

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 water- or foodborne 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 *V. 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. cholerae*; 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 $\mu\text{g/ml}$) and 24.27 ± 0.12 mm (MIC of 100.00 ± 12.82 $\mu\text{g/ml}$) 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.

1. INTRODUCTION

In 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 the developing countries [1]. More specifically, cholera which is a severe form of acute secretory diarrhoea is the second leading cause of mortality worldwide among children under 5 years and also does the cause of morbidity in adults [2,3]. According to the World Health Organization (WHO), 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 *V. 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 cholera toxin) 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 resistant 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 [9–11]. Moreover, the WHO certified that more than 80% of the World's population relies on traditional medicine for their primary health care needs [12]. 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 [13,14]. Different plant species containing a wide ranges 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

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of *Vibrio* spp. [15]. It has been also reported that the plants, such as *Morinda citrifolia*, *Ganophyllum falcatum*, *Leea aequata*, *Lawsonia inermis*, *Saraca indica*, *Syzygium cumini*, *Terminalia belerica*, *Allium sativum*, and *Datura stramonium*, are having better effectiveness against a vibriocidal activity with different solvent extracts [16,17]. Most bactericides have harmful effects on the environments so, natural substances attracted wide interest, while secondary metabolites from higher plants serve as defensive agents against invading microorganisms [18].

Maranta arundinacea L. (commonly known as arrowroot plant) belongs to the family Marantaceae is a medicinally as well as economically important plant. Due to its starchy rhizome, it is widely cultivated in India which yields an easily digestible starch. Rhizome parts are used in confectionery for making of biscuits and weaned foods. Medicinally, the rhizomes are utilized for various digestive disorders, such as abdominal pain, indigestion, acidity, and irritation on the gastrointestinal system [19,20]. The plant is also well known for its anti-inflammatory, antiseptic, and antioxidant properties [21]. It was also reported that the plant is best effective against diarrhoea when applied with castor oil (studied in rat model) [22]; however, no such evidence is found regarding its vibriocidal activity. Hence, the present study is undertaken to evaluate the vibriocidal activity of leaf and rhizome extracts of *M. arundinacea* against *V. cholerae*.

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

Rhizomes of *M. arundinacea* were collected from the coastal fertile belt of Cuttack, Odisha during the month of February 2013 and planted in the garden of Post Graduate Department of Botany, Utkal University (Fig. 1). After the identification by Dr. P.C. Panda, Principal Scientist, Regional Plant Resource Centre (RPRC), Bhubaneswar and deposition of the specimen with accession number BOTU10573, the further work was carried out within the laboratory.

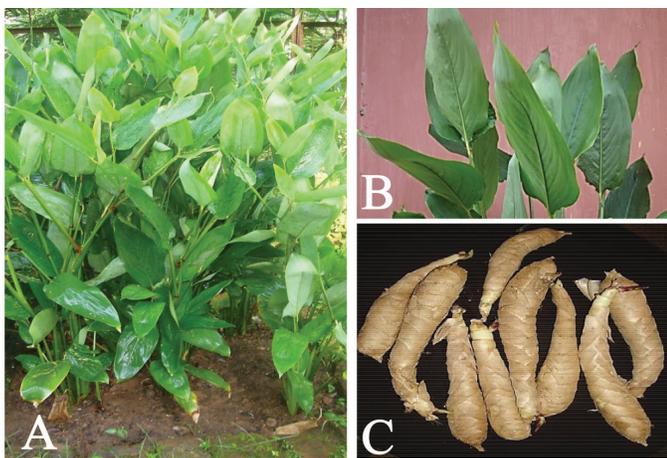


Figure 1: Collection of healthy leaves and rhizomes from garden grown plants of *M. arundinacea*. (A) Healthy plants grown on natural condition; (B) freshly harvested leaves and (C) freshly harvested rhizomes.

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

The healthy leaves and rhizomes were harvested, washed under running tap water, cut into small pieces, and allowed to air dry up to achieve a constant weight. After drying, the plant materials were subjected to extraction by using different solvents systems [aqueous, ethanol (100%), hexane (100%), and methanol (100%)] with the help of Soxhlet apparatus for 48 hours. The obtained crude extracts were concentrated by rotary evaporator at reduced pressure and stored at 4°C for further analysis.

2.3. Microorganisms

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

2.4. Evaluation of Vibriocidal Activity of Plant Extracts

2.4.1. Agar Well Diffusion Assay

Agar well diffusion method initially tried for the screening of vibriocidal activity and was carried out as per the method of Pervez et al. [23]. Mueller Hinton Agar plates were swabbed with sterile cotton swabs by taking broth culture of *V. cholerae*. This procedure was repeated by twice and rotated the plates approximately 60° each time for even distribution of the inoculums. Wells (6 mm diameter) were made in each of these plates using cork borer. Each extract was checked for vibriocidal activity by introducing 250 µl of 100 mg/ml concentration into the wells by using sterile micropipettes. The plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition was measured and expressed in millimeters (mm). Ofloxacin (30 µg) was used as a positive control.

2.4.2. Determination of Minimum Inhibitory Concentration

The evaluation of MIC was determined by applying the method of Akinpelu and Kolawole [24]. The different concentrations of extracts (20–200 µg/ml) were used with the simple dilution processes. Ofloxacin is taken as a standard drug for the vibriocidal activity. MIC was recorded as the lowest extracts of concentration demonstrating no visible growth in the broth.

2.5. Statistical Analysis

All the results are the mean of three independent experimental replicates ($n = 6$) and data are reported as the mean \pm 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* (Table 1). The results are presented by assessing the

inhibition of bacterial growth and compared to that of the 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 the 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 (Fig. 2). Among all the 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 [20,25,26]. Marantaceae plants have been received much more attention since they can produce many complex compounds that are useful for the preparation of herbal medicine [27]. To achieve the effect of vibriocidal activity, various extracts, such as aqueous, ethanol, hexane, and methanol of leaf and rhizome were tried and significant activeness results were obtained against ethanol extracts which indicates that the ethanol extracts may possess more bioactive compounds and chemical constituents which are responsible for strong vibriocidal activity [28,29]. Phytoconstituents mainly phenols, flavonoids, tannins, alkaloids, steroids, and terpenoids are responsible for the antimicrobial activity which was confirmed from our previous study on *M. arundinacea* with ethanolic extracts [20,22].

3.2. MIC Analysis

The MIC values of different solvent extracts of leaves and rhizomes are represented in Table 2, indicating that the variations are dependent not only as per the solvent system but also depend on the strains of *V. cholerae*. Among the various solvent extracts

studied in this assay, ethanolic extract of rhizome exhibited the smallest inhibitory concentration (80.00 ± 10.06 $\mu\text{g}/\text{ml}$, 90.00 ± 10.82 , 100.00 ± 16.11) which indicates the maximum prevention power against SPAB1, SPAB5, and SPAB4, respectively. However, in the case of leaf samples, no such significant variations were observed.

To support the vibriocidal activity of *M. arundinacea*, the MIC values were determined to know the efficacy of concentration against different strains of *V. cholerae* (SPAB1, SPAB4, and SPAB5). The presence of possible constituents was observed even in a low concentration of solvent extracts of leaf and rhizome. Here, the same ethanol yielding extracts were more powerful than others which indicate also might be due to dissolving or diffusing nature of bioactive compounds present within plant extracts [26]. Moreover, the hydrophobic and hydrogen bonding nature of phenolic compounds from plant extracts attach to membrane proteins, followed by the 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* [30,31]. Ethanolic extracts of the leaf as well as the rhizome have exhibited the minimum inhibition activity against different strains of *V. cholerae* which confirms that *M. arundinacea* has capacity to inhibit the growth of diarrhoea causing bacteria.

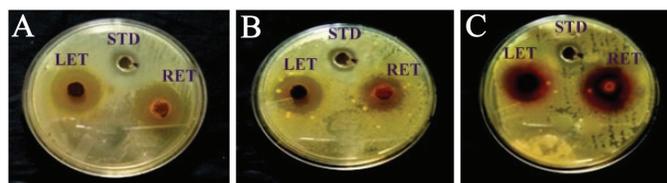


Figure 2: Plate showing zone of inhibition against leaf and rhizome ethanolic extracts of *M. arundinacea* against different strains of *Vibrio cholerae*.

(A) *Vibrio cholerae* strain SPAB1; (B) *Vibrio cholerae* strain SPAB4 and (C) *Vibrio cholerae* strain SPAB5.

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

Extracts	Parts used	Strains of <i>V. cholerae</i>		
		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 \pm SE of replicates ($n = 6$).

Table 2: The minimum inhibitory concentration ($\mu\text{g}/\text{ml}$) of the ethanolic leaf and rhizome extracts of *M. arundinacea* against different strains of *V. cholerae*.

Solvent extracts	Parts used	Different strains of <i>V. cholerae</i>		
		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

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

4. CONCLUSION

The present study concludes that the ethanol extract of rhizome and leaf of *M. arundinacea* is a better and safer 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 the 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 the 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.

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