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Screening of Some Commonly Used Medicinal Plants against Enteric Human Pathogen *Vibrio cholerae*

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Authors' contributions

This work was carried out in collaboration between all authors. Author SC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors LS and MPS managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Present study aims to screen some of the commonly used medicinal plants (*Mangifera indica, Moringa oleifera, Pisidium guajava, Murraya koenigii* and *Ficus infectoria*) and determination of minimum inhibitory concentration (MIC) against enteric human pathogen *Vibrio cholera.*
Place and Duration of Study: Department of Zoology, Ranchi University, Ranchi, between January 2014-July 2014.
Methodology: Plant extraction, antibacterial analysis and phytochemical analysis were Done.
Results: The different methanolic leaf extracts differed in their vibriocidal properties. The MIC values of the plant extracts against test bacteria were in the range of 2-4 mg/mL by a disc diffusion method. *P. guajava* and *F. infectoria* showed promising vibriocidal property at 4 mg/mL concentration. Whereas the other three plants did not show any activity at the highest concentration. Broth dilution method showed 100% inhibition for all the extracts in the range 4-32 mg/mL.

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Conclusion: All the extracts showed positive vibriocidal activity but *P. guajava* and *F. infectoria* showed promising activity and probably can be used as a safe medicine in diarrhea.

Keywords: Vibrio cholerae; diarrhoea; MIC; ZOI.

1. INTRODUCTION

Medicinal plants have been used as traditional medicines for various human diseases for thousands of years. A major cause of morbidity and mortality throughout the world is diarrhoeal diseases which mainly effect the infants and children [1,2]. Resistance to antimicrobial agents, is now an increasing problem throughout the globe [3]. And this antibiotic resistance in bacteria of medical importance the interest is increasing our interest in plants as a source of agents for the treatment of microbial diseases [4]. The most significant bioactive compounds obtained from plants are alkaloids, flavonoids, tannins and phenolic substances [5]. It is a common belief that traditional medicine is cheaper and more effective than modern medicine and are being readily accepted for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components [6]. Research on bioactive substances will definitely lead to the discovery of new compounds, which will result in the formulation of new and more potent antimicrobial drugs, which inturn will overcome the problem of resistance to currently available antibiotics. In India many plants are used traditionally as a medicine for the treatment of gastrointestinal disorders such as cholera, diarrhea and dysentery [7-13]. Various studies have shown that the traditional use of such antidiarrhoeal medicinal plants by investigating the biological activity of extracts of such plants, which suppress gut motility, stimulate water adsorption or reduce electrolyte secretion, have delay intestinal transit, antispasmodic effects [14,15]. Out of the numerous phytochemicals (such as alkaloids, tannins, flavonoids and terpenes etc.) present in active extracts, tannins and flavonoids are thought to be responsible for antidiarrhoeal activity [16-18]. Some of the active ingredients are potentially toxic which should be evaluated for the safety of plant preparations. Few clinical trials have already been evaluated for the safety and tolerability of traditional and herbal medicine preparations which are used to treat diarrhoea and generally indicate that minimal side effects are observed [19].

Vibrio cholerae is the causative agent acute intestinal disease, cholera. This organism has a short incubation period of less than a day, which extends up to five days. The enterotoxin produced by this rod shaped organism causes painless, watery diarrhea leading to vomiting, severe dehydration, and even death if treatment is not prompt [20]. Cholera can spread as an endemic, epidemic, or pandemic. As a treatment antimicrobial agents are administered for 3-5 days; however it is already reported that a single-dose therapy with tetracycline, doxycycline, furazolidone, or ciprofloxacin has been found to be effective in reducing the duration and volume of diarrhea [20].

Several plant and plant products are used in the treatment of cholera traditionally. But most of them have not been investigated pharmacologically so as to demonstrate their antibacterial properties, which could support their use as an anticholera or antidiarrheal remedies in traditional medicine. Therefore the objective of the present study was to evaluate the vibriocidal activities of the extracts of some plants viz. *Mangifera indica*, *Psidium guajava*, *Moringa oleifera*, *Murraya koenigii* and Ficus infectoria used in Indian traditional healthcare system, against enteric human pathogen- *V. cholerae*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The fresh and tender leaves were collected, dried in a shade under room temperature for six to seven days and then crushed into coarse powdery substance by using electric grinder. The coarse powdery substance was dried again and was then sieved to get fine powder using the fine plastic sieve, which was then stored in an air tight bottle in the laboratory until required.

2.2 Extract Preparation

50 g of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature using ~350 mL methanol. The extract obtained was filtered, concentrated after dryness in rotary flash evaporator maintained at 45ºC., percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies.

2.3 Phytochemical Analysis

Freshly prepared extracts of the powdered leaves were subjected to phytochemical analyses to find the presence of the following phyto constituents such as flavanoids, alkaloids, carbohydrates, glycosides, tannins, saponins, steroids, proteins, lipids, oils by standard methods [21,22].

2.4 Anti-bacterial Analysis

2.4.1 Test microorganisms

Vibrio cholerae (MTCC 3906) used during the present experiment was procured from Hi-media which is a potential causative pathogen for different diseases.

2.4.2 Concentrations screened

0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 mg for agar disc diffusion method and for broth dilution method up to 64 mg/mL concentrations were used according to the sensitivity of samples.

2.4.3 Agar diffusion method

Media used consisted of Peptone-10 g, NaCl-10 g and Yeast extract 5 g, Agar 20 g in 1000 mL of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 h. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µL, 104 cfu) and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of samples. The control wells were filled with Gentamycin along with solvent. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zones were noted.

2.4.4 Broth dilution method

Media used consisted of Peptone-10 g, NaCl-10 g and Yeast extract 5 g, in 1000 mL of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 h. The tubes containing above media were prepared, autoclaved and respective concentrations of the samples were added. Each tube was inoculated with 18 h old cultures (100 µL, 104 cfu). A control tube with inoculums and without any sample was prepared along with a sterile media tube as blank. All the tubes were incubated at 37ºC on a shaker with 140 rpm for 24 h and the growth was measured at 660 nm.

3. RESULTS AND DISCUSSION

It was revealed from the results that the leaf extracts showed different degree of inhibition against different microorganisms (Table 1, Fig.1). The diameter of zone of inhibition (ZOI) produced depends on several factors broadly classified as extrinsic and intrinsic parameters. The extrinsic parameters like pH of the medium, period and temperature of incubation, volume of the well, concentration of plant extracts and size of inoculums can be fixed and standardized during experiment, hence no error results due to extrinsic factors [23]. However, intrinsic factors such as nature of medicinal plant including its components, solubility and diffusing property are predetermined. Due to variable diffusibility, the antibacterial with very high potency may not demonstrate ZOI commensurate to its efficacy [23].

The medicinal importance of tannins, alkaloids, saponins, phenols, glycosides and flavonoids recorded in the present study is also common in various antibiotics used in treatment of common pathogenic strains, and these phytochemicals (Table 2) are naturally present in the plant extracts which makes the plant useful for treating different diseases and possess a potential of providing useful and safe drugs and drug leads for human use [24,25].

An ideal antidiarrhoeal agent should possess the ability to reverse (i) the increased luminal osmolarity of osmotic diarrhoea, (ii) the increased electrolyte secretion of secretory diarrhoea, (iii) the decreased electrolyte absorption and (iv) the deranged intestinal motility that causes decrease in transit time [26]. Previous studies have been reported which show that methanolic leaf extract of *M. oleifera* effective against castor-oil induced diarrhoea and significantly reduced the time of diarrhoea and total weight of the faeces and the result obtained establish the efficacy of *M. oleifera* plant extracts as antidiarrhoeal agents [15]. Also reports have shown that the leaf extracts of *Psidium guajava*, *Mangifera indica* possess antibacterial properties and are effective in combating various gastrointestinal disturbances [16].

Table 1. Antimicrobial activity of *Mangifera indica* (M1), *Moringa oleifera* (M2), *Psidium guajava* (M3), *Murraya koenigii* (M4), *Ficus infectoria* (M5) tested against *V. cholerae* by disc diffusion method (Note: NF- MIC not found among the concentrations screened)

Sample	Concentration						
	0.125 mg	0.25 mg	0.5 mg	1.0 mg	2.0 mg	4.0 mg	MIC mg
M1	0	0	0	0	0	0	NF
M2	0	0	0	0	0	0	NF
M3	0	0	0	0	0	2	4
M4	0	0	0	0	0	0	NF
M5	0	0	0	0	0	2	4
Methanol	0	0	0	0	0	0	NF
	25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
Gentamycin	13	15	18	21	23	27	25

Table 2. Phytochemical constituents of of *Mangifera indica* (M1), *Moringa oleifera* (M2), *Psidium guajava* (M3), *Murraya koenigii* (M4), *Ficus infectoria* (M5)

Tests	Samples				
	M1	M2	M3	M4	M5
Carbohydrates	+	+	+	+	+
Glycosides	+	+	+	+	+
Polysaccharides	-	-	-	-	-
Proteins	+	+	+	+	+
Alkaloids	+	+	+	+	+
Steroids	+	+	+	+	-
Triterpenoids	-	+	-	+	-
Flavonoids	+	+	-	-	+
Tannins	-	+	-	-	-
Lipid	+	+	+	+	+
Oils	+	+	+	+	+
Saponins	-	-	-	-	-

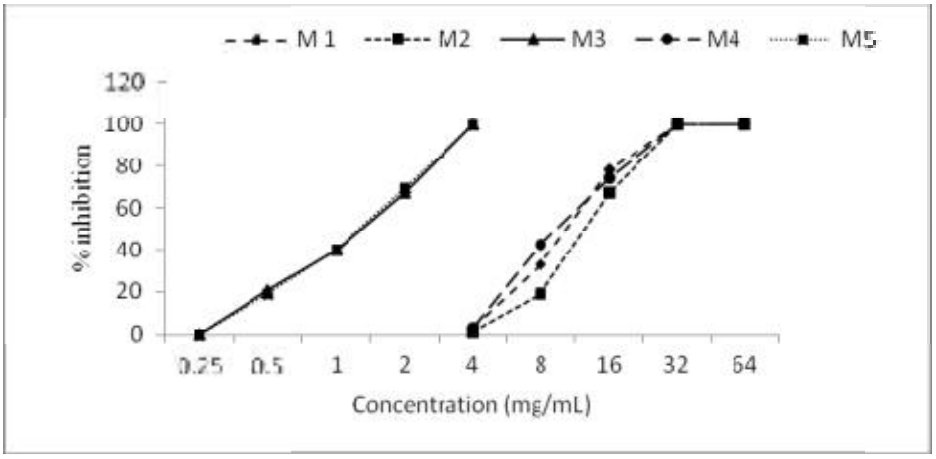

Fig. 1. Inhibition (%) of *V. cholerae* by methanolic extract of *Mangifera indica* (M1), *Moringa oleifera* (M2), *Psidium guajava* (M3), *Murraya koenigii* (M4), *Ficus infectoria* (M5) in broth medium

Alkizim et al. [27] reported that *Mangifera indica* kernel extract inhibited jejunal contractility and with higher doses caused more pronounced effects. This was probably due to increasing availability of agonistic molecules to bind to receptors, hence inhibiting the enteric nervous system (ENS), which inturn inhibit muscularis externa activity.

Out of all methanolic extracts from plants consistently provide more antimicrobial activity than those extracted in other more polar substances [28] and this property of possesing high antibacterial activity of methanol extracts is hypothesized to be due to the polarity of the solvent and to the capability to dissolve or diffuse into the medium used in the assays [28].

This study identified novel role of plant extracts and efficacies in controlling the growth of very challenging human pathogen- *V. cholerae*. For example, Ficus infectoria 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*.

4. CONCLUSION

From the above observations we can conclude that all the plant extracts show promising vibriocidal activities and can be used as a safe medicine in diarrhoea.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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