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Vibriocidal activity of leaf and rhizome extracts of *Maranta arundinacea* L.

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Abstract: Globally, the gastroenteritis or diarrhoea has become a more significant problem today due to infection caused by foodborne/ waterborne pathogen *Vibrio cholera.* In this concern, an investigation was carried out to evaluate the vibriocidal potential of the different solvent extracts of leaf and rhizome of *Maranta arundinacea* under *in vitro* condition. For this, aqueous, methanolic, ethanolic and hexane extracts of both leaf and rhizome of *M. arundinacea* were tested against the pre-isolated strains of *Vibrio cholerae* such as SPAB1, SPAB4 and SPAB5 by agar well diffusion and minimum inhibitory concentration (MIC) method. All the solvent extracts of both leaf and rhizome were found to be active against the tried strains of *V. cholera* however, ethanolic extract showed maximum inhibitory effect against SPAB1 strain with an inhibition zone of 26.23 ± 0.53 mm (MIC of 80.00 ± 10.06 µg/ ml) and 24.27 ± 0.12 mm (MIC of 100.00 ± 12.82 µg/ ml) in rhizome and leaf samples, respectively. Then, the effectiveness was followed in SPAB4 and SPAB5 however, it was not much more significant to that of SPAB1. Therefore, it was suggested that the rhizome and leaf extracts which proved to be potentially effective can be used as the natural alternative for the treatment of diarrhoea caused by *Vibrio* infection.

Keywords: Cholera; *Maranta arundinacea* L.; Phytochemical; *Vibrio cholerae*; Vibriocidal

1. Introduction

In the modern time, human beings are facing a serious threat from various water-or foodborne bacterial diseases. Amongst them, diarrhoea (caused by the intestinal pathogen) is one of the major concerns, as it is responsible for primary causes of infant mortality especially in developing countries [1]. More specifically, the cholera which is a severe form of acute secretory diarrhoea is the second leading

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cause of mortality worldwide among children under 5 years and also does cause of morbidity in adults [2,3]. According to World Health Organization, it is estimated that the more than one million people are reported by acute diarrhoeal cases annually due to *Vibrio cholerae* infection [4]. Cholera caused by *Vibro cholerae* is one of the most notorious enteric pathogens responsible for many cholera outbreaks, which are motile, Gram negative, comma shape belonging to the family Vibrionaceae [5]. There are approximately 200 recognized serogroups, of which serogroup O1 and O139 are associated with cholera epidemics in humans [6]. The cholera toxin (also known as choleragen) is a protein complex which is secreted by *V. cholerae* mainly responsible for causing copious, painful, watery diarrhoea, leading to vomiting, severe dehydration and even death (if the treatment is not so prompt or even if too late) [7,8].

The emergence and spread of multidrug resistance pathogenic bacteria have created the need for the development of novel therapeutic agents. For which, plants are considered as the best sources of natural products as it cures various diseases from an ancient era [9,10]. Moreover, the World Health Organization certified that the more than 80 % of the World's population relies on traditional medicine for their primary health care needs [11]. During the second half of the 20th century, the acceptance of traditional medicine is increased terrifically and become an alternative option in modern health care as it has no/ less side effects [12,13]. Different plant species containing a wide range of natural products are screened for vibriocidal activities and suggested that the plants are the best potential sources to treat cholera caused by some selective strains of *Vibrio* spp. [14]. It has been also reported that the plants like *Morinda citrifolia*, *Ganophyllum falcatum*, *Leea aequata*, *Lawsonia inermis*, *Saraca indica*, *Syzygium cumini*, *Terminalia belerica*, *Allium sativum*, and

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120 *Datura stramonium* are having better effectiveness against vibriocidal activity with
121 different solvent extracts [15,16].

122 *Maranta arundinacea* L. (commonly known as arrowroot plant) belongs to the
123 family Marantaceae is a medicinally as well as economically important plant. Due to
124 its starchy rhizome, it is widely cultivated in India which yields an easily digestible
125 starch. Rhizome parts are used in confectionery for making of biscuits and weaned
126 foods. Medicinally, the rhizome is utilized for various digestive disorders like
127 abdominal pain, indigestion, acidity and irritation on gastrointestinal system [17,18].
128 The plant also possesses anti-inflammatory, antiseptic and antioxidant activity [19].
129 Castor oil-induced antidiarrheal activity was successfully studied in rat the model [20]
130 however, no evidence is found regarding its vibriocidal activity. Hence, the present
131 study is undertaken to evaluate the vibriocidal activity of leaf and rhizome extracts of
132 *M. arundinacea* against *V. cholerae.*

133 **2. Materials and Methods**

134 *2.1. Collection of Plant Material*

135 Rhizomes of *M. arundinacea* were collected from the coastal fertile belt of
136 Cuttack, Odisha during the month of February 2013 and planted in the garden of Post
137 Graduate Department of Botany, Utkal University. After identification by Dr. P.C.
138 Panda, Principal Scientist, Regional Plant Resource Centre (RPRC), Bhubaneswar
139 and deposition of the specimen with accession number BOTU10573, the further work
140 was carried out within the laboratory.

141 *2.2. Preparation of Various Solvent Extracts of M. arundinacea*

142 The healthy leaves and rhizomes were harvested, washed under running tap
143 water, cut into small pieces and allowed to air dry up to achieving a constant weight.
144 After drying the plant materials were subjected to extraction by using different

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145 solvents systems (aqueous, ethanol, hexane and methanol) with the help of Soxhlet
146 apparatus for 48 hours. The obtained crude extracts were concentrated by rotary
147 evaporator at reduced pressure and stored at 4 °C for further analysis.

148 *2.3. Microorganisms*

149 The microorganisms used in this investigation included 3 bacterial strains of
150 *V. cholerae* like SPAB1, SPAB4 and SPAB5. The isolated bacteria were identified
151 based on morphological and biochemical characters. Identification and confirmation
152 of *Vibrio cholerae* at the molecular level was done by 16s r RNA sequencing and
153 submitted to NCBI GenBank having accession number KT985959.1, KT985960.1
154 and KT985961.1 against SPAB1, SPAB4 and SPAB5, respectively.

155 *2.4. Evaluation of Vibriocidal Activity of Plant Extracts*

156 *2.4.1. Agar Well Diffusion Assay*

157 Agar well diffusion method initially tried for screening of vibriocidal activity
158 and was carried out as per the method of Pervez *et al.,* 1990 [21]. Mueller Hinton
159 Agar (MHA) plates were swabbed with sterile cotton swabs by taking broth culture of
160 *V. cholerae*. This procedure was repeated by twice and rotated the plates
161 approximately 60° each time for even distribution of the inoculums. Wells (6 mm
162 diameter) were made in each of these plates using cork borer. Each extract was
163 checked for vibriocidal activity by introducing 250 µl of 100 mg/ ml concentration
164 into the wells by using sterile micropipettes. The plates were incubated at 37 °C for
165 24 hours. After incubation, the zone of inhibition was measured and expressed in
166 millimetres (mm). Ofloxacin (30 µg) was used as the positive control.

167 *2.4.2. Determination of Minimum Inhibitory Concentration*

168 The evaluation of MIC was determined by applying the method of Akinpelu
169 and Kolawole, 2004 [22]. The different concentrations of extracts (20 µg/ ml to 200

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µg/ ml) were used with the simple dilution processes. Ofloxacin is taken as a standard drug for the vibrocidal activity. MIC was recorded as lowest extracts of concentration demonstrating no visible growth in the broth.

2.5. Statistical Analysis

All results are the mean of three independent experimental replicates (n = 6) and data is reported as mean ± standard error.

3. Results and Discussion

3.1. Well Diffusion Analysis

The various solvent (aqueous, ethanol, hexane and methanol) extracts from the leaf and rhizome samples of *M. arundinacea* showed various degrees of the inhibition against three strains of *V. cholerae* using the agar well diffusion method (Table 1). The results are presented by assessing in terms of inhibition of bacterial growth and compared with control (Ofloxacin). The growth inhibition zone measured ranged from 10.04 ± 0.03 - 26.23 ± 0.19 mm for all the strains of *V. cholerae* and for all extracts. However, the maximum inhibitory zone of diameter (24.27 ± 0.12, 26.23 ± 0.53 mm) were observed in ethanolic extracts of both leaf and rhizome, respectively against SPAB1 and then followed in SPAB4 and SPAB5 (Figure 1). Among all tested extracts, ethanol is highly sensitive and then in methanol, aqueous and hexane. Moreover, the efficacy of rhizome is better than the leaf with respect to both solvents as well as strains (Table 1). The above study significantly points out that both leaf and rhizome of *M. arundinacea* possesses a toxicological impact against Cholera.

Presently there is an increasing demand in herbal medicines by the screening of pharmaceutical or bioactive compounds and their ability to treat various diseases [18,23,24]. Marantaceae plants have been received much more attention since they can produce many complex compounds that are useful for preparation of herbal

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195 medicine [25]. To achieve the effectiveness of vibriocidal activity, various extracts
196 such as aqueous, ethanol, hexane and methanol of leaf and rhizome were tried and
197 significant activeness results were obtained against ethanol extracts which indicates
198 that the ethanol extracts may possess more bioactive compounds and chemical
199 constituents which are responsible for strong vibriocidal activity [26,27].
200 Phytoconstituents mainly phenols, flavonoids, tannins, alkaloids, steroids and
201 terpenoids are responsible for antimicrobial activity which was confirmed from our
202 previous study on *M. arundinacea* with ethanolic extracts [18,20].

203 *3.2. MIC Analysis*

204 The MIC values of different solvent extracts of leaves and rhizomes are
205 represented in Table 2, indicating that the variations are dependent not only as per the
206 solvent system but also depend on the strains of *V. cholerae*. Among the various
207 solvent extracts studied in this assay, ethanolic extract of rhizome exhibited the
208 smallest inhibitory concentration (80.00 ± 10.06 µg/ ml, 90.00 ± 10.82, 100.00 ±
209 16.11) which indicates the maximum prevention power against SPAB1, SPAB5,
210 SPAB4, respectively. However in case of leaf samples, no such significant variations
211 were observed.

212 To support the vibriocidal activity of *M. arundinacea,* the MIC values were
213 determined to know the efficacy of concentration against different strains of *V.*
214 *cholerae* (SPAB1, SPAB4 and SPAB5). The presence of possible constituents was
215 observed even in a low concentration of solvent extracts of leaf and rhizome. Here the
216 same ethanol yielding extracts were more powerful than others which indicate also
217 might be due to dissolving or diffusing nature of bioactive compounds present within
218 plant extracts [24]. Moreover, the hydrophobic and hydrogen bonding nature of
219 phenolic compounds from plant extracts attach to membrane proteins, followed by

involvement in the lipid bilayer do inhibition of spreading of tested bacteria as it is a Gram negative in nature and hence acted as a potent bacteriostatic agent to *V. cholerae* [28,29]. *M. arundinacea* leaf and rhizome ethanol extracts have both growth inhibition as well as minimum inhibition activity against different strains of *V. cholerae* which confirms that the tested plant has efficacy towards antidiarrheal activity.

4. Conclusion

The present study concludes that the ethanol extract of rhizome and leaf of *M. arundinacea* is a better option to inhibit the spreading of *V. cholerae*, which is a very challenging pathogen that can cause cholera to humans. For example, is being used for decades as a medicinal plant against various gastrointestinal disturbances, but to our knowledge, this is the first ever report of its efficacy in controlling growth of *V. cholerae*. Moreover, the study indicates that the experimental plant contains some natural anti-vibrio compounds which may need further isolation and characterization and that may be used in future for human health and disease prevention perspective. For that, additional work is essential at the cellular and subcellular level to understand the mechanisms of action of the active plant extracts.

Author Contributions: Conceived, designed and supervised the experiments, J.R.R., S.L.S. and R.D.; performed the experiments and writing-original draft preparation, P.S. and P.T.; analysed the data and wrote the manuscript, P.S., J.R.R. and R.D.; made critical revisions, C.P. and B.K.P.; all authors read, reviewed and approved the final manuscript.

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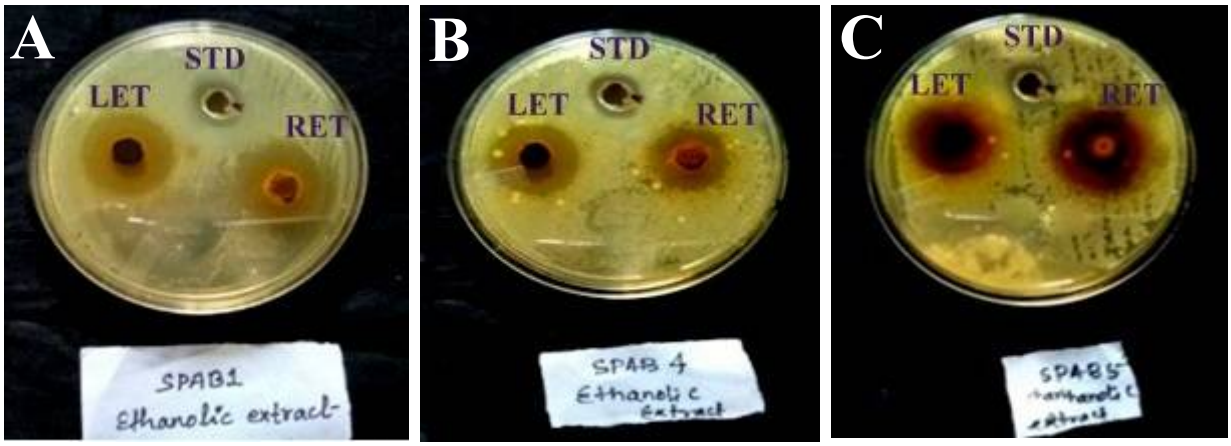

Figure 1. Plate showing zone of inhibition against leaf and rhizome ethanolic extracts of *M. arundinacea* against different strains of *Vibrio cholerae*. **A**: *Vibrio cholerae* strain SPAB1; **B**: *Vibrio cholerae* strain SPAB4; **C**: *Vibrio cholerae* strain SPAB5.

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Table 1: The inhibition zone diameter (mm) of leaf and rhizome extracts of *M. arundinacea* against different strains of *Vibrio cholerae.*

Extracts	Parts used	Strains of *Vibrio cholerae*		
		SPAB1	**SPAB4**	**SPAB5**
AQ	Le	21.08 ± 0.16	20.10 ± 0.28	21.08 ± 0.21
	Rh	22.09 ± 0.78	22.14 ± 0.71	23.16 ± 0.48
ET	Le	24.27 ± 0.12	23.20 ± 0.29	22.15 ± 0.37
	Rh	26.23 ± 0.53	25.20 ± 0.22	24.20 ± 0.12
HE	Le	16.06 ± 0.51	10.04 ± 0.18	18.06 ± 0.54
	Rh	16.18 ± 0.48	15.05 ± 0.46	20.07 ± 0.59
ME	Le	23.20 ± 0.94	24.13 ± 0.38	23.18 ± 0.18
	Rh	25.31 ± 0.41	22.16 ± 0.72	22.18 ± 0.75
Ofloxacin (30 µg)		13.01 ± 0.68	12.06 ± 0.56	11.00 ± 0.33

AQ: Aqueous extract; ET: Ethanolic extract; HE: Hexane extract; ME: Methanolic extract; Le: Leaf; Rh: Rhizome. The data represents mean ± SE of replicates (n = 6).

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381 **Table 2:** The minimum inhibitory concentration (µg/ ml) of the ethanolic leaf
382 and rhizome extracts of *M. arundinacea* against different strains of *Vibrio cholerae*.

Solvent extracts	**Parts used**	**Different strains of *Vibrio cholerae***		
		SPAB1	**SPAB4**	**SPAB5**
AQ	Le	110.00 ± 12.61	120.00 ± 11.55	120.00 ± 12.23
	Rh	100.00 ± 20.17	120.00 ± 13.46	110.00 ± 11.02
ET	Le	100.00 ± 12.82	100.00 ± 14.23	110.00 ± 12.78
	Rh	80.00 ± 10.06	100.00 ± 16.11	90.00 ± 10.82
HE	Le	120.00 ± 12.90	120.00 ± 13.32	120.00 ± 13.22
	Rh	110.00 ± 09.15	120.00 ± 12.26	110.00 ± 12.55
ME	Le	110.00 ± 10.56	120.00 ± 12.44	110.00 ± 12.54
	Rh	90.00 ± 11.15	110.00 ± 09.18	100.00 ± 11.03
Ofloxacin (30 µg)		140.00 ± 11.72	160.00 ± 16.60	140.00 ± 15.91

383 AQ: Aqueous extract; ET: Ethanolic extract; HE: Hexane extract; ME: Methanolic
384 extract; Le: Leaf; Rh: Rhizome. The data represents mean ± SE of replicates (n = 6).
385