



# A Famine food, Rhizomes of *Cyperus rotundus* with anti-cholera activity- An *invitro* study

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## ABSTRACT

From prehistoric times, people have used the rhizomes of the sedge grass as a famine food. It is nutritive and stops dental caries and enteric illness. Many *Vibrio cholerae* isolates are moving towards drug resistance, so searching for alternate medicine from the plant kingdom is in progress. Hence, the food cum medicinal plant *Cyperus rotundus* rhizomes extracts was tested for anti-vibrio activity. The methanol and aqueous extract of the rhizomes of *C.rotundus* was tested against *V.cholerae*. The methanol extracts of *C.rotundus* were effective in inhibiting the growth of *V.cholerae*.The MIC value of the extract was 0.30 mg/ml for *V.cholerae*, and for Neomycin, it was 0.20 mg/ml. At a test concentration, 100 ul selected from the 100mg/ml stock concentration showed a maximum diameter of zone of inhibition [20.33 ± 0.33 mm] . When compared to aqueous extract, methanol extract was highly inhibitory in action. Further study is needed to identify the bioactive compounds and to rejuvenate the food value.

Key words:Cholera,Cyperus,Vibrio , MIC value, Antibacterial activity, Famine food.

## INTRODUCTION

Vibrios are gram-negative, highly motile, facultative anaerobes, with one to three whip-like flagella at one end. Their cells are curved rods and strung together in S-shapes or spirals. Three species of *Vibrio* are common pathogens to humans. This includes *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* causing cholera and other acute enteritis.[1]. Vibriosis causes enteric illness in several thousand people globally as a food-borne illness every day. Morbidity and mortality due to diarrhea continue to be a significant problem in many developing countries, including India and Bangladesh, especially amongst children[2]. Antibiotic resistance developed by the vibrio species is a serious problem in treatment. Drug resistance in *V. cholerae* is regulated by genes, and the genes make changes in the permeability of the antibiotics, influencing translational modifications and hydrolysis of antibiotics[3]. So alternative drugs for drug-resistant *Vibrio cholerae* is searched from natural products [4]. The effectiveness of extracts of some plants used in the traditional healing system of India for the treatment of infectious diseases such as cholera and associated gastrointestinal disorders were reported [5]. The elephant garlic oil was reported to be effective in treating *Vibrio cholerae* [6]. The anti-vibrio activity of the extracts of *Caesalpinia sappan* and *Coptis chinensis* were reported [7]. There are reports on the effect of plant-derived products and *Vibrio spp.*, [8]. In the present study, an oil containing medicinal grass *Cyperus rotundus* Family: Cyperaceae ( Nut Sedge ) extract was tested for anti-*Vibrio cholerae* activity. The rhizomes *Cyperus rotundus* Linn was used as food in ancient times. It is reported as a famine food . There are reports on the antibacterial activity of the extracts of the rhizome of *C. rotundus*[9-12]. In prehistoric days ancient people used rhizomes of this nutsedge as food and were free from bacterial infection[13-15].

## Material and Methods

The rhizomes *Cyperus rotundus* Linn. were Collected from The river Tamirabarani basin in Tirunelveli district of India. Botanical identification was carried out by the experts in SPKC college and authenticated. About 500 g of the rhizomes of the grass was separated from fresh plants and washed well in running water and sterile water. The rhizomes were cut into small pieces and shade dried. After drying, the rhizome pieces were manually macerated, and the powder was extracted using methanol and water in the sohxlet apparatus.

The extracts were concentrated using a rotary evaporator, the powder was dissolved in Dimethyl sulphoxide (DMSO), and a 100mg/ml concentration was prepared.

### Antibacterial Assay

*In vitro*, anti-vibrio activities of methanol extracts were determined by disc diffusion assay. In the Petri plates, the *Vibrio cholerae* isolates [VIBL1] were spread plated on Muller Hinton Agar [In each plate, 100 µl inoculums( $10^6$  CFU/ml)]. From the stock concentration of extracts (100mg/ml), three different volumes, 50 µl, 75 µl, and 100 µl, were prepared to load the sterile disc under aseptic conditions. The plates were incubated at 37°C for 24h. Neomycin (30 mg/disc) was used as standard antibiotic disc for comparison. The experiment was performed in triplicate under strict aseptic conditions. The anti-vibrio activity of each extract was the mean diameter of the zone of inhibition (mm). The diameter of the zone of inhibition was compared with aqueous extract. The MIC value was calculated for methanol extract only.

The MIC of *C. rotundus* extract was determined using the microtitre broth dilution method [16]. The MIC of the *C. rotundus* extract was determined by serial dilution, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. From the prepared stock solution of the extract (DMSO) with a concentration of 100mg/ml, serial dilutions were made ranging from 30 mg/mL to 0.25 mg/mL using Mueller–Hinton broth in 96-well microplates according to CLSI guidelines. The *V. cholerae* [VIBL1] isolates prepared from 24 h fresh culture [ $10^6$  CFU/ml and 100 µl was inoculated into the wells. The microtiter plates were incubated at 37 °C, 24 h and added 30 µl (2 mg/ml) of 0.02% p-iodo nitrotriazolium chloride (INT) and incubated at 37°C for 30 minutes. The INT is an indicator of bacterial growth. The bacteria metabolized it and changed it into pink color. If the wells show no change in color after the addition of INT, it indicates the inhibition of the growth of *V. cholerae* and that concentration of the extract were taken as MIC value. Neomycin drug was used as the positive control. 10% DMSO and plant extracts without bacterial suspension were used as the negative controls.

### Statistical analysis

All experiments were performed with three independent replications, and data are expressed as mean value + standard error (mean + SE). Statistical analysis was made with a one-way analysis of variance (ANOVA).

## RESULTS

Antibacterial activity of methanol extract of *Cyperus rotundus* L. rhizomes against *Vibrio cholerae* [VIBL1]

Concentration of extract	Diameter of Zone of inhibition[mm] in Aqueous extract exposure	Diameter of Zone of inhibition[mm] in methanol extract exposure	Diameter of Zone of inhibition[mm] in Neomycin (30 mg/disc )
50 $\mu$ l	9.09 $\pm$ 0.33	17.67 $\pm$ 0.33 *	21.33 $\pm$ 0.33 **
75 $\mu$ l	11.63 $\pm$ 0.33	19.33 $\pm$ 0.33*	
100 $\mu$ l	13.44 $\pm$ 0.33	20.33 $\pm$ 0.33 **	

Values are expressed as Mean  $\pm$  SEM (n=3); \*p<0.01 \*\*p<0.001. The negative control has shown no antibacterial activity; Neomycin as positive control; SEM = Standard Error of mean.



Fig. 1. Photograph showing the diameter of the zone of inhibition expressed by the action of different concentrations of the extracts on *Vibrio cholera*

## Disc Diffusion Assay

The extracts of the rhizome of *C.rotundus* showed a significant inhibition zone in all the concentrations tested.[Table 1 and Figure 1]. Maximum zone of inhibition was observed in 100 µl of the extract ( $20.33 \pm 0.33$  mm), and minimum zone of inhibition was noted in the 50 µl aqueous extract ( $9.09 \pm 0.33$  mm). The inhibitory potential of the methanol extract was superior to the aqueous extract. Also, a concentration of extracts dependent effectiveness of the inhibition was seen. A comparison of diameters obtained in the disc diffusion assay for methanol extract of the rhizomes of *C.rotundus* is close to standard antibiotic tested. The MIC value is the lowest concentration of an antibiotic at which bacterial growth is completely inhibited. The minimum inhibitory concentration was used to determine the effectiveness of the methanol extract. The extract demonstrated a MIC of 0.30 mg/ml for *V.cholerae*, and for Neomycin, it was 0.20 mg/ml.

## DISCUSSION

In this era of increasing drug resistance, efforts are taken to find alternative medicine solutions. In alternate medicine, ethnobotanical knowledge is given a priority. Several bioactive compounds in medicinal plants are rich in antibacterial activity. In the current study, an attempt is made to evaluate the anti-vibrio action of the rhizome extract of the medicinal plant *C.rotundus*. The methanol extract of the plant was compared with aqueous extract, and methanol was found as a good solvent to bring out the antibacterial bioactive compounds present in *C.rotundus*

There are limited data on the antimicrobial activity of *C.rotundus* on many human pathogens, particularly *Vibrio* sp. causing cholera. However, there are reports on antidiarrheal, anti-inflammatory, and antipyretic activities of *C. rotundus* Linn.[17],The essential oil in *C. rotundus* is responsible for bactericidal action against Gram-positive bacteria. The major constituents present in *C. rotundus* are essential oil, triterpenes, polyphenol, alkaloids, and flavonoids[18]. *C. rotundus* has limited activity against different forms of infectious diarrhea due to its selective activity against diarrheal pathogens. In the absence of a marked antimicrobial activity, this plant seems to exhibit antidiarrheal action because of its action on some features of bacterial virulence [19-21]. The present observation shows less anti-vibrio activity in the aqueous extract as suggested by earlier workers[22]. Compounds extracted during the extraction procedure are mainly dependent on the type of the solvent. Water is the primary solvent used in traditional medicine.

But the compounds extracted in organic solvents have exhibited more significant antibacterial activity compared to those extracted in water [23]. A novel compound peroxysesquiterpene glucoside was isolated from the methanolic extracts of rhizomes of *Cyperus rotundus*. This compound was responsible for a good bactericidal, fungicidal, and cytotoxic potential[24].

The MIC value is approved to be the best available parameter to reflect the effectiveness of an antibiotic against bacterial strain. In the current study MIC value observed for *C.rotundus* is almost nearer to the MIC value shown by the standard drug neomycin, the positive control used for the study. It is reported that if the MIC value is equal to or lower than 0.50 mg/ml, it indicates a strong microbial inhibition[25].

## Conclusion

The famine food source *C.rotundus* was found with a good inhibitory activity against the cholera causing *V.cholerae*. The chosen rhizome can be used as food as well as medicine to remediate enteric diseases. The present study concludes that less polar compounds exhibit more antibacterial activity than polar compounds. The less polar methanol was able to extract high concentrations of many phytochemicals like polyphenol compounds, alkaloids, tannins, and saponin with antibacterial action. That may be the reason for the antimicrobial activity of methanol extract. The anti-vibrio action of bioactive compounds in the rhizomes of *C.rotundus* needs to be explored further to identify the compound in the extracts using molecular docking.

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