10}O_2$	
4.	Cetylpyridinium chloride	18.410	0.14	340.0	$C_{21}H_{38}ClN$	
5.	Nonylcyclopropane	18.538	2.54	168.32	$C_{12}H_{24}$	
6.	Diethyl Phthalate	21.291	0.53	222.24	$C_{12}H_{14}O_4$	
7.	1-Dodecanol	22.873	1.37	186.33	$C_{12}H_{26}O$	
8.	Ethanol, 2-(dodecyloxy)-	23.558	6.04	230.39	$C_{14}H_{30}O_2$	
9.	Decanoic acid	24.340	16.64	172.26	$C_{10}H_{20}O_2$	
10.	Mome Inositol	24.595-25.670	46.89	180.16	$C_6H_{12}O_6$	
11.	Methyl alpha-D-mannopyranoside	24.775	7.84	194.18	$C_7H_{14}O_6$	
12.	Bis (2-ethylhexyl) phthalate	25.880	1.47	390.6	$C_{24}H_{38}O_4$	

13.	(Z)-9-Hexadecenoic acid, methyl ester	26.380	0.99	254.41	$C_{16}H_{30}O_2$	
14.	Hexadecanoic acid, methyl ester	26.664	1.04	270.4507	$C_{17}H_{34}O_2$	
15.	cis-11-Eicosenoic acid	26.910	0.19	310.5145	$C_{20}H_{38}O_2$	
16.	n-Hexadecanoic acid	27.186	1.98	256.4241	$C_{16}H_{32}O_2$	
17.	Diethylene glycol monododecyl ether	27.558	0.89	274.4394	$C_{16}H_{34}O_3$	
18.	2H,8H-Benzo[1,2-b:3,4-b']dipyran-2-one, 8,8-dimethyl-	27.748	0.45	228.2433	$C_{14}H_{12}O_3$	
19.	7-Oxabicyclo[4.1.0]heptan-2-one, 6-methyl-3-(1-methylethyl)-	28.334	0.52	168.2328	$C_{10}H_{16}O_2$	
20.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	28.787	0.23	294.4721	$C_{19}H_{34}O_2$	
21.	9-Octadecenoic acid (Z)-, methyl ester	28.858	0.50	296.4879	$C_{19}H_{36}O_2$	
22.	trans-13-Octadecenoic acid, methyl ester	28.930	0.16	296.5	$C_{19}H_{36}O_2$	
23.	Phytol	29.010	0.29	296.5310	$C_{20}H_{40}O$	
24.	Methyl stearate	29.159	0.26	298.5038	$C_{19}H_{38}O_2$	
25.	cis-Vaccenic acid	29.320	0.84	282.4614	$C_{18}H_{34}O_2$	

26.	Octadecanoic acid	29.583	0.68	284.4772	$C_{18}H_{36}O_2$	
27.	1, 2-Benzenedicarboxylic Acid	33.201	0.33	166.1308	$C_8H_6O_4$	
28.	γ-Sitosterol	34.225	0.30	414.7067	$C_{29}H_{50}O$	
29.	Octadecanoic acid, 2,3-dihydroxypropyl ester	34.525	0.67	358.5558	$C_{21}H_{42}O_4$	

Graphical Abstract



The *Mirabilis jalapa L.* leaves contains various phytochemical constituents also exhibited effective anti-vibriocidal activity and DPPH free radical scavenging activity. The GC-MS analysis of crude methanolic leaf extract shown various chemical constituents and some are with different pharmacological activities.

Conclusion

In conclusion, the methanolic leaf extract of *Mirabilis jalapa* revealed efficient anti-vibriocidal activity and DPPH free radical scavenging activity. GC-MS analysis revealed 29 bioactive compounds or phytochemicals, Out of which Mome Inositol shown highest peak percentage. Many of these compounds are polyphenols and having broad spectrum biological activities. There are fewer compounds on which the biological activity was not revealed yet. These identified bioactive compounds can be useful in the development and formulation of antimicrobial drugs.

Abbreviations

AHPND: Acute Hepatopancreatic Necrosis Disease
EMS: Early Mortality Syndrome
Pir: Photorhabdus insect related
GC-MS: Gas Chromatography-Mass Spectrometry
DPPH: 2, 2-Diphenyl-1-picrylhydrazyl
NIST: National Institute Standard and Technique library

Declarations

Ethics approval and consent to participate
Not Applicable

Consent for Publication
Not Applicable

Competing interests
The authors declared no competing interest

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Authors Contributions
AM were involved in supervision and conceptualization of project. PVN and AMR were responsible for methodology, investigation, resources and writing the original draft manuscript. SRM, PBK, RA, IJNP, BSK and SF were involved in data curation, editing and reviewing of the manuscript.

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